WHO/V&B/99.12
ENGLISH ONLY
DISTR.: GENERAL

Procurement of vaccines for public-sector programmes

A reference manual

USAID

BASICS

path

DEPARTMENT OF VACCINES AND OTHER BIOLOGICALS

World Health Organization
Geneva

The Department of Vaccines and Other Biologicals thanks the donors whose unspecified financial support has made the production of this document possible.

This document was developed jointly by
the **Access to Technologies Team** of the
Department of Vaccines and Other Biologicals
and
USAID, BASICS and PATH

Ordering code: WHO/V&B/99.12
Printed : May 1999

This document is available on the Internet at:
http://www.who.int/gpv-documents/

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ACKNOWLEDGEMENTS

This manual is the result of a joint effort between the United States Agency for International Development (USAID), through the Basic Support for Institutionalizing Child Survival (BASICS) contract (HRN-C-00-93-00031-00), the Program for Appropriate Technology in Health (PATH) and the World Health Organization (WHO) Global Programme on Vaccines-Vaccine Supply and Quality (GPV/VSQ) unit.

Reviewers and collaborators have included the United Nations Children's Fund (UNICEF), Pharmaceutical Research and Manufacturers of America (PHARMA) and representatives of the United States pharmaceutical industry, the United States Department of Commerce, the United States Food and Drug Administration (USFDA), International Federation of Pharmaceutical Manufacturers Association (IFPMA) and representatives of the European pharmaceutical industry. USAID, the BASICS Project, PATH and WHO/GPV/VSQ wish to thank members of the Zimbabwe Expanded Programme on Immunization for participating in the initial field trial of this document and for their valuable observations.

Major funding was provided by the United States Agency for International Development through the Bureau for Global Programs, Field Support and Research, Office of Health and Nutrition and the Bureau for Europe and the New Independent States, Office of Democracy, Government and Social Reform. WHO wishes to gratefully acknowledge the funding provided by the government of Ireland to support the development of this manual.

Many ideas for an earlier manual upon which this joint document has been based were inspired and/or corroborated by three principle publications for which the authors express grateful appreciation:

- Managing Drug Supply, Management Sciences for Health, 1986
- International Procurement, A Training Manual, Gosta Westring, 1985, sponsored by ITC, UNITAR and The World Bank
- United Nations Commission on International Trade Law Yearbook, Volume XX: 1989

The opinions expressed herein are those of the authors and do not necessarily reflect the views of USAID nor WHO.

Special thanks is extended to Jan J.R. Wolters of Merck Vaccine Division for the following viewpoint:

Precepts for Procurement of Vaccines for Public Sector Programs

The historical role of vaccines in disease prevention is unprecedented in the reduction of devastating epidemics and suffering throughout the world. The substantial investments in research and development of vaccine-based disease prevention have gone hand-in-hand with

investments in public health infrastructure to procure, store and deliver these life-saving medicines. The success of global immunization efforts has depended on leadership and cooperation among many different organizations, including private industry, inspection and testing laboratories, public health ministries, health care providers, bilateral and international donor institutions and non-governmental organizations.

It is appropriate for a document that details the complex procedures and safeguards in managing vaccines procurement, quality assurance and distribution, that the community of organisations involved in these processes reflect on some key precepts for continued success in global immunization efforts. Four fundamental principles can guide and encourage ongoing immunization programs and the development of new vaccines for new and re-emergent diseases.

Strong global leadership for immunization activities that incorporates efficient managerial capacities will provide the fundamental basis for success. This leadership must consist of the top level executives of all the relevant partners in global programmes. Public and private cooperation among health care providers, academia, technical laboratories, public health and finance ministries, the pharmaceutical industry, donor institutions and private sector charitable organisations is essential. It is important for these partners to set up the working mechanisms to provide the strategic planning and review of issues and lessons learned in successful immunization efforts.

Governments, private-sector charitable organisations and foundations and donor organisations must be committed to sustained and increased financial resources for vaccines procurement and immunization programmes. The benefits of immunization in disease prevention must be clearly articulated and quantified in these organisations so that the cost-effectiveness is understood by finance ministries and other health care payers. This is particularly relevant to the development of new vaccines for such worldwide health problems as tuberculosis, malaria and HIV.

The community of partners in global immunization activities must recognise the work of appropriate pricing and tendering in the development, quality assurance and delivery of vaccines. The costs of innovative research, vaccines approval, inspecting and testing procedures, administrative infrastructure in procuring vaccines and adequate storage and distribution require education and training of those involved in the procurement process. Governments and donor organisations have an important role to play in helping countries develop this essential infrastructure.

Both public- and private-sector entities should work towards promoting a general environment that preserves the incentives to invest in research and development of vaccines for the diseases most prevalent in developing countries. These organisations will need to enforce prod-

uct quality and safety through the elimination of corruption which contributes to the high and growing problem of substandard and counterfeit pharmaceuticals. Governments, donor organisations and private industry can play a vital role in strengthening local drug regulatory agencies, customs, storage, distribution systems and patent and judicial offices.

There is every reason to believe that ongoing immunization efforts can be strengthened and that new resources and commitment by global leaders can continue to reduce disease and death throughout the world. The leveraging of knowledge and financial resources through the global partnership of public and private institutions can be assured of success through agreement on common precepts and the will to continue working together on preventing the most destructive infections affecting people throughout the world.

PREFACE

Many government ministries and agencies that, in the past, received vaccines through international donor agencies or a central authority are now assuming responsibility for the procurement and financing of their national immunization programmes. The personnel charged with obtaining vaccines and related supplies often have very little knowledge or experience of international purchasing procedures and are in need of practical advice, particularly with regards to purchasing vaccines on the international market.

The purchase of safe, effective vaccines is a process that requires special expertise and knowledge. Although producers of vaccines in industrialised countries have quality assurance systems and national regulatory bodies that ensure their vaccines meet international standards, nearly half of the world's vaccine supply is produced by manufacturers in countries that have yet to establish the necessary quality assurance procedures. Vaccines produced under these conditions do not always meet the requirements of safety, potency and efficacy. In addition, most vaccines lose their potency if they are transported and stored in conditions where temperature is not strictly controlled. Vaccines that have lost their potency and vaccines of uncertain quality are dangerous. The former do not protect the recipient from the disease and the latter can cause dangerous, sometimes fatal side effects.

Because of the risks related to the use of a vaccine, quality is the overriding consideration governing its purchase. The need for absolute certainty with regard to quality, coupled with the fact that there are a limited number of companies producing vaccines of known good quality, mandates that the purchase of vaccines is undertaken using procedures different from those established for the purchase of pharmaceuticals and other commodities or equipment.

This manual is designed to be an easy-to-use guide and compendium of information on how to purchase vaccines for public-sector immunization programmes. It will be most effective when used in conjunction with the support of specialised technical assistance. Much of the information in the manual can be applied to the purchase of other products but the focus throughout is specific to vaccines.

The procedures selected and described are based on those widely used in public-sector international tendering with a number of variations that are tailored to the special requirements of vaccines. The guidance provided must be used in the context of the legislation in the purchasing country with particular reference to legal and economic systems and administrative structures. However, it must be clearly understood that **in order to ensure the purchase of potent, safe and effective vaccine**, no public-sector purchasing entity should consider purchasing vaccines unless the purchaser's country has established, through appropriate legislation, a regulatory authority for vaccines that is independent, competent and carries out all the essential functions. The manual is therefore mainly directed to the purchase of vaccines by countries that have established such a regulatory authority. Advice also is provided to countries that have not yet estab-

lished a regulatory authority for vaccines on what steps they should take to acquire the necessary vaccines (see page 3)[1].

World Bank procurement guidelines have been used as a basis for the procurement procedures described in the manual because governments are increasingly purchasing critical medical supplies with the financial assistance of the Bank as they move from donor commodity support to financial independence. In addition, many governments have adopted national procedures similar to those of the Bank. It should be borne in mind that the Bank requires adherence to its guidelines when purchases of goods or services are made using IBRD loans or IDA credits.

[1] Advice is provided to countries on what steps they should take to acquire the necessary vaccines if they do not have a National Control Authority with biologicals expertise.

TABLE OF CONTENTS

INTRODUCTION 1

OVERVIEW 5

PART I - OPTIONS 11

A. Procurement Methods 12
B. Procurement Transaction Participants 15
C. Procurement Entities 19
D. Selection Tools 21

PART II - PROCEDURES 27

Introduction 28
A. Planning and Forecasting 29
B. Pre-qualification 35
C. Preparation for Procurement 47
D. Preparation of Bid Documents for Selective Tender 57
E. Preparations for Bid Evaluation 93
F. Solicitation and Receipt of Offers for Selective Tender - LIB 97
G. Evaluation and Comparison of Bids 105
H. The Selection Process 115
I. The Award Process 123
J. The Contract 135
K. Financial Arrangements 139
L. Contract Performance 147
M. Shipment 153
N. Customs Clearance and Delivery 157
O. Closeout Activities 161

PART III - SPECIFICATIONS 165

Introduction 167
A. General Requirements and Standards 168
B. BCG Vaccine (Bacillus Calmette Guerin) 239
C. DTP Vaccine (Diphtheria, Tetanus, Pertussis) 273
D. Measles Vaccine 336
E. OPV (Oral Polio Vaccine) 406
F. Hepatitis B Vaccine 457
G. Yellow Fever 507
H. Tetanus Toxoid 556

PART IV - QUALITY ASSURANCE 567

A. Role of the World Health Organization 568
B. Information for Procurement Personnel 568
C. Donations and Special Offers of Vaccine 571
D. Pre-shipment and Compliance Programmes 573
E. Guidelines for National Authorities on Quality Assurance for Biological Products 583
F. Regulation and Licensing of Biological Products in Countries with Newly Developing Regulatory Authorities 600
G. WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce 608
H. National Control Authority: Guidelines for Assessment of Vaccine Quality in Non-Producing Countries (WHO/VSQ/95.1) 620
I. GPV Policy Statement (WHO/VSQ/97.05) 639

PART V - THE UNICEF OPTION 643

A. UNICEF Standard Procurement Services 644
B. Vaccine Independence Initiative 645
C. UNICEF Purchase Agreement/General Conditions 645
D. Minimum Requirements for Vaccines to be Supplied for UNICEF and Model Inserts 653
E. UNICEF Quality Assurance Program 662
F. UNICEF Application of Vaccine Vial Monitors 677
G. UNICEF Pricing 677

PART VI - GENERAL INFORMATION 679

A. Price and Cost Data 681
B. Vaccine Sources and Supply Survey 692
C. Payment Options 705
D. International Commerce 712
E. International Shipping Documents 721
F. Transportation and Storage 750
G. Vaccine Temperature Monitoring 782
H. Vaccine Vial Monitors 799
I. Vaccine-Related Products 806

PART VII - PROCUREMENT UNDER WORLD BANK PROCEDURES 827

A. What The Bank Is 828
B. What The Bank Does 828
C. General Conditions of a World Bank Loan 828
D. How Bank Projects Are Developed 829
E. How Bank Loans are Linked to Bank Projects 829
F. How Bank Projects Are Implemented 830
G. How Bank Projects Are Monitored 830
H. What The Bank Usually Monitors in the Area of Procurement 830
I. Why The Bank Is Concerned About Procurement Methods and Procedures 831
J. General Timeframes Associated With World Bank Projects 831
K. Important Publications Associated With Procurement Under World Bank Projects 831
L. Special Issues of Vaccine Procurement Under World Bank Projects . 832
M. Special Clauses for Vaccine Procurement Under World Bank Projects 832

ACRONYMS 951

GLOSSARY 953

GENERAL INDEX 969

INDEX OF FORMS, SAMPLES AND SUPPLEMENTS 975

INDEX OF WHO DOCUMENTS 979

The Department of Vaccines and Other Biologicals was established by the World Health Organization in 1998 to operate within the Cluster of Health Technologies and Pharmaceuticals. The Department's major goal is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases.

The Department replaces the former Global Programme for Vaccines and Immunization. Five teams implement its "bench-to-bush" strategy, which starts with the establishment of norms and standards, focusing on major vaccine and technology issues, and ends with implementation and guidance for vaccination programmes. The work of the teams is outlined below.

The **Quality Assurance and Safety of Biologicals Team** ensures the quality and safety of vaccines and other biological medicines through the development and establishment of global norms and standards.

The **Vaccine Development Team** coordinates and facilitates the development of new vaccines and immunization-related technologies.

The **Vaccine Assessment and Monitoring Team** assesses strategies and activities for reducing morbidity and mortality caused by vaccine-preventable diseases.

The **Access to Technologies Team** endeavours to reduce financial and technical barriers to the introduction of new and established vaccines and immunization-related technologies.

The **Expanding Immunization Team** develops policies and strategies for maximizing the use of vaccines of public health importance and their delivery. It supports the WHO regions and countries in acquiring the skills, competence and infrastructure needed for implementing these policies and strategies and for achieving disease control and/or elimination and eradication objectives.

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INTRODUCTION

The user of this guide to the procurement of vaccines may wonder why it has been necessary to create such a substantial document. A wealth of text has been written about the theory and practice of procurement in general but very little is available to explain, in detail, how to go about doing this work. In addition, vaccine procurement requires specialised knowledge and a careful approach. The authors have attempted to deliver, in one comprehensive document, a step-by-step model for vaccine procurement, critical background information and basic reference materials of importance to procurement personnel in developing and transitional country programmes.

Vaccine quality issues

Vaccine quality cannot be assured by all suppliers. At present, no more than 25 manufacturers in the private sector produce vaccines of a sufficient quality and quantity suitable for use in immunization programmes in any country around the world. There are a number of public-sector producers making high quality vaccines, but most of these do not export their products. On the other hand, there are many producers of vaccines of variable quality in countries with inadequate regulation of both the conditions of manufacture and the quality of the product. With vaccines, quality is critical.

Vaccine production is a biological process, using living organisms as raw material. Thus, the character of each batch is subject to variation and the quality of the finished product cannot be determined solely by laboratory testing. Quality control requires full compliance with Good Manufacturing Practices (GMP) and with Good Laboratory Practices (GLP). It is essential that quality is ensured from the first steps in the production process to the final packing of the product.

Vaccines are heat sensitive and must be stored and transported in a “cold chain”. Certain vaccines are damaged by freezing and light as well. Even under the most favourable conditions, vaccines have a very limited shelf life (a maximum of two years).

Vaccine quality is not only important in and of itself; the credibility of an immunization programme is highly dependent on it. Children immunized with vaccines of poor quality may die from the disease that the vaccine should have prevented. Such deaths could destroy public confidence in an immunization programme and thus place even more lives at risk.

Vaccines have a national impact on public health. Immunization programmes are designed to reach all children and all women of child-bearing age in a country. Vaccination is generally mandatory, so the “consumer” has no choice. Vaccination is also preventive and therefore administered to a healthy person with the expectation of preventing illness. The consumer cannot judge the quality of the protection provided. It is therefore the responsibility of the immunization programme to ensure that the vaccine purchased is in compliance with

internationally and nationally accepted standard requirements and that the quality and efficacy of the vaccine is maintained throughout its receipt, transport, storage and use.

The need for competent procurement

The above factors show that effective vaccine procurement requires specialised knowledge and a careful approach. Vaccines should always be purchased by a competent procurement entity following established procedures, with due regard for their special nature. Ideally, countries should be able to buy safe, effective vaccines at reasonable prices; if not, they should have a plan in place to upgrade their staff skills. In the absence of vaccine procurement expertise, a country may need to use a purchasing agent. Options here include:

- purchasing vaccines using a commercial agent, with instructions from the National Regulatory Authority and Expanded Programme on Immunization (EPI) programme staff,
- purchasing vaccines using the guidance of competent procurement staff of another country, or
- purchasing through the United Nations Childern's Fund (UNICEF) or World Health Organization (WHO).

A competent regulatory authority

It is essential that any country in which vaccines are manufactured have a regulatory authority. It is equally important for non-producing countries to have a regulatory authority to manage vaccine imports, whether the vaccines are purchased by a government procurement entity or by international agents.

In order to ensure the quality of the product purchased, it is important that no government undertake the procurement of vaccines unless they have established, through appropriate legislation, a national regulatory authority which includes competency for vaccines. Such an authority, which is often an autonomous part of a national drug regulation body, must be properly staffed and have the authority to license vaccines for use in the country, to establish procedures for lot release and to create a post-marketing surveillance structure.

The following summary of the functions of such a regulatory body provides a general context for the links between the regulation of vaccines and the purchase of the product. More information on the functions and responsibilities of a national regulatory authority can be obtained from WHO publications (see Part IV—Quality Assurance, page 567).

Critical Control Functions

Vaccine Source	Licensing	Surveillance	Lot Release	Laboratory Access	GMP Inspection	Clinical Evaluation
UN Agency	√	√				
Procure	√	√	√	√		
Produce	√	√	√	√[1]	√	√

The terms of reference of the regulatory authority established by government legislation should provide the authority to:

- define the criteria for assessing the licensing of vaccines to be used in the country;
- issue, vary or withdraw licenses for the importation and use of vaccines on the basis of safety, potency and efficacy;
- continually oversee the quality of the vaccines by releasing each lot intended for use in the country, using the manufacturer's Summary Lot Production Protocol as the minimum basis for review and
- monitor the impact of vaccines in use through a well-functioning surveillance system that provides for the possibility of taking regulatory action if problems are detected.

The procurement entity should work closely with the national regulatory authority for vaccines since the main responsibility for specifications and for quality management rests with this body. The importance of the regulatory authority will become even more evident as the user of this manual makes use of the procedures described.

Different countries use different terminology to describe the regulatory authority. Throughout this manual the term "National Control Authority"[2] has been used generically as the abbreviation "NCA". Whatever the term used, the functions are the same and should be ensured by appropriate legislation.

Where there is limited or no regulatory authority for biologicals

Ideally, every country should have a biologicals committee within its national pharmaceutical regulatory authority. If it does not, it should have, at a minimum, a system in place for registering vaccine imports, reporting adverse events and recalling vaccines if required by the manufacturer. Surrogate regulatory controls may need to be considered. These include:

- purchasing vaccines following the advice of WHO as to acceptability of the vaccine in principle,

[1] A regulatory authority in a country with no vaccine production might arrange for collaboration with a laboratory in another country.

[2] See page 17.

- purchasing vaccines using the guidance and oversight of a national control authority of another country (for example, the United States Food and Drug Administration—USFDA), or
- purchasing vaccines through UNICEF or WHO.

The responsibilities of the EPI manager

In addition to the responsibilities of managing the implementation of the immunization programme, the EPI manager must ensure that the specifications of the vaccines will meet the needs of the programme. While the technical specification of the vaccine is the responsibility of the regulatory authority, the EPI manager must specify physical characteristics, such as vial size (i.e., number of doses per vial most suited to the programme strategy), the packaging and the information contained in the package insert and on the vial label, including the language used in the insert and label. These requirements can vary from year to year depending upon the immunization strategy being used to combat a particular disease. It is therefore essential that full consultation on the programme needs take place before the tender for a new supply of vaccine is launched.

If EPI programme management is inadequate

Purchased vaccines must meet programme needs, with detailed specifications that match training materials, cold chain performance and other programme realities. A country should be able to determine its own needs; however, if it cannot, it should have a plan in place to upgrade its staff. In the absence of this expertise nationally, surrogate expertise must be sought, such as:

- purchase following WHO advice as to the acceptability (in principle) of the vaccine,
- purchase using the guidance of competent EPI staff of another country or
- purchase through UNICEF or WHO.

These options will help to ensure the provision of standard products that are suitable for most programmes.

Conclusion

Vaccine procurement clearly requires specialised approaches. The supply of good quality vaccine depends on careful procedures, a national authority overseeing the quality of vaccines to be purchased and an EPI manager to ensure that vaccines meet programme needs. The strongest of links is between the procurement entity and these two other bodies. The various sections that form Part VI of the manual contain additional information on vaccine procurement from the regulatory authorities of major producing countries, from major producers of vaccines and from the Global Programme for Vaccines and Immunization (GPV) at WHO headquarters in Geneva.

OVERVIEW

About this manual...its architecture and its contents

Procurement of vaccines for public-sector programmes - A reference manual is made up of two volumes, divided into seven parts. They are designed to fit into standard A-4, two-hole binders. Volume One (Parts I, II and III) contains basic introductory and operations material and vaccine specifications. Volume Two (Parts IV, V, VI and VII) contains a compendium of frequently needed reference material and supplementary information, as well as a glossary and indices.

A summary table of contents appears at the beginning of Volume One, outlining the basic information contained in both volumes. A separate table of contents appears at the beginning of each of the seven sections, outlining the contents of that section in more detail. Pages are numbered continuously from the beginning of Volume One through the end of Volume Two.

A one-page table of the manual layout appears on page 6, followed by a brief description of the contents of each of the seven Parts.

A system of Reference Boxes located to the right of the main text alerts the reader to the existence of related information elsewhere in the manual and gives its location. See additional discussion under Part II below.

Advice to new vaccine buyers on how to use this manual

Make yourself familiar with the architecture and basic features of the manual.

- Review the one-page table and brief description of the seven Parts.
- Review the main Table of Contents.
- Locate the beginning of each Part and review its detailed Table of Contents.
- Notice that supplementary material is located between sections of text.
- Pick a Reference Box in the main text (Part II contains many) and locate the additional material it mentions.
- Locate the Glossary and notice the words that have been included.
- Locate the Index and notice some of the phrases that have been included.
- Look at several of the flow charts in Part II located at the beginning of each section. Notice the column headings—Funder / Supplier, Procurement Team, Ministry / Senior Programme Management, Resources—and the activities listed under each.

Read Parts I, IV and V.

Use Part II as an operations model.

Procurement of Vaccines for Public-Sector Programmes*

Acknowledgements
Preface
Table of Contents
Introduction
Overview

Part I	**Part II**	**Part III**	**Part IV**	**Part V**	**Part VI**	**Part VII**
Options	**Procedures**	**Specifications**	**Quality Assurance**	**UNICEF**	**General Information**	**World Bank**
Page 11	Page 27	Page 165	Page 567	Page 643	Page 679	Page 827
Volume 1	Volume 1	Volume 1	Volume 2	Volume 2	Volume 2	Volume 2
Methods	Step-by-step A-O with Supplementary Information and Implementation Tools	General Requirements	Role of WHO	Procurement Services	Price Data	What the Bank Is
Participants		BCG	Information for Procurement Personnel	VII	Sources	What the Bank Does
Procurement Entities		DTP	Donations and Special Offers	Purchase Agreement	Payment Options	General Loan Conditions
Selection Tools		Measles	Pre-Shipment Compliance	Minimum Requirements	Letter of Credit	Developing Bank Projects
		OPV	Collection of WHO Documents	QA Programme	INCOTERMS	Linking Loans to Projects
		Hep B		VVM	Shipping Documents	Project Implementation
		Yellow Fever		Pricing	Transportation and Storage	Project Monitoring
		TT			Related Products	Procurement Monitoring
						Procurement Concerns
						General Timeframes
						Procurement Guidelines
						Vaccine Procurement
						Special Clauses

Acronyms
Glossary
Index

*Approximately 1000 pages contained in 2 volumes.

Use Parts III, VI and VII for reference.

Rely on page numbers to find the beginning of specific Parts or documents.

PART I - OPTIONS

Several methods of procurement are available to public-sector entities, as well as choices as to who will actually execute procurement transactions. Part I explains these variables.

PART II - PROCEDURES

Part II, the main focus of this reference manual, contains a step-by-step guide for international competitive procurement of vaccines. In order to meet the needs of both those seeking only a brief overview of international procurement and those requiring in-depth information, the material is presented on three increasingly detailed levels:

- At the most basic level, the face page for each section A through O shows a flow chart summary of steps within each task, roughly divided into two main columns according to who is normally expected to execute the step, i.e., the Procurement Team or Ministry / Senior Programme Management / EPI. In addition, a column at the right indicates resources the procurement team may need to call upon and a column at the left shows points of contact with the funder and the supplier.
- The main content of each section A through O provides the next level of detail. Task steps indicated by the flow chart are expanded and presented in outline form along with supporting directions, justifications and considerations.
- The third level of information is contained in Forms, Samples and Supplements. **Forms** are blank documents to be copied and used as is or modified for specific applications. **Samples** are completed forms. In most cases, they are actual examples useful for guidance in filling out forms; they are not intended as a case study with a single vaccine procurement process. **Supplements** contain additional information on a particular topic.

  Forms, Samples and Supplements are labelled with the corresponding Part, Section and paragraph numbers of the text that they augment.

  Boxes located to the right of main text alert the reader to the existence of related Forms, Samples or Supplements and provide a key to locating it, usually at the end of the Section, but sometimes in an entirely different Part of the manual.

  Example:
  The Box to the right of this sentence indicates that there is a related *supplement* at the end of Part II, Section D, Paragraph 6a.

Supplement II.D.6a., page 102

Boxes to the right of main text also are used to indicate the location of related *text*.

Example:
The Box to the right of this sentence indicates that there is related information in the *text* of Part VI, Section C.

Part VI.C.

PART III - SPECIFICATIONS

Part III contains reference information and sample "procurement requirements" for the WHO EPI vaccines, including hepatitis B and yellow fever vaccines. It also contains a general section relevant to all vaccines.

The purchasing entity will find this Part useful in developing product specifications for bidding documents.

PART IV - QUALITY ASSURANCE

Part IV provides information on techniques that can be used by a procurement entity to help ensure that the products it buys are safe and effective. Part IV includes information about sampling and testing vaccines prior to purchase, prior to shipment and upon delivery. It also includes information about national regulatory controls on vaccines and manufacturers.

The purchasing entity will find this Part useful in developing specifications and contracts, as well as understanding basic quality assurance issues associated with vaccines.

PART V - THE UNICEF OPTION

Part V explains vaccine procurement through UNICEF. It includes a description of UNICEF vaccine procurement services and the related quality assurance program.

PART VI - GENERAL INFORMATION

Part VI contains a collection of information on sources, prices and payment options, as well as model instructions on packing, shipping and storage of vaccines. It also provides information on vaccine-related products, such as syringes and temperature markers.

The purchasing entity will find this Part useful for planning and executing procurement activities.

PART VII - WORLD BANK

Part VII discusses World Bank bidding procedures and contains the *Standard Bidding Documents for Procurement of Pharmaceuticals and Vaccines* issued by the World Bank as a "Trial Edition" in September 1993[3]. In addition, it suggests appropriate vaccine-specific wording for use with these documents.

[3] This document has not been released in a finalised form. However, it represents current Bank practice at the writing of this manual.

PART I - OPTIONS

A. Procurement Methods 12
Competitive procurement 12
Sole-source procurement 14
B. Procurement Transaction Participants 15
Definitions and summary of roles 15
Purchaser's rights and seller's expectations 18
C. Procurement Entities 19
Procurement by in-house staff 19
Using a procurement agent 19
Using UNICEF/ WHO as procurement agents 21
D. Selection Tools 21

PART I - OPTIONS

In the public sector, procurement is generally conducted on a competitive basis, although direct, sole-source contracting can be practised under special circumstances. There are several types of competitive procurement and many choices as to who will execute procurement transactions on behalf of a public-sector entity. This Part explains the options available and provides several tools to assist decision makers.

A. Procurement Methods

Should an agency purchase vaccines through competitive procurement or by direct, sole-source contracting? The choice may vary with individual purchases, depending not only on the vaccine and cost / quantity factors, but also on delivery date requirements, product continuity requirements, availability of foreign currency, restrictions on the use of funds and conditions attached by donor agencies or financing organisations. The experience and skills of the procurement staff can also have an impact.

Competitive procurement

Competitive procurement takes place when prospective suppliers bid against one another for an order or contract.

Competitive procurement requires that the purchaser take initiative in contacting potential suppliers.

Competitive procurement is appropriate when several supply sources are available and reasonably large quantities and / or a large expenditure is involved. Many governments and multilateral agencies require formal competitive procedures in procurements executed with their funds. The key features of formal competitive bidding are summarized in Supplement I.A., Ten Principles of Competitive Bidding, located at the end of this section.

Supplement I.A., page 23

In addition to encouraging the lowest possible prices, competitive procurement provides a degree of protection from bias and favouritism in the award of supply contracts. Procurement of vaccine and related commodities through annual or semi-annual purchase is a common public-sector practice, providing an opportunity for additional cost savings through lower prices for large quantities.

Competitive procurement is not appropriate when only one acceptable source of supply is available, when the volume or total value of the order is minimal or the purchase is in response to an emergency situation.

The main types of competitive procurement are as follows.

- **International Competitive Bidding (ICB) (unrestricted bidding or open tender)**

  The objective of International Competitive Bidding is to provide all eligible prospective bidders with an equal opportunity to participate in the tender. It is generally initiated with a widely advertised notice of the bidding opportunity. Sealed bids[4] are required, based on clearly stated product specifications and performance expectations. Submissions are evaluated on their technical, commercial, contractual and financial merit, with awards going to the supplier making the most advantageous and cost-effective offer. All bids are final and no negotiation is allowed, except in regard to minor contractual points, after selection of a winning bid.

  ICB is the most complex of the procedures at the country level; however, when quantities are large, this process generally ensures the lowest price. ICB is not suited to the purchase of vaccines as these products are subject to shelf life limitations and stringent, difficult-to-control quality requirements. The public announcement of a tender for vaccines could result in the receipt of many unacceptable offers. Under ICB rules all offers must be considered and can be eliminated only during the examination and adjudication phases of the tender. In the case of vaccines this is a technically complex, costly and time-consuming process. Samples of vaccine provided at the time of the tender are not relevant because vaccine, as a biological product, must be tested on a lot-by-lot basis at the time of production. In addition to expense and the impossibility of providing quality guarantees, the ICB process can require up to 17 months to complete. This is unacceptable for immunization programmes.

- **International Competitive Bidding (ICB) with Pre-qualification**

  Pre-qualification of bidders is often employed when there is concern that advertised tender notices will elicit a large number of unacceptable offers and the procuring entity has only limited resources with which to perform detailed evaluations.

  Pre-qualification focuses on the technical and financial capabilities of prospective suppliers. An invitation for pre-qualification is advertised in place of the tender notice, responses are evaluated, samples are inspected and may be tested and a short list of qualified suppliers is developed. Firms on the short list are invited to submit priced bids. If deemed necessary, site visits may be made at this stage in order to more fully evaluate the technical capabilities of the responders.

  Public-sector purchasing entities should use a modified ICB process as the advertised opportunity for producers to offer specific vaccines for licensing. It should be noted that the licensing of a vaccine by the NCA of the purchasing country does, in effect, pre-qualify that specific vaccine. The role of the NCA in vaccine procurement is discussed further below.

[4] A formal process where bids are submitted in sealed envelopes and held unopened until an appointed date and time, then opened and read out in public, with bidders in attendance.

- **Local Competitive Bidding (LCB), sometimes called National Competitive Bidding (NCB)**

  Although not generally applicable to vaccine procurement because of the very limited number of good quality local producers, Local Competitive Bidding is the most efficient and economical way of procuring goods which, by their nature or scope, are unlikely to attract foreign competition. LCB may be the preferred method when the contract values are small or a reputable product is available locally at prices below international market rates.

  Procedures for LCB are broadly consistent with International Competitive Bidding, but advertising may be limited to the national press or an official publication.

- **Limited International Bidding (LIB) (restricted bidding or selective tender)**

  Limited International Bidding is essentially International Competitive Bidding by direct invitation without open advertisement. It may be the appropriate method of procurement where the contract values are small, there are only a limited number of qualified suppliers or other exceptional reasons justify departure from ICB procedures.

  Under Limited International Bidding procedures, the procurement entity seeks bids from a list of potential suppliers broad enough to ensure competitive prices; a total of seven to eight is often recommended.

  Used in conjunction with pre-qualification and licensing of vaccines, LIB is the preferred method for procurement of vaccines when there is a range of suitable suppliers.

- **Request for Quotation (RFQ), sometimes called International Shopping**

  The Request for Quotation process compares quotations from several suppliers, usually at least three, to ensure competitive prices. It is an appropriate method for the procurement of vaccines provided that quotations are solicited only from suppliers of pre-qualified vaccine. This method is appropriate for other commodities when procuring ready-made, readily available goods or standard specification commodities that are small in value. Quotes can be submitted by fax, telex or e-mail.

  Although less competitive, a Request for Quotation is sometimes used instead of the more formal sealed-bid process when the prospective supplier base is small and / or when time is limited by an urgent requirement for the commodity. Some degree of negotiation may be included in the determination of final price, terms and conditions.

Sole-source procurement

Sole-source procurement occurs when the purchaser does not ask for or consider offers from more than one source. Although the final price, terms and conditions are negotiated, this practice normally yields per-unit prices that are higher than

those obtained through competition. It is an appropriate method for the procurement of vaccines only if the subject vaccine has been pre-qualified.

Sole-source procurement is used primarily for small-volume purchases, proprietary products or emergency needs. Sole-source contracting may also be useful during the transition from donor-supplied products to government financing and procurement, or when brand continuation plays an important role in a programme's continued success. It is particularly appropriate when an existing contract can be extended for additional goods of a similar nature, where no advantage would be obtained by further competition and where prices on the extended contract are reasonable.

In practice, sole-source procurement often occurs without appropriate justification when the purchaser is passive, responding only to the initiative of a supplier. Resulting purchases are based on convenience and factors other than economic prudence.

B. Procurement Transaction Participants

- PURCHASER for public-sector vaccine programmes can be a government procurement entity, a qualified procurement agent, UNICEF or WHO.
- SELLER should be a manufacturer or a manufacturer's licensed representative (agent).

Definitions and summary of roles

- **Manufacturer**

  Producer of bulk vaccine from raw materials and, sometimes, a firm that fills vials and packages bulk vaccine.

  a. produces (or fills vials and packages) one or more vaccines
  b. develops new vaccines (optional)
  c. maintains production facilities and equipment
  d. establishes and maintains a quality control program
  e. conforms with requirements of the NCA
  f. sells vaccine
  g. ships vaccine
  h. advises recipient, agent or representative of shipping details

- **Regulator (in country of <u>manufacture</u>)**

  Body established and backed by national legislation to assure the quality of medicines and vaccines; i.e., National Control Authority (NCA) (see discussion in Part IV, page 569). Ideally, a specialized Biologicals Committee is attached to the NCA.

a. establishes licensing criteria, including review of clinical performance
b. monitors field performance and investigates adverse events
c. operates a lot release system
d. maintains appropriate laboratory capability
e. ensures regular GMP inspections

- **Manufacturer's Representative**

Individual who has a formal, legal relationship with a manufacturer to sell that manufacturer's vaccine and usually receives a commission on the sale (may be salaried).

a. represents manufacturer
b. sells manufacturer's product
c. earns commission on sales, but may be salaried

- **Independent Middleman**

Individual or organization that obtains vaccine from multiple sources and resells it to make a profit; usually has no legal agreement with manufacturers of the vaccine; often wholesalers and discounters.

a. purchases vaccine from various sources
b. resells vaccine at a profit
c. may ship vaccine
d. may advise recipient of shipping details

Responsible procurement entities should not purchase vaccine from independent middlemen who have no legal, verifiable relationship with the manufacturer of the vaccine. Although pre-qualified, licensed vaccine—or what is represented to be pre-qualified, licensed vaccine—may be offered for sale by independent middlemen, it should be considered unsafe without the manufacturer's direct acknowledgement and assurance of identity, quality, safety, remaining shelf life and cold chain integrity. It will be necessary to carry out laboratory testing of vaccine purchased from middlemen unless the manufacturer and the quality of the vaccine can be clearly established.

This recommended source limitation seeks to ensure that no counterfeit, mishandled or short-dated vaccine reaches consumers.

- **Procurement Agent (commercial or state)**

Organisation (or individual) hired by another organisation that wishes to buy vaccine but does not have the time or capability necessary to effect the transactions; the procurement agent represents the purchaser.

a. follows purchaser's instructions
b. obtains vaccine on behalf of the purchaser
c. charges the purchaser a fee for services rendered

- **UNICEF**

  United Nations agency that contracts annually with WHO-assessed manufacturers for large quantities of vaccine on behalf of government immunization programmes.

  a. estimates annual needs of all prospective customers
  b. purchases vaccines from producers "pre-qualified" by WHO to a standard set of specifications
  c. processes orders from government organisations ("customers") or donates vaccine to countries in greatest need
  d. requires advance payment including:
     estimated cost of vaccine
     administrative fees
     estimated cost of freight
     contingency amount
  e. directs supplier to ship vaccine
  f. advises recipient of shipping details
  g. refunds any unused portion of advance payment

- **Regulator (in purchaser's county)**

  As described above, a body established and backed by national legislation to assure the quality of medicines and vaccines; i.e., NCA.

  a. establishes procedures for assuring biological products intended for use in the country are of adequate quality, safety and efficacy
  b. issues, varies and revokes licenses for vaccines and other biological products on the grounds of safety, potency and efficacy
  c. monitors imports and locally produced products (lot release) and has the ability to recall, if necessary
  d. investigates adverse events (post-marketing surveillance system)
  e. collects fee from manufacturer to cover costs of licensing
  f. may inspect or arrange for inspection to monitor GMP compliance as part of pre-qualification process
  g. responsible for storage and distribution standards

- **Purchaser**

  Any country, organisation or person that contracts with a seller for the supply of vaccine for an agreed price.

  a. identifies the type and quantity of vaccine needed (EPI manager or programme staff)
  b. establishes specifications and draft contract requirements (NCA and procurement entity)
  c. informs prospective suppliers of opportunity to sell vaccine and how they should go about making offers (procurement entity)

d. selects the winning bid or offer (procurement review committee made up of representatives from immunization programme, procurement entity, ministry of health, ministry of finance, NCA and others as necessary)
e. arranges for inspection and, if needed, testing (NCA)
f. makes financial arrangements (procurement entity, ministry of health, ministry of finance)
g. receives and stores vaccine, maintaining appropriate temperatures (ministry of health or immunization programme)
h. distributes vaccine, maintaining appropriate temperatures (ministry of health or immunization programme)
i. monitors use; reports adverse events (procurement entity, programme personnel, NCA)

Purchaser's rights and seller's expectations

Purchaser has the right to:

- Decide exactly what is required and ask for it; for example:
  a. vaccine type
  b. shelf life
  c. quantity and delivery date
  d. vial size, packaging
  e. marking and packing
  f. terms and conditions for the contract
  g. shipping mode
  h. route for shipment
  i. payment mode
- Set a maximum budget and reject offers that exceed it
- Decide what will comprise proof of quality (NCA)
- Set the rules for the purchase process
- Establish how and when the purchase will proceed
- Establish evaluation criteria and method for review and comparison of bids and priorities
- Seek and consider competing offers
- Reject offers that do not meet basic criteria
- Reject goods that do not meet specification or otherwise comply with the contract
- Insist on free replacement of defective goods

Seller expects that:

- The purchaser has the funds needed to pay for the purchase
- The purchaser will want assurances of quality

- The purchaser has described exactly what he wants
- The purchaser wants the most advantageous price
- The purchaser understands that there is an obligation to pay extra for items and services that are requested in addition to what is specifically included in the contract

C. Procurement Entities

Who should carry out the procurement activity? The choice of a procurement entity depends upon the existing level of procurement staffing and expertise available and conditions attached to grants or loans by donor or financing organisations. More than one procurement entity may be chosen by a public-sector programme, depending on the complexity of the transactions to be managed and the entity's special expertise.

Public-sector programmes generally choose among three options: procurement by in-house staff, procurement contracted out to a commercial or state procurement agent and purchases through a United Nations agency such as UNICEF / WHO or a similar public-sector supply service.

Procurement by in-house staff

Staff requirements for competitive procurement operations include knowledge of international and local procurement procedures, product specifications, inspection procedures, regulatory and financial requirements, international shipping and trade documentation. Additional knowledge of international and local vaccine sources and price levels is desirable. Staff should have access to sufficient office space and equipment, such as typewriters, computers, copiers and communications equipment. Specialised technical assistance should include an outside assessment of staff procurement capabilities.

Using a procurement agent

A public-sector programme that lacks appropriate internal capabilities and experience for the proposed procurement activities may employ a commercial or state procurement agent to act on its behalf. It is important that the agent be well qualified for the particular type of procurement to be undertaken, and that programme control over procurement transactions and decisions is retained.

In practise, it is not uncommon for local middleman suppliers to label themselves as "procurement agents". These "agents" offer vaccine from pre-arranged sources and do not initiate competitive procurement on behalf of the purchaser. WHO recommends against the use of independent middlemen for the purchase of vaccines (see discussion in Part I.B., page 16).

A procurement agent can be local or international and is defined as one who:

- Enters into a formal contract with the purchaser to act on his behalf

- Solicits quotations from multiple sources for vaccine and related products meeting the required specifications
- Involves the purchaser and NCA in selection of the supplier
- Is responsible to the purchaser for his actions
- charges the purchaser an agreed fee for each procurement service based on a percentage of the cost of the goods

Unlike procurement of goods, selection of a procurement agent does not involve a competitive process with the award being made to the lowest evaluated bidder. Financial terms must not dominate selection to the detriment of effective execution of the procurement transaction. The competence and relevant experience of the firm and the personnel to be assigned are the principal factors to consider.

Qualifications of the procurement agent:

- Verifiable relevant training and experience of the personnel to be assigned to the procurement activity
- Verifiable record of successful performance of similar services involving vaccine and cold chain requirements
- Verifiable list of major contracts handled over the last five years
- Current business license or registration in country of legal domicile
- References with contact information

Contracts with procurement agents:

- Terms of reference
  a. precise statement of the objectives of the assignment
  b. scope and timing of the required services
  c. inputs to be provided by the contracting party
  d. particulars of the output including documents and reports
- Budget for the anticipated purchases
- Fees and payment terms

  Fees paid to procurement agents are generally in accordance with the level of effort required and may range from 3 percent to 10 percent, or more, of the cost of the goods purchased.
- General terms and conditions of the contract, for example:
  a. notifications
  b. governing language
  c. legal authority
  d. force majeure

e. expiration

f. termination

- Signatures

Using UNICEF / WHO as procurement agents

UNICEF

The United Nations Children's Fund (UNICEF) carries out vaccine procurement on behalf of countries and public-sector programmes, usually for a fee of six percent of the value of the supplies. Because it is oriented primarily towards children, UNICEF concentrates on the WHO / EPI vaccines, but it may provide other vaccines on a case-by-case basis. Purchases for countries and programmes are normally made against large-quantity, annual supply agreements with vaccine manufacturers that have been established through limited international competitive bidding. Only manufacturers who have been assessed by WHO for supply of vaccines to United Nations agencies for use in immunization programmes in developing and transitional countries are eligible for these contracts.

From the standpoint of the purchasing entity, procurement through UNICEF resembles a sole-source process. UN agencies rarely bid for contracts to supply goods but will act as procurement agents in certain circumstances. Using the Procurement Services system, UNICEF offers its services as an agent for the purchase of vaccines and other commodities for which it has the necessary expertise. Further information can be obtained from UNICEF Supply Division in Copenhagen, either directly or through the country UNICEF Representative. Additional information may be found in Part V - "The UNICEF Option", page 643.

WHO

The procurement arm of the World Health Organization (WHO) will act as an agent and purchase non-standard vaccines for governments. Each purchase is made following the issue of a specific bid established for the purpose. WHO charges a fee equal to three percent of the value of the vaccines and payment is required in advance. Further information can be obtained from the WHO Supply Division in Geneva.

D. Selection Tools

Supplement I.D.1., page 25

Several tools are available to assist in choosing the best procurement method for a specific situation. The chart titled "Comparison of Purchasing Methods" shows the effect on price, delivery and workload of open tenders (competitive bidding), selective tenders (limited bidding), negotiated procurement (request for quotation) and direct sole-source procurement. It also offers an opinion on the relative need for a merit rating of suppliers under each of the procurement options and lists conditions favouring the use of each.

Supplement I.D.2., page 26

A generic outline of the individual tasks that must be accomplished during the procurement process along with an estimate of time requirements is presented for planning purposes. The "Sample Procurement Activity Timeline" details tasks and timespans for an average 9 ½-month procurement process. Public-sector competitive procurement is a complicated undertaking that can require up to 17 months from the time a need is identified until the time goods are available for distribution. It is affected by the manufacturer's "lead time", the mode of shipment and the degree of formality in the solicitation and adjudication process. It is also affected by the government approval process and the period allowed for prospective vendors to respond to a solicitation. The sample timeline should be revised according to individual circumstances.

Ten Principles of Competitive Bidding[5]

1. Suitable Package

Design tender requirements to attract the interest of as many suppliers as possible. For vaccines, ensure that tender requirements do not exclude any manufacturers of pre-qualified vaccines.

2. Early Warning

Allow at least 45 days for bidders to submit offers.

3. Non-discrimination

The tender must be open to all qualified manufacturers. When selective tendering is used in place of unrestricted advertised solicitation for the purchase of vaccine, invite only manufacturers of pre-qualified vaccines.

4. Accessibility

Ensure wide access to competition by setting reasonable costs for bid documents and bid securities. For vaccines, it is unlikely that the value of the contract will justify the purchase of bid documents or the use of bid securities.

Respond to all written questions and requests for additional information from each bidder; provide identical information to all other bidders without identifying the source of the inquiry.

5. Neutrality

Use generic terms in the specification rather than brand names.

Do not show preference for a specific brand or manufacturer in bid specifications; include the phrase “or equivalent” if a brand name must be used.

6. Formality

Require that bids be in writing, signed and received in sealed envelopes before a stated date and time.

7. Confidentiality

Do not open bids until the assigned date and time.

Restrict all bid information to authorised parties.

[5] Based on the essential elements of World Bank tendering.

8. Consistency

Evaluate all bids against the same criteria.

Do not ask or permit a bidder to change the substance of the bid unless equal opportunity is given to all bidders within the competitive range.

9. Objectivity

Determine if bid is “substantially responsive” by checking for errors, correct signatures and adherence to basic tender requirements.

Select the most advantageous bid considering both the price and the evaluation criteria announced in the bidding documents.

10. No Negotiation Before Award

Encourage the lowest responsible offer from each bidder through the process of tender competition; minor contractual adjustments may be allowed after selection of the most advantageous bid.

Comparison of Purchasing Methods[6]

Purchasing Method	Brief Description	Effect on Price	Delivery Times	Workload on the Procurement Unit	Need for a Merit Rating of Suppliers	Conditions Favouring Use
Open Tender	Bids are accepted from all interested suppliers	Very favourable	Long	Very high	Very high	• Many reputable suppliers are available • May be necessary at beginning of programme to open supply channels • For vaccine use, open tender only for pre-qualification
Selective Tender	Suppliers bid against each other for the contract, but participation of suppliers is limited to those who have registered with the government or who have met established prerequisites	Favourable	Moderate	Moderate	Moderate	• Where only a few reputable suppliers are available • Products where bioequivalence, sterility, precise formulation is important • Large volume, standard items for which all reliable suppliers are known • For vaccines, use only pre-qualified sources and products
Negotiated Procurement	The buyer approaches a small number of selected potential suppliers and bargains with them for specific price or service arrangements	Moderately favourable	Short	High initially, low later	Only initially	• Bulk buying of single-source drugs • Special terms or specifications are required by the buyer • For vaccines, use only pre-qualified sources and products
Direct Procurement	Purchase is made directly from a single supplier at his quoted price	Unfavourable	Very short	Very low	No need	• Low price, small volume items • Emergency items where negotiation not possible • Items from international non-profit groups • For vaccines, use only pre-qualified sources and products

[6] Based on original work by Management Sciences for Health.

PART II - PROCEDURES

Introduction 28
A. Planning and Forecasting 29
B. Pre-qualification 35
C. Preparation for Procurement 47
D. Preparation of Bid Documents for Selective Tender 57
E. Preparations for Bid Evaluation 93
F. Solicitation and Receipt of Offers for Selective Tender 97
G. Evaluation and Comparison of Bids 105
H. The Selection Process 115
I. The Award Process 123
J. The Contract 135
K. Financial Arrangements 139
L. Contract Performance 147
M. Shipment 153
N. Customs Clearance and Delivery 157
O. Closeout Activities 161

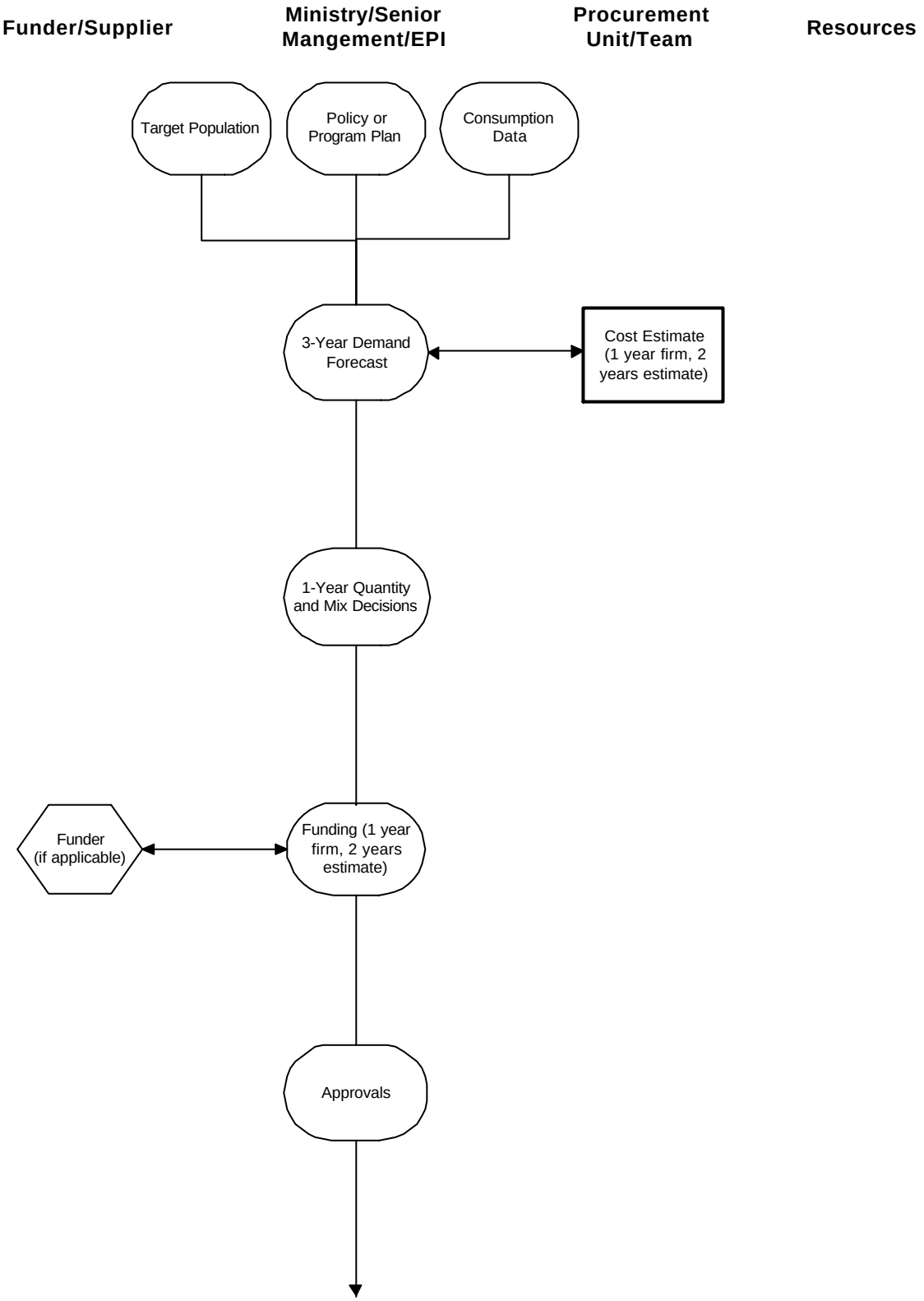
PART II - PROCEDURES

Introduction

Part II is the main focus of this reference manual. It contains a step-by-step guide for international competitive procurement of vaccines with emphasis on activities that are normally assigned to the Procurement Unit or other personnel assigned to the execution of purchasing tasks.

The flow chart preceding the text of each section of Part II is organised in columns to show the activities and responsibilities of the Procurement Unit or Procurement Team and the activities and responsibilities of Ministry and Senior Programme Management / EPI (including Tender Review Committee) in the middle columns. In addition, a column at the far right indicates resources the Procurement Team may need to call upon and a column at the far left shows points of contact with the Funder and the Supplier. Procurement Unit / Team activities and responsibilities are symbolized by a rectangle. Ministry and Senior Programme Management / EPI and Tender Review Committee activities and responsibilities are symbolized by an oval. Resources are shown as circles and Funder / Supplier contacts are shown as hexagons. In the Procurement Unit / Team column, rectangles drawn with light lines indicate optional activities.

A. Planning and Forecasting



A. Planning and Forecasting

1. Supply decisions.

Supply decisions are programme activities that must be undertaken at a high managerial level with input from appropriately trained specialists.

Supplement II.A.1., page 31

2. Procurement staff participation.

Procurement staff often does not participate in the supply decision making process, except to provide commodity cost history and current pricing. However, their participation has advantages because they bring additional knowledge to the supply process in the area of forecasting and logistics and have a role to play in ensuring that storage and distribution channels are established. They can introduce a valuable element of monitoring and cross check on supply needs calculations. In addition, later purchasing decisions will be made on a broader base of knowledge and understanding.

3. Required components:

- Stock on order
- Current physical inventory of stock on hand
- Estimate of wastage by vaccine type taking into account immunization programme policy
- Vaccination schedule
- Determination of supply needs, including type of vaccine, quantity and delivery date: 1-year firm, 2 years estimate
- Cost estimate for aggregate annual supply requirements
- Confirmation that annual funding is arranged
- Approval for expenditure
- Authorised request for procurement

Forecasting Vaccine Needs

An accurate forecast of requirements ensures that vaccines required by the immunization programme are available when needed and that preventable wastage due to overstocking or the expiration of vaccines is minimised. Poor forecasting, or no forecasting, can lead to temporary suspensions of immunization services and ultimately to outbreaks of vaccine-preventable diseases.

Obtaining an accurate forecast of vaccine needs requires reliable estimates of target populations and the expected immunization coverage. Past years' usage, end-of-year balances and wastage rates are also useful for accurately forecasting needs. Requirements for special vaccination campaigns should be calculated separately and then added to the requirements for routine immunization. Forecasting the theoretical demand for a campaign should be modified according to existing stock to avoid depleting supplies for routine services or overstocking from vaccine left over after a campaign.

Where is the forecast made?

Forecasting and distribution follows either a "pull" or a "push" system. These two words describe where the demand forecast is generated. In a pull system, the most peripheral level in the delivery chain, the local level, estimates its needs and makes requests for vaccines. In other words, it "pulls" the vaccine to its level. The health facility and district totals are then consolidated centrally to arrive at the national forecast. Vaccine is distributed according to the requests from the field and districts. The pull system may be appropriate in countries where the health workers are well trained on vaccine supply management and where local information systems and data management are reliable.

In the push system, the national programme usually estimates the needs for the districts and health facilities based on available consensus data and population projections and / or on past usage. In some countries, the districts provide the estimates to the central level, which then adjusts these according to central management estimates and experiences. Countries without capacity for micro planning at the health centre or district levels may benefit more by using the push system. In some instances, a push system might be more efficient for accelerated and timely disease control initiatives requiring mass immunization. This is especially true when time is not sufficient to collect information from the field. The push system may also be appropriate for unanticipated situations, such as a high influx of refugees. However, to the extent possible, a well-planned effort should involve peripheral levels in estimating vaccine requirements.

Calculating needs

Vaccine stock records should always be maintained in terms of doses. Using doses allows for easier calculations based on population data; that is, the number of individuals to be immunized. Records by doses also prevent confusion, should different vial sizes be provided in the future, and allow for easier calculation of vaccine wastage or usage rates. Keeping records by doses avoids one additional step and consequently another potential source for error: the division required to convert doses into the number of vials.

In the absences of more precise data, vaccine needs for a given vaccine can be estimated in doses by using the following formula:

Target Pop. x Expected Coverage x Number of Doses per Child (woman) x Estimated Wastage Factor

If certain EPI vaccines will be shared with the adult community, the dose requirement forecast will need to be increased.[7]

When ordering vaccines, the time period for which the forecast applies must include the lead time until the next order is due. The next shipment should cover the time period until the following shipment arrives and therefore should include a time safety factor to compensate for unanticipated variations in demand or shipping delays. A rule of thumb for deliveries would be an extra 30 to 50 percent of stock, although this figure should be reviewed each time an order is placed to avoid overstocking.

Wastage

Vaccine "wastage" may occur because of a cold chain failure, expiration of vaccine or vaccine remaining at the end of an immunization session which cannot be used for another session. The "wastage factor", which is also called the index of vaccine use, vaccine usage rate, or doses used per immunization, can be obtained from records on the number of immunizations given and on the amounts of vaccine used during a certain period (e.g., 1, 3, 6 or 12 months). Although the term "wastage" is widely used, this term can be misleading. A vial of vaccine does not always provide the manufacturer's stated number of doses. For example, a vial labelled as "20 dose" may actually contain only enough vaccine to immunize 18 children. However, in theoretical and mathematical terms, the two doses which were not available appear as "wasted vaccine" when making general estimates of wastage. In campaign situations, vaccine which is left over after the campaign can be incorrectly considered as "wastage" if there is no systematic attempt to record the unused balance remaining. Furthermore, unused vaccine which must be discarded because only a few children could be immunized is not necessarily "wasted" if this results in the timely immunization of children who are difficult to reach and who would otherwise not have been immunized.

Wastage rates may vary by vaccine and by location (e.g., national store, district store, health facility). Wastage often exceeds 50 percent and may be as high as 90 percent for some vaccines. Wastage rates vary according to immunization policies, vial sizes, delivery strategies and the number and size of immunization sessions. For example, some countries require that a vial should be opened even for a single child, while other countries advocate opening a vial only for a minimal number of children. Sessions with low attendance might have higher wastage rates, although higher wastage may not necessarily be undesirable in situations where children and mothers can only infrequently attend immunization sessions due to limited access to health facilities. It is therefore important that programme management estimates the level of wastage for each vaccine with as much precision as possible. Wastage rates for mass campaigns tend to be much lower than wastage during routine immunization services because of the high volume of immunizations and the short time period in which they are given. Wastage rates during routine

[7] The recommended adult dose for BCG and some hepatitis B vaccine is double the infant dose.

immunization sessions can be reduced by effective planning and communication to ensure that session attendance is optimal for the vial size in use.

The wastage factor, which is needed to calculate vaccine requirements, can be estimated as follows:

$$\textbf{Wastage Factor} = \frac{\textbf{number of doses of vaccine used during a certain time period}}{\textbf{number of immunizations given during the same time period}}$$

Wastage also occurs when too much vaccine is ordered and especially when vaccine is ordered without considering the balance of vaccine in the entire system, i.e., regional and district stores and health facilities. System-wide wastage can be significant and is indeed preventable. An efficient supply management system provides accurate information on the balance of vaccine in the entire system, not just at the central store.

The WHO open vial policy

WHO's open vial policy stipulates that opened vials of vaccines which do not need to be reconstituted and which are properly kept in the cold chain can be used in subsequent immunization sessions. For EPI, the open vial policy only pertains to the following vaccines: OPV, DPT, DT, TT and hepatitis B vaccines. However, depending on national policies and local situations, some countries specify that opened vials which have been taken out of a health facility for immunization activities, such as for outreach or National Immunization Days (NIDs), should be discarded at the end of the day. Any vial suspected of contamination should be discarded. If properly implemented, the introduction of the open vial policy can significantly reduce vaccine wastage. Details on the use of opened vials of vaccine can be found in the following WHO documents: WHO/EPI/LHIS/96.01, WHO/EPI/LHIS/96.04 and WHO/EPI/LHIS/95.01.

Worldwide supply and demand

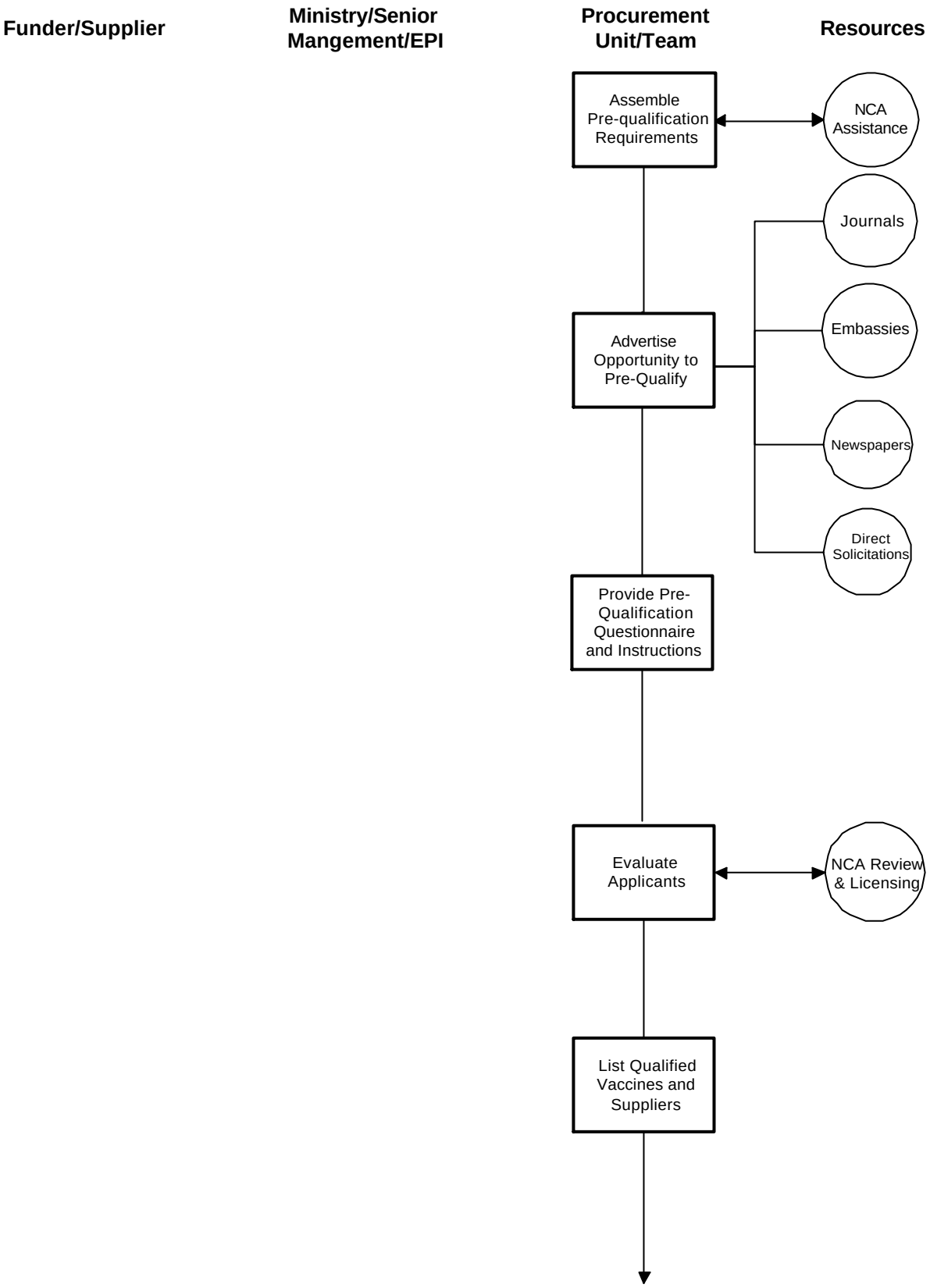
The volume of vaccines supplied to national immunization programmes has risen during the last decade from approximately 2 billion to over 3.5 billion doses per year. This increase results primarily from purchases by UNICEF and other donors for immunization programmes in nonindustrialised countries. In addition, there has been expansion in local production of some vaccines, but this expansion has been limited to supplying local needs. Without accurate forecasting from every country, it will become more and more difficult for manufacturers to respond to the increase in demand. The high volume and timely provision of vaccines for national immunization campaigns adds to the importance of accurate vaccine forecasting.

Market knowledge is necessary for manufacturers to carry out efficient planning and for deciding on the level of investment required for vaccine production. It is essential that private-sector manufacturers are aware of future demand so that production resources are not diverted away from the high-volume vaccines for the public sector to the higher price paid by the private sector. The increased demand, combined with the increased emphasis on sustainability and reliance on governments to purchase vaccines, makes accurate forecasting even more essential.

Conclusion

Careful demand forecasting is indispensable for effective and sustainable immunization programmes. Consistent forecasting methods and accurate calculations will provide reliable information on vaccine needs, storage requirements and cost requirements at all levels. A forecast is only as accurate as the system used to collect the information. Therefore, the reliability of the reporting and tracking system must be given particular attention.

B. Pre-qualification



B. Pre-qualification

Pre-qualification for vaccine procurement differs from conventional pre-qualification exercises in that it focuses first on the quality and acceptability of the product and second on the commercial and technical suitability of the supplier. In addition, it is divorced from specific procurement contracts.

First level of pre-qualification—vaccine

The objective of vaccine pre-qualification is to yield a list of acceptable vaccines, each defined in relation to its specific manufacturer and particular technical attributes. The preferred method of vaccine pre-qualification is through licensing by the purchasing country's National Control Authority. The procurement entity does not take part in licensing activities. Ministries, agencies, facilities and immunization programmes within a country may purchase from the list of licensed products at any time.

Because licensing is at the initiative and expense of the manufacturer, a procurement entity may encounter licensed vaccines that do not respond to the needs of the immunization programme. Such vaccines can be eliminated by adding programme requirements to licensing requirements for pre-qualification criteria.

Alternate methods of vaccine pre-qualification might be necessary if procurement activity must commence before formal licensing can be accomplished. These include selecting from the list of vaccines pre-qualified in principle by WHO for supply to United Nations (UN) agencies, or obtaining a Statement of Known Good Quality from WHO, or by selecting vaccines produced and licensed in countries with recognised, well-functioning NCAs such as the USFDA. Pre-qualification, in this case, must be approved by an appropriate authority of the Ministry of Health. It may be necessary for the procurement staff to initiate this activity; however, the approval process is the responsibility of the immunization staff within the Ministry of Health.

Part VI.B.1., page 692
Part IV.B., page 568

Second level of pre-qualification—supplier

The second level of pre-qualification for vaccine procurement is the assessment of commercial and technical suitability of prospective vaccine suppliers. This pre-qualification is also divorced from specific procurement contracts. The aim is to develop a list of reliable, reputable and technically competent suppliers of each vaccine from which the procurement entity can solicit bids for specific vaccines on an as-needed basis.

Unless the purchasing entity has reason to doubt the commercial viability of a vaccine manufacturer, this list will automatically include each manufacturer of vaccine that has been licensed or pre-qualified by the appropriate health authority of the purchaser's country, but only for that specific vaccine. The list may also include prospective suppliers who are not manufacturers from which licensed vaccines may be purchased. (Appropriate quality assurance documents from the manufacturer will always be required.)

Senior procurement staff and Ministry officials normally participate in the assessment of potential non-manufacturing vaccine suppliers. The business and management aspects of this second level pre-qualification are not appropriate areas of concern for NCAs; however, they must be consulted on all technical matters.

Pre-qualification should not be carried out each time a contract is to be placed. Once a vaccine and its producer have been pre-qualified, they may remain so unless a particular reason arises. It is appropriate that a review is undertaken every three to four years. Pre-qualification is highly recommended when a new vaccine appears on the market.

Pre-qualification opportunities may be advertised on a periodic basis by the procurement entity. Pre-qualification questionnaires that have been approved by the NCA of the purchaser's country may be sent to known, reputable manufacturers and suppliers of specific vaccines required by the immunization programme, as well as to prospective suppliers that respond to the advertisement.

The sample pre-qualification questionnaire in this manual (see Sample II.B.6.) combines inquiries appropriate to manufacturers as well as non-manufacturing suppliers. It is designed to reveal the exact activities of each applicant, as these can sometimes be unclear and may be deliberately misrepresented.

1. Determine whether a pre-qualification exercise is necessary (procurement entity).

- To increase the number of licensed vaccines of the required types (licensing of vaccine by the purchaser's NCA is required under WHO guidelines)
- To increase the number of qualified suppliers for each vaccine
  - Manufacturers
  - Manufacturer's licensed representatives
- To eliminate unsuitable potential suppliers
- To eliminate offers of inferior products
- To reduce the number of tenders to be evaluated and compared
- To provide a basis for rejecting unsolicited offers

2. Develop pre-qualification criteria for vaccines, which should include at least one of the following:

- Product licensed / registered by the NCA in the purchaser's country, or
- Product assessed and pre-qualified, in principle, by WHO for supply to UN agencies, or
- Product produced and licensed in a country with a recognised, well-functioning NCA that carries out all six critical national control functions, such as USFDA.

Part IV.G., page 608

3. **List documentation that will be required to establish the product's eligibility for pre-qualification. Examples include:**

- Product file for each vaccine (obligatory)
- Proof of current vaccine licensing / registration with NCA of purchaser's country (obligatory)
- Copy of vaccine license / registration issued by recognised, well-functioning NCA of exporting country (optional)
- Statement of Known Good Quality obtained from WHO (optional)
- Evidence of pre-qualification, in principle, by WHO for supply to UN agencies (optional)
- Evidence of registration by recognised, well-functioning NCAs of other importing countries (optional)

4. **Develop pre-qualification criteria for suppliers who are not manufacturers of the licensed vaccine (with advice from the purchasing entity's NCA if applicable). For example:**

- Direct relationship with manufacturer of pre-qualified vaccine (essential)
- In business for at least five years (desirable but not mandatory)
- Financially stable
- Currently registered as business entity in country of domicile
- Currently registered as business entity in country of purchaser
- History of satisfactory business with at least five similar customers

5. **List documentation that will be required to establish supplier's eligibility. For example:**

- Proof of legal representation agreement with manufacturer
- Business history
- Financial references and annual report
- Proof of business registrations
- Customer references

6. **Develop a pre-qualification questionnaire and instructions for use in initial contact with unregistered vaccine manufacturers and non-manufacturing suppliers.**

See Sample B.6., Pre-Qualification Questionnaire.

Sample II.B.6., page 41

7. Advertise pre-qualification opportunity as required.

a. Provide basic information about the pre-qualification.

- Name and address of the pre-qualification authority
- Criteria for pre-qualification, including licensing requirements
- Place and means for obtaining the pre-qualification documents
- Place and deadline for submitting the application to pre-qualify

b. Provide basic information about anticipated procurement opportunities.

- Name(s) of the authorised purchasing authority
- Description of vaccine to be purchased
- Estimated annual quantity and delivery schedule

c. Use Pre-qualification Announcement as model.

Sample II.B.7.c., page 45

8. Provide pre-qualification questionnaire to respondents.

9. Transmit pre-qualification announcement and questionnaire directly to manufacturers and suppliers that are known to provide the required vaccine, who have not responded to the advertisement; identify additional potential vaccine suppliers through:

Part VI. B., page 691

- Prior market knowledge
- National and international registers and publications
- International non-governmental organisations (particularly WHO)
- Foreign embassies, commercial attachés
- Chambers of Commerce
- Donors

10. Evaluate technical and commercial merit of applicants.

a. Review completed pre-qualification questionnaire and attachments.

b. Check references.

c. Seek assistance / concurrence from National Health Authority.

11. Notify qualified applicants.

a. Advise, as necessary, on next steps for licensing / registration of vaccines offered for sale.

b. Advise as required on any steps required for formal licensing or registration of supplier.

12. Notify unqualified applicants.

Pre-Qualification Questionnaire

All information provided in response to this questionnaire will be held strictly confidential and will not be revealed to any persons other than those directly responsible for the supply of vaccines to the Government of _____.

Section A. Business Information

1. Business name, mailing address, fax and e-mail address of applicant

2. Address to which bid forms and correspondence are to be mailed

3. Type of organisation (manufacturer, manufacturer's licensed representative, etc.)

4. Name and address of parent company and / or subsidiary or associated companies (include brief summary of ownership relation)

5. Name and address of branches

6. If incorporated, indicate location(s)

7. Names of officers, members or owners of applicant firm (Chief Executive Officer; Directors or Vice Presidents of Sales, Finance, Marketing; Secretary of Company)

8. Names and titles of individuals authorised to sign bids and quotations in firm's name

9. Names and titles of individuals to contact on matters concerning bids and quotations

10. Number of personnel employed by applicant firm

    Total: Technical: Production: Administrative:

11. Floor space (square feet or square meters) for the following areas:

    Manufacturing:

    Quality control:

    Warehouse:

    Sales and administration:

12. Number of years in business

Section B. General Information

1. Which, if any, government agencies are responsible for inspecting and / or licensing your company's facilities and operations

2. List names, dates, numbers and expiration dates of current licenses and permits

3. Date of last GMP inspection (if applicable)

4. List government or international agencies currently purchasing vaccine and related products from your company and include length of time doing business with each

5. If your company has not done business with any governmental agencies, list names, addresses and fax numbers of at least five other current customers

6. List financial references (name and address of bank)

7. State total sales turnover in previous three years (give figures for each year separately) split between exports and domestic sales and into the various major operating divisions of your company

Section C. Product Information

1. List bulk vaccines actually manufactured at the facility designated in Section A

2. List bulk vaccines not manufactured, but filled or labelled at this facility, showing the name and address of the bulk manufacturer for each

3. List vaccine offered for sale but not manufactured, filled or labelled at this facility; show the name and address of manufacturer for each vaccine

4. List manufacturing country's health authority or National Control Authority (NCA) registration status and basic data for each vaccine offered for sale

5. List countries to which packaged or bulk vaccines are presently exported and indicate for each vaccine the names of countries in which it is licensed for sale or is registered by the NCA

6. List vaccines offered for sale that have been pre-qualified, in principle, by WHO for sale to UN Agencies

Section D. Quality Control Information

1. List names and addresses of any quality control laboratories used in addition to or in lieu of your company's own laboratory

2. Have there been any product recalls of vaccine produced or sold by your company during the last three years? List and include reason for recall

3. Does your company perform quality audits of its suppliers?

4. Explain the type of control your government enforces on your company's establishment and on the products it offers for sale

Section E. Required Attachments

1. Annual Report or equivalent (including most recent audited financial statement)
2. Product file for each vaccine offered for sale
3. Document confirming existing registration with health authority or NCA of purchasing country for each vaccine offered for sale, or one of the following:
   - Signed request to NCA or health authority of purchasing country for application and instructions to register / license each vaccine offered for sale, or
   - Statement of Known Good Quality obtained from the World Health Organization (GPV / VSQ)
4. The purchaser may elect to provide instructions for registration / licensing with the pre-qualification form and require a signed application (and payment of an appropriate fee) as part of the required attachments

Section F. Certification

I hereby certify that the information contained herein is complete and correct and that I will co-operate with any official of _____ in making a personal inspection of relevant facilities and records.

Signature_____ Date_____

Name_____

Title_____

Pre-Qualification Announcement

……[name]…… Date:

……[address]……

……[telephone / fax]……

invites applications to pre-qualify for the supply of vaccine to the national immunization programme of ……. ……

Vaccine Types	Estimated Annual Requirement
……	……
……	……
……	……

Vaccine Requirements:

• Licensed by health authority or NCA of……[purchaser's country]……

Supplier Requirements:

• Verifiable business and financial stability

• Manufacturer of licensed vaccine, or

• Direct legal relationship with manufacturer of licensed vaccine

• Registration as business entity in country of domicile and _____

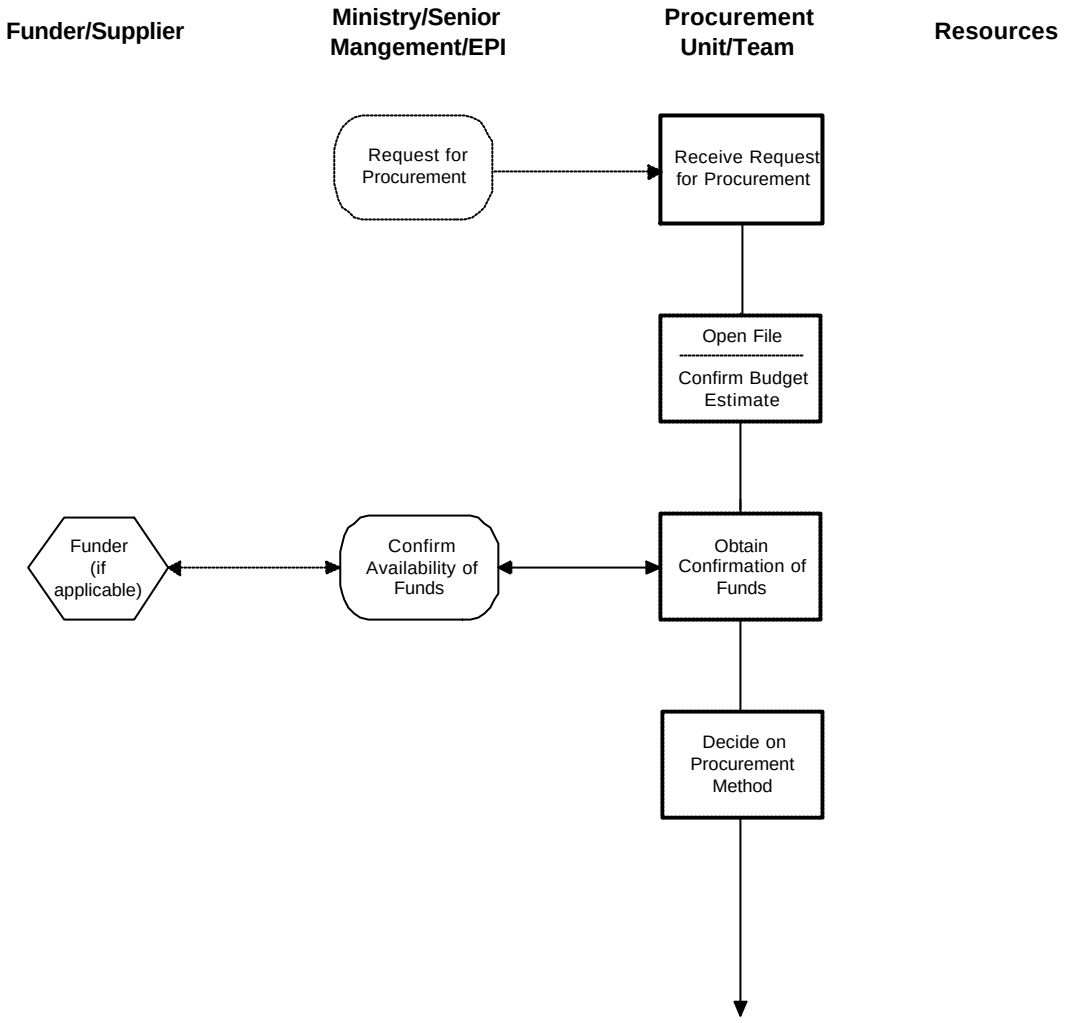
• History of satisfactory business dealings with at least five similar customers

Complete sets of supplier pre-qualification documents may be obtained by any responsible party upon application to the above address.

Application and instructions for licensing may be obtained by manufacturers or their representatives from NCA of _____ at _____.

Applications for pre-qualification must be received by _____.

C. Preparation for Procurement



C. Preparation for Procurement

1. Open procurement file(s), one set per commodity request.

Each set of files should eventually contain:

- Official signed request for procurement
- Specification
- Budget estimates
- Procurement request form
- Official approvals
- Bidding documents
- Bidders lists (for vaccine, this will be limited to suppliers of pre-qualified vaccine)
- Record of advertisements (advertisement not appropriate for vaccine procurement)
- Meeting minutes
- Correspondence
- Adjudication materials
- Contracts
- List of responsibilities for contract performance

Sample II.L.1.a., page 150

- Timelines
- Protocols for inspection and testing
- Inspection and test reports
- Copy of Letter of Credit
- Shipping and marking instructions
- Copy of shipping documents
- Receiving report from warehouse
- Supplier history form

Form II.O.2., page 163

2. Receive official request for procurement.

Responsibility is passed to the Procurement Unit from a ministry or from senior immunization programme management.

3. Verify estimate of funds required.

Use the budget estimate worksheet.

Form II.C.3., page 52
Sample II.C.3., page 53

4. **Confirm through official channels the availability of sufficient funds for procurement.**

   - Amount and source in local currency
   - Amount and source in freely convertible currency

5. **Assemble summary description of procurement requirements.**

   Summary will include:

   - Brief description of product
   - Unit of measure for pricing (vial / ampoule or dose)
   - Quantity
   - Confirmed budget
   - Date needed
   - Date of request
   - Shipping terms
   - Payment terms
   - Name and address of consignee
   - Project identification numbers
   - Accounting identification numbers
   - Special conditions / requirements of contract
   - Special marking requirements for shipping boxes
   - List of authorisations required
   - Source of request / signature of requesting party
   - Source of funds

6. **Design a form to include the above items and ensure that pertinent information is gathered and consolidated in one place for easy reference.**

Form II.C.6., page 54
Sample II.C.6., page 55

   a. The description should include reference to any applicable standards and licenses required, as well as vial size, language for labels and package inserts, primary packaging and other detail.

   b. The units of measure for vaccine should include doses per vial.

   c. All vaccine should be shipped by air. The shipping terms should be noted as CIP (Carriage and Insurance Paid) or Ex Works with airfreight pre-paid and added. See INCOTERMS 1990, Part VI.D.

Part VI.D., page 712

   d. The payment terms should be noted as L / C (Letter of Credit); Documentary Collection; Net 30 (30 days after billing); etc.

7. Determine criteria for selecting potential bidders; obtain approval from Tender Committee if necessary.

- Limit to suppliers of vaccine that has been pre-qualified for use in the purchaser's country
- Determine if bids will be invited or accepted from suppliers that are not the vaccine manufacturer (for example, a manufacturer's licensed representative)
- Determine if bids will be invited or accepted from potential suppliers who have not been pre-qualified as an entity and are not the vaccine manufacturer

8. Decide on method of procurement.

Part I.A., page 12

a. Options for competitive tendering:

- Open tender (unrestricted bidding, known as International Competitive Bidding or "ICB" in World Bank procedures):

  Bids are solicited by means of a widely advertised invitation directed to all suppliers wishing to participate in the tender proceedings. **This method of procurement is not appropriate for vaccine.**

- Selective tender (restricted bidding, known as Limited International Bidding or "LIB" in World Bank procedures):

  Bids are solicited only from certain suppliers selected by the procuring entity. **This is the preferred method for vaccine procurement** (bidding is restricted to manufacturers and suppliers of pre-qualified vaccine).

b. Alternate competitive procedure for special circumstances:

- Negotiated procurement (also known as Request for Quotation - "RFQ", and international shopping in World Bank procedures)

  Proposals are solicited from a limited number of manufacturers and suppliers of pre-qualified vaccine; price and content are discussed and negotiated; contract is awarded based on lowest negotiated price and most advantageous offer.

c. Sole-source procurement:

- Price and terms are negotiated with one chosen supplier, without benefit of competition (**the supplier must offer pre-qualified vaccine).**

d. Factors that help determine method of procurement:

- Source of funds (for example, World Bank)
- Government regulations (often require open tender)
- Internal policy

- Number of suppliers available
- Quantities required
- Total estimated value of procurement
- Delivery date(s) required

Form II.C.3.

Budget Estimate Worksheet

Date _____ Approved Budget _____

Supply Year _____

Estimated Cost of Commodities Required

Commodity _____

Quantity _____ x Cost / unit _____ = _____

Shipping Cost _____

Insurance Cost _____

Inspection Cost _____

Testing Costs _____

Customs Duties _____

Clearance Fees _____

Other _____

Total Required _____

Convertible Currency Required _____ (amount and currency)

Convertible Hard Currency Available ____ yes ____ no

Budget Estimate Worksheet

Date *January 30, 1996*

Approved Budget US*$650,000*

Supply Year *1997*

Estimated Cost of Commodities Required

Commodity *OPV*

Quantity *7 M doses*	x	Cost / unit *$0.08*	=	*$560,000*
		Shipping Cost		*44,800*
		Insurance Cost		*1,540*
		Inspection Cost		*2,800*
		Testing Costs		*0*
		Customs Duties		*0*
		Clearance Fees		*600*
		Other: *airport storage*		*300*
		Total Required		*$610,040*

Convertible Currency Required *US$610,040*

Convertible Currency Available *X* yes ____ no

Procurement Request Form

Date _____ **Consignee:**

Requested Delivery Date _____
(Do Not Specify "ASAP")

Requested by _____

Project Manager Approval _____

Charge Code _____

Partial Shipment Permited? _____ **Budget Allowance** _____
(Verify for items over $100)

Is this a transshipment? _____

Line Item	Description/Justification (Include part or model # if available)	Quantity	Unit of Measure	Estimated Unit Cost	Estimated Total Cost
	Quote _____ (date and reference)				
				Applicable tax:	
				TOTAL	

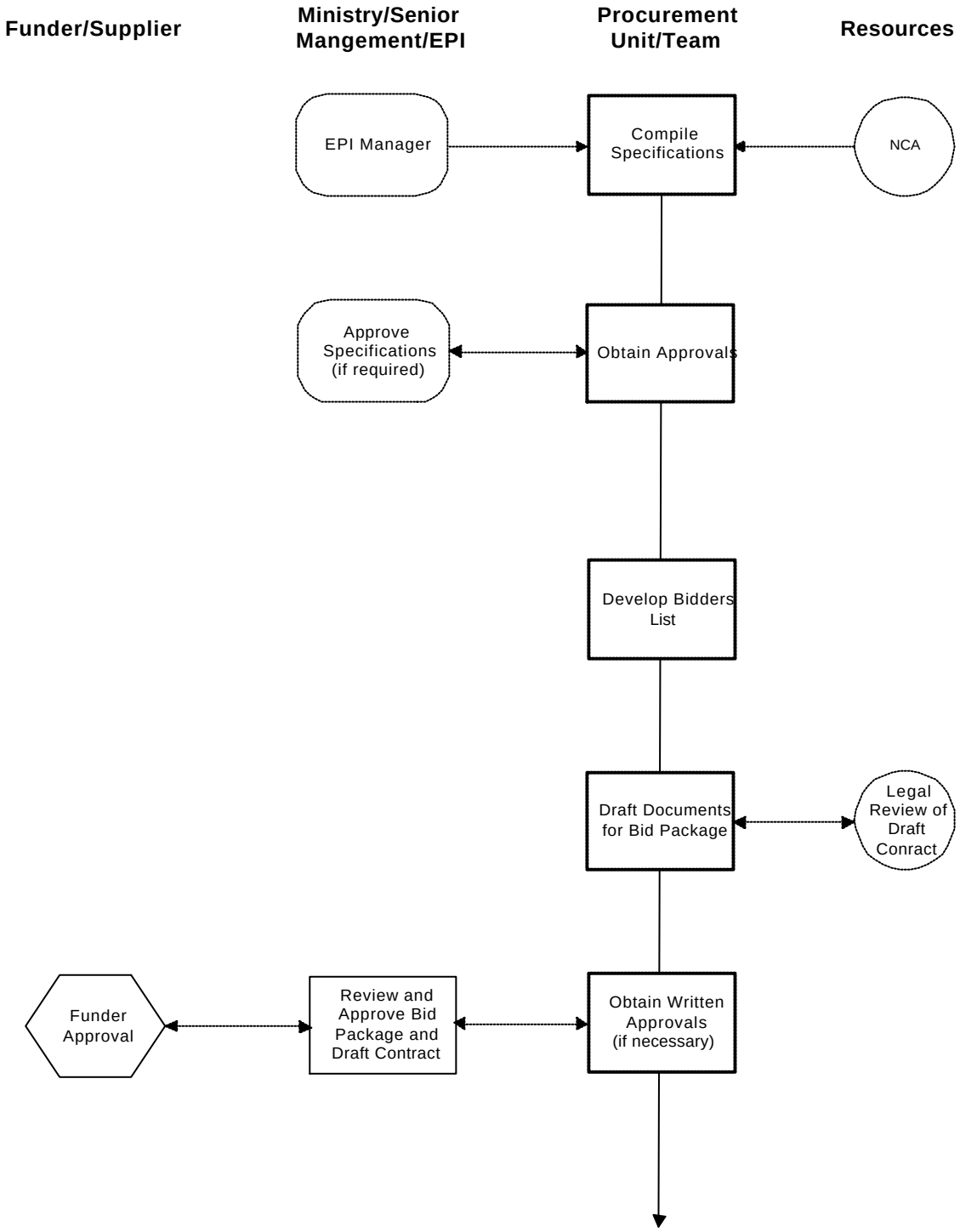
Vendor:
(inclu
address, phone
and contact)

Alternate Vendors:
(if over $1,000, minimum
2 additional required)

Delivery Date _____ **Delivery Terms** _____

Payment Terms _____ **Method of Shipment** _____

D. Preparation of Bid Documents for Selective Tender (LIB)



D. Preparation of Bid Documents for Selective Tender

Care must be taken to ensure that the documentation required to cover all aspects of the contract and all the technical requirements for the vaccine are included in the bid "package" because the bids received in response will form the basis for the contract(s). A comprehensive list of the necessary documentation is included in item number 8 below.

1. Compile specifications and obtain approvals.

Part III, page 165

a. Thoroughly describe product, quantity, packaging, external packing and marking requirements and delivery dates. They must be technically accurate and complete.

- Seek advice and input from vaccine and cold chain professionals and the NCA as appropriate
- The resulting specification will become the basis for the contractual obligation of the supplier to the purchaser

b. Elements to be included differ according to the product. For vaccine:

- Technical specifications (obtain from NCA, if possible)[8]
  - ◊ Name and type of vaccine
  - ◊ Properties such as:
    potency, stability, innocuity, concentration of preservative or adjuvant, strain
  - ◊ Requirements such as:
    testing standards, storage temperature, licensing, vaccine vial monitors (for polio vaccine)
- Programme requirements, including:
  - ◊ Quantity
  - ◊ Description of intended use
  - ◊ Shelf life remaining (at receipt in country)
  - ◊ Dosage size
  - ◊ Dose package
    Type (vials or ampoules)
    Vial (or ampoule) size (in doses per vial)
    Filling volume
    Labelling
    Vial closures
  - ◊ Printed materials

[8] These will normally have been established at the pre-qualification stage. If a vaccine which is not pre-qualified is required, special procedures should be undertaken as detailed in Part IV, page 568.

- Packing and marking requirements
  - ◊ Packing
    Inner boxes
    Over-packing
    Cold chain monitors
    Exterior shipping cartons
  - ◊ Marking
    Inner boxes
    Exterior shipping cartons
- Quality assurance requirements
  - ◊ Warranty
  - ◊ Documentation
  - ◊ Pre-shipment inspection (of physical attributes)
  - ◊ Pre-shipment sampling and testing (for analysis of suspect products)

c. Refer the final specification back to the requesting department or ministry and NCA for confirmation and approval prior to further action.

Sample specifications for use with bid documents may be found in Part III. They are labelled "Sample Procurement Requirements".

2. Develop bidder's list.

a. List names, addresses, fax numbers and appropriate contact information for manufacturers of required, pre-qualified vaccine.

b. List names, addresses, fax numbers and appropriate contact information for suppliers of required, pre-qualified vaccine who are not manufacturers.

3. List documentation to be presented by the bidder to confirm <u>bidder's</u> eligibility to participate in tender and establish any preferences[9] mentioned in instructions to bidders.

a. Typical eligibility documentation:

- Nationality (applicable to World Bank procedures)
- Valid license or registration with national health authority (pre-qualification)[10]
- Agent or representative agreement with manufacturer
- Valid tax registration number

[9] Preferences normally do not have a bearing on product quality or supplier credibility, but are included to establish convenience factors.

[10] If not currently pre-qualified, invited bidders may be asked to submit completed application with bid.

- Current facility registration or business license
- Verifiable business and financial references (if potential bidder has not already been pre-qualified)

b. Examples of preference documentation:

- Supplier's product line literature on other items of interest to Ministry of Health
- Description of communications capability (languages, local representatives, telephone, fax, e-mail)
- Location / visiting information for manufacturing facility

4. List documentation to be presented by the bidder to establish vaccine's eligibility for use in the purchaser's country.

Any of the following may serve as evidence of pre-qualification by the health authority of purchaser's country:

- Copy of product license or registration issued by the NCA of purchaser's country
- Product pre-qualified in principle by WHO for supply to UN agencies
- Statement of Known Good Quality, obtained from WHO
- Copy of current license or certificate of free sale or equivalent issued by NCA of country with recognised, well-functioning NCA, such as USFDA.

5. Determine documents to be submitted with bids to establish product's conformity with specifications. For example:

- Package inserts
- Product file

6. Determine pre-shipment compliance programme, including rights and requirements to be included in the contract. Each type of commodity and situation will have slightly different requirements; seek technical advice.

Part IV., page 573

For example:

- Documentary review to confirm product's conformity with specifications
  - ◊ Lot release letter or certificate from the NCA of the country of manufacture
  - ◊ Certificate of analysis and batch records
  - ◊ Package insert

- Visual inspection to confirm product's conformity with specifications
  - ◊ Product and labelling
  - ◊ Packaging
  - ◊ Cold chain packing
  - ◊ Cold chain monitoring devices
  - ◊ Shipping marks

- Sampling and testing to confirm product's conformity with specifications is reserved for unusual circumstances (see Part IV.D.2. and IV.D.4.)

Part IV.D.2., page 573
Part IV.D.4., page 574

- Authorisation for shipment (see Part II.M.6.)

Part II.M.6., page 154

- Payment dependent upon proof of compliance

7. Determine government requirements, practices and procedures applicable to imports in general.

These may include:

- Pre-shipment inspection programme
- Import licensing
- Physical inspection and sampling at port of entry
- Currency exchange regulations
- Customs duties (or exemptions)
- Value added tax (or exemptions)
- Registration of pro-forma invoices

8. Determine documents required for import of vaccine, including number of originals and number of copies.

- Always required by government:
  - ◊ Commercial invoice
  - ◊ Air waybill
  - ◊ Packing list

- Sometimes required by government:
  - ◊ Certificate of origin
  - ◊ Certificate of clean findings
  - ◊ Certificate of analysis
  - ◊ No objection certificate
  - ◊ Sanitary certificate

- ◊ Manufacturer's quality certificate
- ◊ Import license number
- ◊ Product license or registration certificate
- ◊ Customs clearance agreement
- ◊ Product approval document issued by importing country
- ◊ Lot release certificate from regulatory authority of manufacturer's country

- Often required by purchaser
  - ◊ Insurance certificate

Form II.D.9.a., page 66
Sample II.D.9.a., page 68

9. Draft documents for bid package[11].

a. Invitation to bid

- Specifies who is issuing the invitation
- May specify source of funding
- Indicates how to get further information

b. Instructions to bidders

- Indicates if and how much bid security is required and acceptable financial instruments
- Repeats information found in the Invitation to Bid
- Explains the use of the bidding documents
- Gives detailed information on preparation of the bids
- Specifies documents required to establish bidders' eligibility and respond to purchaser's stated preferences
- Specifies documents required to establish goods' eligibility and conformity to bidding documents
- Gives rules for submission of bids
- Gives rules for bid opening
- Gives rules for evaluation of bids
- Gives rules for award of the contract

c. General conditions of contract

Supplement VII.K.1., page 833

- General clauses dealing with overall contract issues such as warranty, termination, force majeure, governing language and notices

Supplement II.D.9.c., page 72

[11] Selective tender procedures (Limited International Bidding).

d. Special conditions of contract.

- Adds to and / or supersedes General Conditions of Contract; prevails over General Conditions in event of conflict
- Specific to unique requirements of the procurement

  For example:
  - ◊ Pre-shipment compliance programme: rights and requirements (such as inspection)
  - ◊ Unacceptable trans-shipment points
  - ◊ No deliveries on public holidays (list) or weekends

e. Schedule of requirements.

- Items required for purchase
- Quantities
- Delivery schedule

f. Specifications.

Part III, page 165

- Complete description of the product
  - ◊ Properties
  - ◊ Standards
  - ◊ Licenses
  - ◊ Programme requirements
  - ◊ Packing and marking requirements
  - ◊ Quality assurance requirements

g. Bid form.

Part VII.K., page 831

- To be completed and signed by the bidder
- Binds successful bidder to conditions set out in the bidding documents
- Becomes a temporary contract upon notification of award

h. Price schedules.

Part VII.K., page 831

- Includes itemised charges for unit price of goods in specified currency
- Includes itemised charges for freight and insurance in specified currency
- May include itemised charges for domestic value added (not applicable for vaccine)
- May separate foreign and domestic bidders in order to calculate a margin of preference for locally manufactured products (not applicable for vaccine)

i. Bid Security Form.

Part VII.K., page 831
Form II.D.9.i., page 75

- Form used by a bank to guarantee financial compensation to purchaser if bidder fails to sign a contract after selection and award
- Bidder arranges and pays all costs
- Only used if required by the funding source

j. Contract form.

Part VII.K., page 831

- May be used to bind the purchaser and supplier until a formal contract can be signed
- Incorporates:
  - ◊ Bid form
  - ◊ Price schedule
  - ◊ Schedule of requirements
  - ◊ Specifications
  - ◊ General conditions of contract
  - ◊ Special conditions of contract
  - ◊ Purchaser's notification of award

k. Performance guarantee form.

Part VII.K., page 831
Form II.D.9.k., page 76

- Used by the guaranteeing bank
- Certifies the arrangement between the supplier and the bank with regard to performance of the contract
- Purchaser will be paid the guaranteed amount by the bank if supplier is in default under the contract

10. Prepare transmittal letter for Invitation to Bid.

- Reference pre-qualification
- Include basic information such as:
  - ◊ Purchaser's name and address
  - ◊ Type and quantity of vaccine required
  - ◊ Closing date of bid

11. Submit draft copies of "Bid Package" including proposed bidders list to Tender Committee (and funder, if applicable) for comment and approval.

Part II.E., page 93

12. Prepare draft evaluation and selection criteria, and submit to concerned ministries, senior programme management, NCA, and funder (if applicable) for comment and approval (often done only once and then considered "standard" unless special needs arise).

13. Alternate procedure for negotiated procurement.

a. Draft a Request for Quotation including[12]:

- Specification
- Expected contract terms
- Instructions and requirements for submitting offers

Form II.D.13.a., page 77
Sample II.D.13.a., page 79

b. Draft a preliminary contract to use as a basis for final negotiations. This draft should be made available, on request, to solicited suppliers.

Sample II.D.13.b., page 85

c. Develop prospective bidders list.

- Under WHO guidelines for vaccine procurement, the prospective bidders list should be limited to suppliers of pre-qualified vaccine
- Promote competition by including as many qualified suppliers as reasonable

d. Document justification for negotiated procedures.

- Procurement under a certain value
- Goods available from one source only
- Emergency
- Limited number of qualified suppliers

e. Secure approval, if necessary, for use of negotiated procedures (many national regulations permit negotiated procurement only in exceptional cases).

[12] Form II.D.13.a., page 77, contains basic wording. Sample II.D.13.a., page 79, is based on an actual, two-step Request for Quotation (RFQ) exercise conducted by fax, where EPI managers and the procurement team wished to compare the cost per vaccination among several different presentations before deciding on a vial size and soliciting final prices.

General and Special Conditions for Supply Contracts[1]

Format

As a matter of convenience, contract conditions are generally divided into two groups:

1. General Conditions of Contract

   All clauses are numbered and listed.

   - Clauses which are common to all contracts of a purchasing authority.
   - Clauses containing general statements of a topic which will be addressed further in Special Conditions.

2. Special Conditions of Contract

   Clauses are numbered and correspond with General Conditions of Contract; only applicable clauses are included.

   - Modifications to the general conditions that address unique requirements related to the specific purchase; supersede or add to the corresponding general condition.

Content

The following topics are appropriate for supply contracts:

Applicable Law	Usually, laws of the purchaser's country.
Assignment	Procedures for assignment; usually contract cannot be assigned to someone else without purchaser's prior written consent.
Change Orders	What may be allowed and cost adjustment provisions.
Contract Amendments	Procedures; amendments are usually required in writing.
Country of Origin	Requirements and limitations.
Definitions	Purchaser, Supplier, Goods, Contract, Contract Price, etc.; additional definitions may be included in Special Conditions.
Delays in Supplier's Performance	Provisions for notices, penalties or extensions.
Delivery and Documents	General Conditions may prescribe use of trade / shipping terms such as INCOTERMS; Schedule of Requirements and / or Special Conditions will contain specifics.

[1] Adapted from World Bank Sample Bidding Documents.

Force Majeure	Definition and procedures.
Governing Language	Required language of bid, contract, correspondence and other documents.
Inspections and Tests	General Conditions give the right to inspect and test; specific requirements are included in Special Conditions or Technical Specifications.
Insurance	Type, value and beneficiary.
Liquidated Damages	Conditions under which they may be imposed.
Notices	Rules regarding the method of sending notices and effective dates.
Packing	General Conditions might mention “adequate to prevent deterioration and damage during transit;” specific details are included in Special Conditions or Technical Specifications.
Patent Rights	Indemnities against third party claims.
Payment	General Conditions will usually include how to request payment, the time allowed to pay and currency requirements. The specific method of payment is addressed in Special Conditions.
Performance Security	If required, the amount and acceptable mechanisms such as performance bond. (May be waived in the case of reputable multinational suppliers.)
Resolution of Disputes	Procedures.
Standards	Minimum requirements and alternatives; specifics such as ISO are generally listed in Technical Specifications.
Subcontracts	Requirements for notification to purchaser if any part of contract will be performed for the supplier by someone else.
Taxes and Duties	Who is responsible for payment.
Termination for Convenience	Whether or not purchaser will be allowed this option; may include provision for payment based on completed or delivered goods.
Termination for Default	Conditions under which contract may be terminated for default.
Termination for Insolvency	Whether or not purchaser has a right to terminate in event of supplier bankruptcy.

Transportation	Carrier requirements such as nationality; who is responsible for payment; unacceptable trans-shipment points.
Use of Contract Documents and Information	Limitations on disclosure.
Warranty	Required period; usually 12 months from receipt or 18 months from shipment.

Bid Security Form

Standard Form of a Bid Bond

Letter by the Guarantor to the Purchaser

We, the undersigned _____ (bank, insurance company), in order to allow _____ to submit a bid for _____ (project, subject matter of contract), hereby irrevocably and independently guarantee to pay to you without delay any amount up to a total of _____, waiving all objections and defences.

We shall effect payments under this guarantee on your first written demand, which must be accompanied by your confirmation that you have accepted the above-mentioned bid and that _____ are not prepared to abide by their bid.

This guarantee shall expire not later than _____ by which date we must have received any claims by registered letter, telegram or telex.

It is understood that you will return this guarantee to us on expiry or after settlement of the total amount to be claimed hereunder.

_____ _____

Place, date Guarantor

Performance Guarantee Form

Standard Form of a Performance Bond

Letter by Guarantor to the Purchaser

On _____ you concluded with _____ ("Contractor") a contract for _____ (project, subject matter of contract) at a price of _____. According to the provisions of the contract, the Contractor is obliged to provide a performance bond in the order of _____ percent of the contract price.

We, the undersigned _____ (bank, insurance company), waiving all objections and defences under the aforementioned contract, hereby irrevocably and independently guarantee to pay on your first written demand any amount up to a total of _____ (in words _____) against your written declaration that the Contractor has failed to perform the aforementioned contract.

In the event of any claim under this guarantee, payment shall be effected to _____.

This guarantee shall expire not later than _____, by which date we must have received any claims by registered letter, telegram or telex.

It is understood that you will return this guarantee to us on expiry or after settlement of the total amount to be claimed hereunder.

_____ _____

Place, date Guarantor

Request for Quotation
(basic wording)

Request for Quotation

Company Name **Date**

Address

City State

Country

The Government of _____, Ministry of Health requests your quotation for the supply of _____doses _____ vaccine. This vaccine will be distributed as part of the _____programme sponsored by _____. Below is information regarding product specifications, quantity requirements and delivery schedules along with instructions for submitting offers. A draft of the contractual terms and conditions under which these goods are expected to be supplied is enclosed for your reference. Please address any questions you may have in writing to _____. General clarifications and supplemental information provided to you as a result of your inquiry also will be provided to the other invited participants in this Request for Quotation.

A. Requirements

1. Specification: _____

   _____

   _____

   _____

2. Licensing: _____
3. Quantity required: _____
4. Delivery date required: _____
5. Shipping terms: _____
6. Payment terms: _____
7. Shipment via: _____

8. Documents to be provided with each shipment:

   a) Certification for the release of the vaccine lot by the National Control Authority in the country of manufacture

   b) Manufacturer's batch / lot information including protocols, certificate of analysis, test summary sheets and approval and release records signed by the regulatory affairs manager for the manufacturing facility.

   c) Copy of package insert translated into _____[language].

B. The following rules should be followed in preparing your quotation:

1. Prices must be itemised as follows:

   a) Goods
   b) Freight
   c) Insurance
   d) Handling fees
   e) Documentation fees
   f) Export packing
   g) Inspection
   h) Inland transportation
   i) Other _____

2. Offers must be valid for 90 days from the date of the quotation.

3. Offers must be signed by an authorised representative of the offering firm in order to be considered valid.

4. Five sample vials per type and size with package insert must accompany each offer.

5. Offers must be presented to this office on or before _____. Submissions via fax are acceptable provided they are followed within 10 days by a signed original.

C. If you are not currently registered with _____ as a pre-qualified supplier of licensed vaccine, you must submit a completed and signed Pre-qualification Questionnaire with your quotation.

   ◊ questionnaire is available on request from _____

   ◊ questionnaire is attached _____

The Ministry of Health looks forward to receiving your offer.

Sincerely,

Purchasing Officer

Request for Quotation
(practical example with expanded wording)

Request for Quotation

FAX TRANSMISSION

To: Date:

Fax #:

REQUEST FOR QUOTATION [*reference number*]

The Ministry of Health, [*country*] requires substantial quantities of vaccine in order to supply its national immunization programme. The Ministry of Health requests your quotation or proposal of price and availability for any or all of the items and quantities shown in Annex One. Detailed Procurement Requirements for the vaccines known to match your product line appear in Annex Two[13]. The Ministry of Health [*country*] will provide its Procurement Requirement for any of the remaining items upon your request.

Sections A and B of the Procurement Requirements (Annex Two) for vaccines are unique to the vaccine type required; Sections C through F (pages three through six) are common to all vaccines required by this solicitation. We are transmitting only one copy of Sections C through F. Please apply these sections to each vaccine Procurement Requirement you receive.

Contracts are expected to be signed during [*date or quarter of year*] with initial deliveries commencing immediately thereafter. Payment terms are negotiable or by irrevocable Letter of Credit; fully convertible currency is available for this purchase. An outline of applicable Terms and Conditions appears in Annex Three.

Instructions for Response:

Please indicate prices for all items you wish to offer by completing the table in Annex One. Your response should also include the following:

1. Payment terms offered, including currency of payment. For the purpose of price comparison, all offers will be converted to U.S. dollars at the rate effective on the day indicated as the last day for receipt of quotations.

2. Availability.

   a. items and quantities available for shipment to [*country*] by delivery date(s) indicated in Schedule of Requirements (Annex One)
   b. estimated dates for availability of remaining balance

[13] Annex Two (procurement requirements) has not been reproduced in this sample. See Part III for detail.)

3. Information on manufacturer's standard packaging configurations.

   a. number of vials per package and per shipping container
   b. technical description of shipping containers for each type of vaccine proposed for shipment to [*country*] including: gross weight, dimensions, insulation, and type and quantity of refrigerating material

4. Copy of package inserts normally shipped with vaccine or syringes and copy of customary vial labelling.

5. Information on product approvals.

   Please indicate if the vaccine has been pre-qualified by the World Health Organization for sale to United Nations agencies, or approved for use by any other international agencies, or licensed by the National Control Authority of other countries. Submit list.

6. Copy of product registration or licensing in country of manufacture.

7. Copy of recent batch protocol and certificate of analysis pertaining to each of the products offered.

   Note: this does not necessarily need to be from the batch proposed for shipment to [*country*];

8. Business information and customary financial data.

   a. name and address of production facility
   b. type of organisation
   c. affiliations, parent company or subsidiary relationships
   d. number of years in business
   e. countries to which products are presently exported
   f. approximate annual sales in U.S. dollars

9. Sample of each vaccine in each size offered, forwarded separately on date of fax response. Cold chain packing is not required. Please include all package inserts.

Upon receipt of the completed Price Offer in Annex One, the Ministry of Health will select vial sizes, indicate options of interest and request your final CIP price by fax.

Offers shall be made in English and will be translated into [*language*] upon receipt in [*country*]. Alternately, prospective suppliers may provide translations with their offers.

Offers shall be valid for 90 days from date of initial response.

Please send your response by international courier or fax to:

[*name, address and fax number of ministry and name of responsible person*]

Telephone inquiries in English may be made through:

[*name and telephone number of contact person, if required*]

The Ministry of Health looks forward to receiving your offer before:

[*closing date*]

Sincerely,

[*name and title of responsible person*]

Ministry of Health Solicitation

Annex One
Request for Quotation Number_____

Ministry of Health
[*country*]

SCHEDULE OF REQUIREMENTS

ml/dose	**HEP B 0.5**	**Mumps 0.5**	**OPV**	**BCG 0.05**
Quantity in doses*	144,100	199,800	128,000	464,000
Date for delivery	1-Aug-96	1-Jun-96	1-Aug-96	1-Aug-96
No. of shipments	one	one	one	one
Shelf life**	18 mo	12 mo	18 mo	12 mo

*from one production lot
**remaining on date of delivery

PRICE OFFER: Vaccines (Ex Works)

	HEP B				
Price/vial	**Plasma**	**DNA**	**Mumps**	**BCG**	**OPV**
1 dose vial					
2 dose vial					
5 dose vial					
10 dose vial					
20 dose vial					

Please enter prices in U.S. dollars for vials in appropriate boxes. Purchaser will calculate price per dose.

Period of validity:_____
Signature:_____ Date _____
For _____
(name of company)

Annex Three
Request for Quotation Number_____

Ministry of Health
[*country*]

TERMS AND CONDITIONS

SHIPPING TERMS:	CIP [*name of city, country and port of entry*]
TRANSPORT MODE:	Air
TRANS-SHIPMENT:	[*designated international airport*]
PARTIAL SHIPMENTS:	By Agreement
FINAL DESTINATION:	[*name of city and country*]
PAYMENT TERMS:	Negotiable, or by Irrevocable Letter of Credit
INSPECTION AND TESTING:	Pre-Shipment Compliance Programme The Ministry of Health of [*country*] reserves the right for a designated representative to inspect, sample and test, or cause to be tested, each lot of goods proposed for shipment to [*country*] for conformance with contract requirements BEFORE said shipment leaves the manufacturing facility.
DOCUMENTS FOR EACH SHIPMENT:	A. Quality Documents Lot release letter from the government regulatory authority in the country of manufacture. Evidence of product and facility registration / licensing in the country of manufacture. Certificate of Analysis for lot(s) being supplied. Manufacturer's batch / lot information including protocols, test summary sheets and approval and release records signed by the regulatory affairs or quality assurance manager for the manufacturing facility. THE ABOVE DOCUMENTS SHALL BE PROVIDED THREE WEEKS PRIOR TO SCHEDULED VACCINE SHIPMENT.

B. Commercial Documents
Commercial Invoice, Bill of Lading or Air Waybill, Packing list, Certificate of Origin, Insurance Certificate.

PACKING: Vaccine must be packed in accordance with the Procurement Requirements in Annex Two for each type of vaccine supplied and the WHO EPI Guidelines on the International Packaging and Shipping of Vaccines.

MARKING: The exterior of all shipping cartons or containers must be marked in accordance with the Procurement Requirements in Annex Two, including cautionary wording regarding temperature range.

Other pertinent shipping label information will be provided prior to shipment.

NOTIFICATION: The Supplier will keep the [*Ministry of Health*] informed of any circumstances that might affect the delivery schedule.

Supplier shall confirm shipping date to [*Ministry of Health*] three weeks prior to dispatch and provide flight details as early as known.

WARRANTY: The supplier shall warrant that the goods supplied to the Government of [*country*] comply with all provisions of the Procurement Requirement and related documents.

CONTRACT AWARD: Contracts will be awarded on the basis of the most advantageous and lowest evaluated cost offer which conforms to this Request for Quotation. In addition to price, criteria for evaluating the offers will include technical merit, commercial merit, contractual merit and convenience.

The Ministry of Health reserves the right to award separate contracts for different portions of the goods covered by the RFQ, or to combine different items into one contract.

A copy of the purchaser's draft contract is available upon request.

DRAFT MODEL

CONTRACT FOR SUPPLY OF VACCINES

CONTRACT No. _____

between _____ (country) Ministry of Health and

_____

Date: _____

The Seller **The Buyer**

Ministry of Health, (country)
represented by (name of official)

1. SUBJECT

The Seller undertakes to sell and the Buyer to purchase goods in accordance with the terms stipulated in Annex 1 of the present contract and the attached specification[14].

2. NAME OF GOODS

Vaccines in accordance with Specification Number _____.

3. PRICE

The prices will be in [*currency*], CIP [*City, Country*]. The prices include the cost of the goods, transportation, packing, marking and insurance, delivered without payment of customs fees and taxes. The prices are firm and not subject to revision during the whole term of the contract. The total value of the contract is _____.

4. TERMS OF DELIVERY

The vaccine designated in this contract shall be delivered to [*City*] in equal quarterly shipments over a period of one year. Seller is obliged to deliver the shipments after payment by Buyer on account of Seller as mentioned in paragraph five (5.) below.

Seller must inform the Buyer by fax or telex ten (10) days in advance of each shipment of goods. The Government of [*Country*] reserves the right for a designated representative to inspect and test the goods for conformance to contract requirements prior to shipment. The date of the first shipment shall

[14] Specification has not been reproduced in this sample. See Part III for details.

not be more than _____ weeks after the date funds are made available to Seller at _____ Bank.

Seller must ensure that delivery does not take place on a weekend or during a public holiday in the country of delivery.

5. TERMS OF PAYMENT

The Buyer will deposit funds equal to _____ percent of the value of each shipment in the account of Seller at _____ Bank, Account Number _____ upon receipt of specified documents according to the following schedule:

$ _____ not earlier than _____ (date)

$ _____ not earlier than _____ (date)

$ _____ not earlier than _____ (date)

$ _____ not earlier than _____ (date)

The Buyer will transfer the remaining balance due for each shipment's value to the account of Seller at _____ Bank upon receipt of conforming goods.

6. QUALITY

6.1 The Seller is responsible for the quality of supplied vaccine according to the technical standards set by the manufacturer's Certificate of Quality.

6.2 The vaccines supplied to the Government of [*Country*] must meet internationally recognised standards for safety, efficacy and quality, and must strictly comply with the terms of Annex 1.

6.3 The remaining shelf life of vaccine on the date of delivery must not be less than 18 months or 75 percent of its original shelf life as designated in the manufacturer's technical standards.

6.4 For each vaccine supplied to the Government of [*Country*], quality assurance documentation from the manufacturer and from the National Control Authority of the country of manufacture must be provided according to the terms of the Annex of this Contract.

7. LABELLING

7.1 The label on each vial or ampoule of vaccine shall conform to the requirements of the country of use and shall appear in the _____ language.

7.2 Each vial or ampoule label must include:

- the generic name of the vaccine and the manufacturer's name
- the trade name of the vaccine (if applicable)
- the lot number and expiration date of the vaccine
- the composition and concentration of the vaccine
- recommended dosage and mode of administration

7.3 All labelling must withstand immersion in water and remain intact.

8. PACKAGING

8.1 _____ (number) individual vials or ampoules will be contained in sturdy cardboard boxes outfitted with individual segments for protecting and separating each vial or ampoule. Storage volume[1] occupied per infant dose of vaccine shall not exceed storage volume standards recommended by WHO in EPI Guidelines on International Packaging and Shipping of Vaccine, EPI / CCIS / 81.4 rev. 6.

8.2 Each inner box mentioned in 8.1 above will be marked as follows:

- Contract No.
- [*Government, Ministry of Health*]
- the generic name of the vaccine or medical product
- the commercial name (if made under license)
- the manufacturer's name
- the presentation (vial, ampoule) and quantity
- the lot / batch number and expiration date

8.3 Each inner box must contain a package insert with a full description of the vaccine and instructions for use in the [*Country language*] and in [*English*]. Photocopies of [*Country language*] translation are acceptable. If necessary, Buyer will provide text translation to Seller.

9. PACKING AND MARKING

9.1 Packing and marking of vaccines and medical supplies must strictly comply with international export package standards. Vaccine must be packed in cartons / containers suitable for export shipment and be in accordance with WHO EPI Guidelines on the International Packaging and Shipping of Vaccines, EPI / CCIS / 81.4 rev. 6, including all measures needed to maintain required temperatures for 72 hours.

[1] Storage volume includes the vaccine vial, the packet containing the vaccine vial and any intermediate parcelling of vaccine packets.

9.2 Each insulated shipping container must include appropriate temperature monitoring devices designated by the Buyer.

9.3 The Seller must reimburse the Buyer for any loss due to improper packing.

10. SHIPPING MARKS AND DOCUMENTATION

Shipping marks, documentation and notification must be in accordance with the Annex of this Contract.

11. ACCEPTANCE

11.1 The goods will be received by [*agent*] for the Ministry of Health , postal address _____
_____

11.2 For quantitative and damage claims, the Buyer must present to the Seller a written claim drawn up by [*Chamber of Commerce or similar independent body*] of the Buyer's country.

11.3 All quantitative and damage claims shall be settled within _____ weeks of submission.

12. DEFECTIVE GOODS

12.1 If, after receipt in [*Country*], the vaccines are believed not to conform to the specifications mentioned above and in the referenced annexes, Seller will be contacted and asked to verify the conformity of the vaccine. The Seller will advise on the preferred mechanism for return of suitable samples for testing. The Buyer will ensure that the total shipment of vaccine is stored under the correct conditions during the period of testing.

12.2 If it is established that the vaccine does not comply with the contract specifications, Seller must replace the defective goods with new ones at his own expense not more than _____ weeks after the date of the claim, and pay all transport costs and other expenses connected with the replacement or return of the defective goods including transportation costs in the territory of the Buyer's country.

12.3 If the Seller does not replace the defective goods with new ones within _____ weeks from the date of the claim, the Buyer has the right to cancel the contract. In this case, the Seller must reimburse the Buyer for his payments (if any).

13. PLACE OF DESTINATION

[*City airport - Country*]

14. FORCE MAJEURE

Each party to this agreement may be excused from fulfilling its obligations under the Contract in the event of force majeure circumstances such as military operations, blockades, prohibition of export or import and catastrophic loss from natural disaster, fire, strikes and changes in legislation.

In such cases, the party claiming force majeure must inform the other party in written form within fifteen (15) days after the beginning of the force majeure circumstances and the existence of these circumstances must be confirmed by the Chamber of Commerce of the claiming party. If force majeure circumstances last more than four months, each party has a right to refuse to fulfil its obligations under the contract and none of the parties has the right to claim compensation for possible losses from the other party.

15. ARBITRATION

In case disagreements arise in the course of the Contract, all disputes will be settled finally in accordance with the Rules of Conciliation and Arbitration in the International Chamber of Commerce.

16. OTHER CONDITIONS

16.1 The present contract falls under [*the law of the Buyer's country*].

16.2 All correspondence between the two sides must be sent to [*addresses*].

16.3 The Seller guarantees having all the patents and other rights pertaining to the vaccine.

16.4 The cost of repeating any pre-shipment inspection or test is borne by Seller.

16.5 The Seller completes all formalities and pays all export taxes in his country.

16.6 The Buyer completes all customs clearance formalities and pays all import taxes in his country.

16.7 All vaccine arriving in [*Country*] on or after [*date*], must bear a license or registration number issued by the National Control Authority of [*Country*].

16.8 All annexes to this contract are integral parts of it.

16.9 All alterations and amendments to this contract are valid only if they are in written form and signed by the authorised representatives of the parties.

16.10 Neither of the parties has the right to assign its obligations on the contract to third parties without a written consent of the other party.

16.11 From the moment of signing the contract, all the previous correspondence and negotiations will become invalid.

16.12 The date of delivery will be the date of arrival at [*City*] airport.

16.13 In the case of an address change or a change in bank, each side must inform the other within 15 days of the change.

17. BANKS OF THE PARTIES

for the Seller: for the Buyer:

_____ _____

The present contract is written in English and _____ languages and signed in _____[*City*] _____ [*number*] original copies that have equal force.

For and on behalf of the Seller

(signature and stamp)

Name: _____
Title: _____
Address: _____
Date: _____

For and on behalf of the Buyer

(signature and stamp)

Name: _____
Title: _____
Address: _____
Date: _____

Contract No. _____

ANNEX I

1. Vaccine Documents:

   The following documents must be included with each lot (numbered batch) of vaccine shipped to [*Country*]:

   a. Certification for the release of the vaccine lot by the National Control Authority in the country of manufacture.

   b. Manufacturer's batch / lot information including protocols, certificate of analysis, test summary sheets and approval and release records (protocols) signed by the regulatory affairs manager for the manufacturing facility.

   c. Certificate of registration or licensing of the vaccine in the country of manufacture.

   d. Copy of package insert translated into [*Country*] language.

2. Parties Receiving Documents:

   A complete set of documents identified in Section 1 above, plus normal and customary international shipping documents, i.e.,

   a. Air waybill.

   b. Commercial invoice.

   c. Packing lists.

   d. Insurance certificate.

   must accompany each shipment and an identical set must be sent in advance of each shipment by international courier or fax to the following party:

   Name:

   Address:

   Telephone:

   Fax:

3. Marking Instructions:

   The exterior of all outer shipping cartons / containers shall note the quantity and description of contents. All outer shipping containers and accompanying standard documents must also bear:

   a. Lot number.

   b. Expiry date.

   c. Emergency contact telephone number.

   d. The following cautionary wording in English and [*Country*] language:

      "Perishable Material, Keep Refrigerated (2-8 degrees centigrade)". Add as appropriate: "KEEP FROM FREEZING".

4. Shipping Instructions:

   a. Commercial air cargo.

   b. Trans-shipment allowed at [*designated airport*] only.

   c. Each vaccine shipment should be consigned as follows:

      Name:

      Address:

      Telephone:

      Fax:

   d. All invoices and shipping documents must carry the markings as stated in Section 3 above.

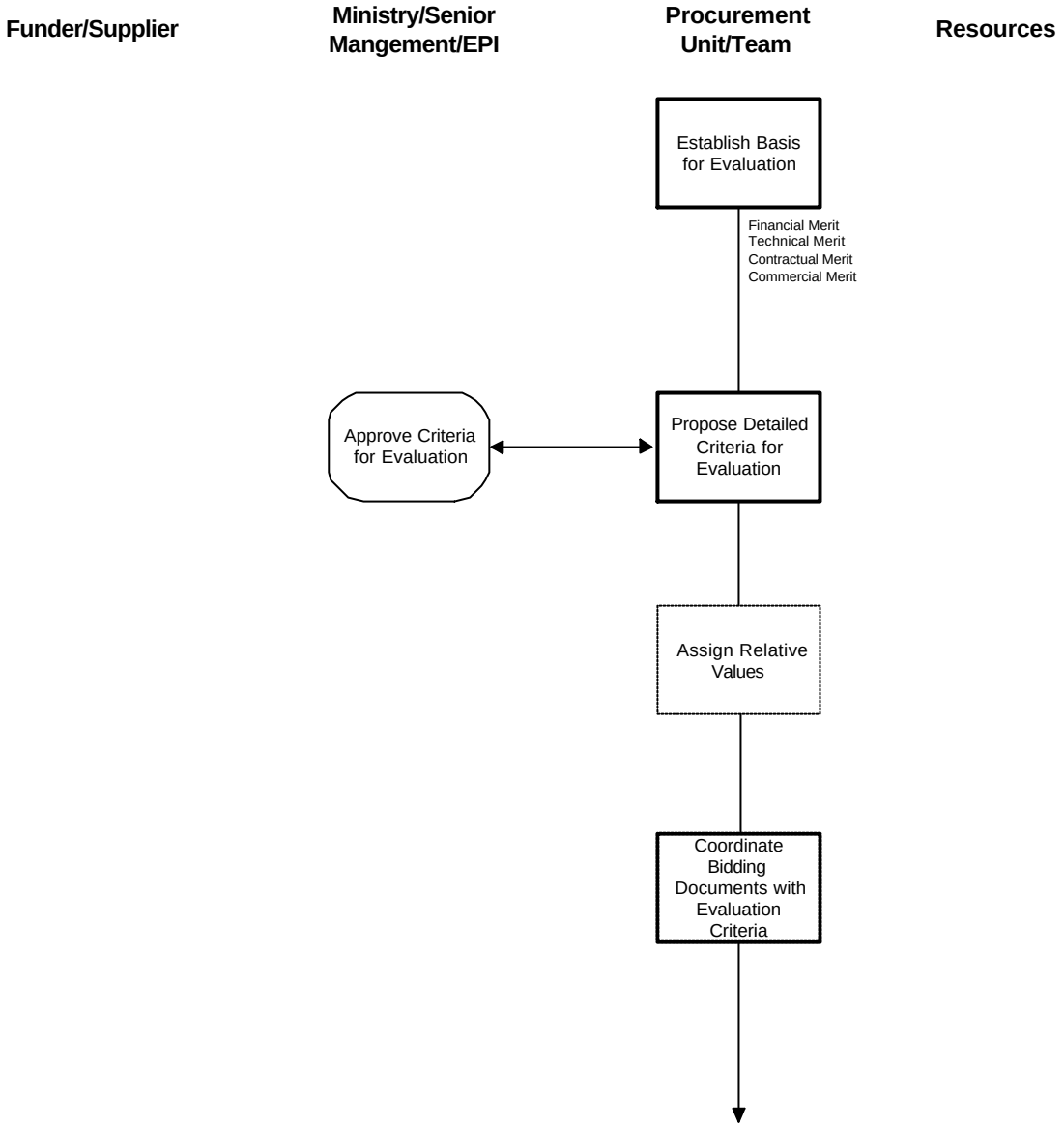
5. Delivery Information:

   a. Prior to shipment, the Seller will fax the following information to the parties identified in 4.b above.

      i. Airline, flight number and departure time
      ii. Estimated time of arrival (ETA) in [*City*]
      iii. Total quantity and description of commodity
      iv. Total number and description of containers
      v. Dimensions of each piece
      vi. Accurate net and gross weight per piece
      vii. Total gross weight and cube of entire shipment

6. The Seller will keep the Ministry of Health, Government of [*country*] informed of changes in the production / delivery schedule.

E. Preparations for Bid Evaluation



E. Preparations for Bid Evaluation

This step is performed before the bidding documents are distributed to prospective bidders.

A Procurement Team is often formed to propose evaluation criteria to the Tender Review Committee. This team will usually include procurement staff, programme staff and at least one ministry official, all with intimate knowledge of programme requirements and the bidding documents. As it undertakes this activity, it may uncover important points that should be addressed in the bidding documents, but were inadvertently omitted. This exercise allows an opportunity to correct omissions and other points that may be unclear, as well as facilitate the work of the Tender Review Committee.

1. **Propose basis for evaluating and comparing offers.**

   Categories for adjudication usually include:

   - Financial merit
   - Technical merit[15]
   - Contractual merit
   - Commercial merit

2. **If a merit point system for selecting the winning bid will be used, propose relative values for each category. For example:**

   - Financial merit: 35%
   - Technical merit: 35%
   - Contractual merit: 15%
   - Commercial merit: 15%

   Total 100%

3. **Propose detailed criteria for analysis of the *subjective* components of each offer, e.g. technical, contractual and commercial merit. (Financial merit based on bid price is an *objective* factor that requires no analysis.)**

   a. Select elements in each category that are most important to the Purchaser and immunization programme. For example:

      - Technical merit:
        - ◊ Product attributes
        - ◊ Packaging (e.g., vial size, closures)
        - ◊ Cold chain packing
        - ◊ Package inserts
        - ◊ Shelf life

[15] Limited to variable programme requirements and preferences, i.e., criteria that are not part of a national licensing requirement.

- Contractual merit:
  - ◊ Delivery schedule
  - ◊ Payment terms
  - ◊ Warranties
  - ◊ Shipping terms
  - ◊ Inspection rights

- Commercial merit:
  - ◊ Bidder pre-qualified by National Control Authority for supply of vaccines to immunization programme
  - ◊ Direct relationship with manufacturer of pre-qualified vaccine
  - ◊ Product line includes other items of interest to Ministry of Health
  - ◊ Ease of communication with manufacturer or representative

b. Make a list of specific characteristics, requirements and preferences as described in the Invitation to Bid for each selected element in each category. For example: Product has 18 months remaining shelf life when it arrives in country.

c. Evaluation of offers received in response to the solicitation will be based on how well the elements of each offer match the selected characteristics, requirements and preferences described in the Invitation to Bid.

4. Tender Committee meets to:

a. Review and approve "Bid Package" prepared by procurement staff, e.g., the bidding documents.

b. Review, modify as necessary and approve evaluation criteria proposed by procurement team.

5. If a merit point system will be used, propose relative values for selected elements within each category in order to establish a framework for subjectively quantifying how well the elements of each offer match the corresponding characteristics, requirements and preferences described in the Invitation to Bid. For example:

Technical merit (35 points):

10 points	Product attributes
5 points	Packaging (e.g., vial size, closures)
10 points	Cold chain packing meets
5 points	Package inserts
5 points	Shelf life

In an adjudication of bids offering licensed vaccine, the criteria for analysis of technical merit are based on programme issues, i.e., factors that are not part of the license requirements and may, in fact, vary from purchase to purchase depending upon how the vaccine will be stored, distributed and

used. Examples would be presentation (vial or ampoule and size), extended shelf life, additional filling volume, optional labelling (such as "for public-sector use only"), specific language labelling (such as Russian) on inserts and vials, special packaging or packing such as placement of carton labels on a narrow end rather than a wide side, extra cold chain monitor cards, special monitoring equipment, such as re-usable, electronic time / temperature recorders. In a merit point system, the programme itself decides the relative importance of each issue by assigning an appropriate value.

6. **Make sure that bidding documents request information corresponding to the evaluation criteria and include information about the basic criteria to be used in selection of a winning bid (2 and 3 above).**

7. **Provide proposed evaluation criteria to Tender Committee for review and approval.**

F. Solicitation and Receipt of Offers for Selective Tender (LIB)

Funder/Supplier	Ministry/Senior Mangement/EPI	Procurement Unit/Team	Resources
		Transmit Invitations to Bid	
		Receive and Hold Bids Unopened	
		Open and Read Bids	
		Collect and Record Bid Security	
		Record Bids	

F. Solicitation and Receipt of Offers - Selective Tender

1. Send Invitations to Bid and bidding documents to as many prospective suppliers as practical.

Part II.D.2., page 59

See "Bidder's List" (suppliers that offer the specific pre-qualified vaccine).

2. Respond to inquiries on the substance of the bidding documents via fax, e-mail or other means.

a. Copy clarifications to all invited bidders.

b. Amendments to the bid documents may be appropriate.

3. Hold bids unopened in a secure location until the designated date and time.

4. Hold formal bid opening on date and at time and place specified on the bid documents.

a. Bidders and bidders representatives may be present when opening takes place.

b. Record the attendance of any bidders or bidders' representatives present.

- Name, address, phone, fax, e-mail
- Company representing
- Manufacturer
- Signature of attending representative

c. Open bids; read bids aloud.

- Name of each tenderer
- Tender price, total or unit, shipping terms
- Other relevant aspects of the tender, for example, delivery schedule

d. Record the bid opening and set aside for further examination.

Form II.F.4.d., page 100

5. Bid examination.

a. Examine bids to determine responsiveness to tender requirements.

A bid is *substantially* responsive when it is presented in the required manner and appears to include all required information, samples, statements, securities, signatures, forms and supporting documentation.

b. Any bid that is not substantially responsive should be rejected. In the case of vaccine, this would include vaccine that has not been licensed by

the NCA of the purchaser's country, provided that requirement is stated in the bidding documents.

c. Examine bids to determine if they are free of computational error; make corrections based on unit price.

6. Collect and record bid securities (not necessary for vaccine purchases unless required by funder).

Appropriate vehicles are:

- Insurance document (bid bond)
- Certified check
- Letter of Credit
- Bank draft
- Bank guarantee
- Other negotiable bank document

Responsibility for record keeping and preservation of funds must be clearly defined; also, systems should be established and provision made for safeguard of the securities.

7. Complete and sign Record of Bid Examination.

Form II.F.7., page 101
Sample II.F.7., page 103

Record of Bid Opening

Invitation to Bid Number: _____

Bidder: _____

Total Bid Amount: _____

Opening Date: _____

Opening Location: _____

Currency Equivalent: _____ @ _____ Date _____

Manufacturer: _____

Country of Origin: _____

Number of Bid Copies Submitted: _____

Bid Security Submitted: Yes _____ No _____

Name of Issuer: _____

Reference Number: _____

Amount: _____

Expiry Date: _____

Record of Bid Examination

Bidder: _____ Invitation to Bid Number _____

1. Bid documents submitted in __(language)__; Language required:_____
2. Bid form included? ___yes ___no ___n / a[1] Bid signed? ___yes ___no
3. Price schedule included? ___n / a[1] ___yes ___no
4. Documents regarding bidder's eligibility to participate
   a. ___yes ___no
   b. ___yes ___no
5. Documents regarding product's eligibility
   a. ___yes ___no
   b. ___yes ___no
6. Documents regarding product's conformity to specification
   a. ___yes ___no
   b. ___yes ___no
7. Bid security provided ___n / a[1] ___yes ___no ___ ____% of total bid
   _____% required

   Instrument _____bid bond
   _____bank draft
   _____certified check
   _____letter of credit
   _____other

   Period of validity_____

   Required Period of validity_____

[1] Not applicable.

This bid is substantially responsive to tender requirements. ___yes ___no

This bid is free of computational error. ___yes ___no

Are the bid documents submitted without inter-lineations or overwriting.

___yes ___no

Date_____ _____

Authorised Procurement Official

Record of Bid Examination

Bidder: ***XYZ Company*** Invitation to Bid Number: ***F27***

1. Bid documents submitted in: ***English*** Language required: ***English***
2. Bid form included? ___yes ___no _x_ n/a Bid signed? _x_ yes ___no
3. Price schedule included? ___n/a _x_ yes ___no
4. Documents regarding bidder's eligibility to participate
   a. ***proof of registration as supplier to MOH*** _x_ yes ___no
   b. ***current business license*** _x_ yes ___no
   c. ***completed and signed pre-qualification questionnaire*** ___yes _x_ no
5. Documents regarding product's eligibility
   a. ***proof of licensing with NCAB of purchaser's country*** _x_ yes ___no
   b. ***statement of Known Good Quality from WHO*** ___yes _x_ no
   c. ***current certificate of registration and licensing of the vaccine in the country of manufacture*** _x_ yes ___no
6. Documents regarding product's conformity to specification
   a. ***product file*** _x_ yes ___no
   b. ***package insert*** _x_ yes ___no
7. Bid security provided _x_ n/a ___yes ___no _____% of total bid

   _____% required

   Instrument _____bid bond

   _____bank draft

   _____certified check

   _____letter of credit

   _____other

   Period of validity_____

   Required Period of validity_____

This bid is substantially responsive to tender requirements. _x_ yes ___no

This bid is free of computational error. _x_ yes ___no

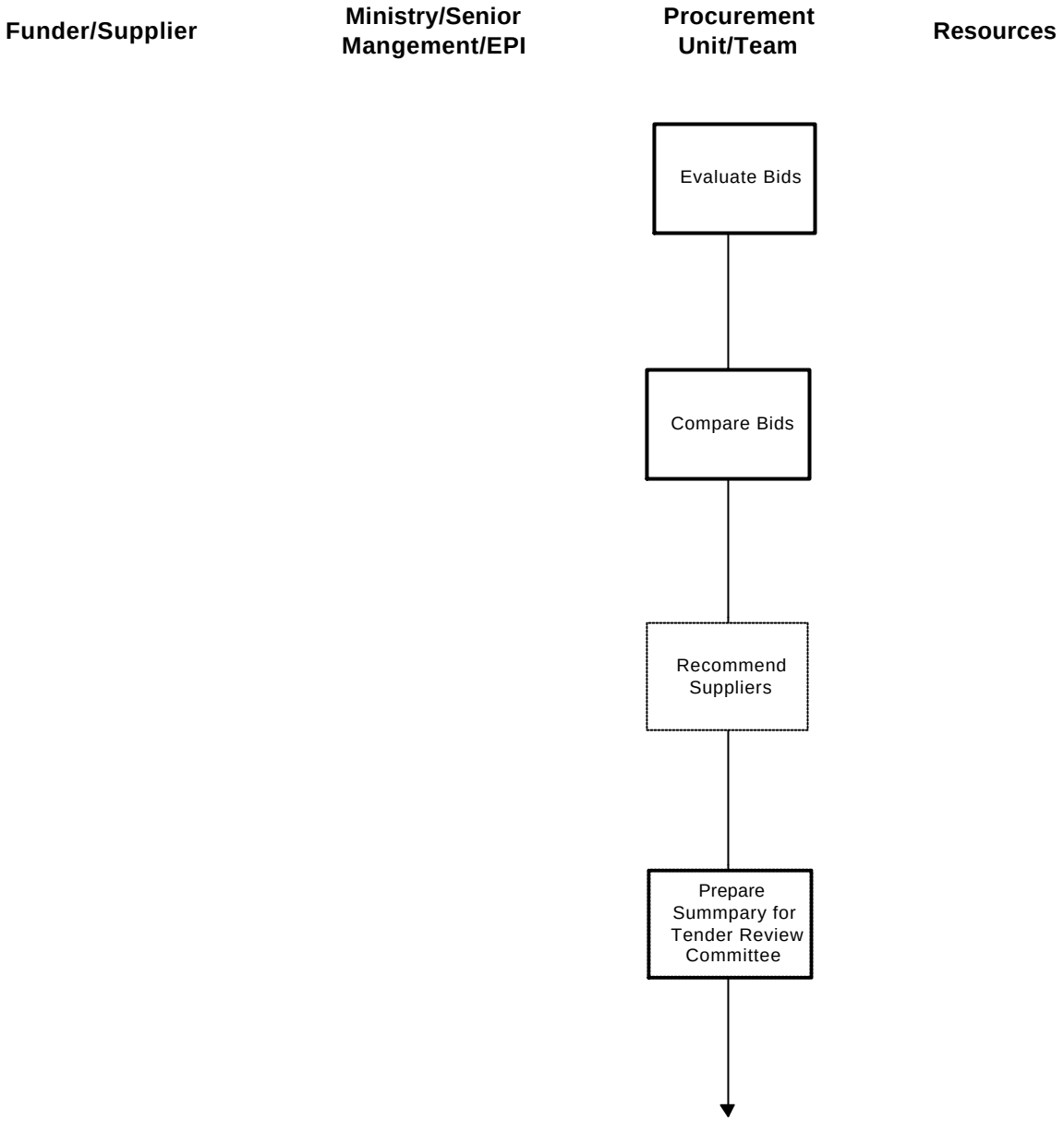
Are the bid documents submitted without inter-lineations or overwriting.

_x_ yes ___no

Date ***September 1, 1997*** _____*[signature]*_____

Authorised Procurement Official

G. Evaluation and Comparison of Bids



G. Evaluation and Comparison of Bids

Section G. "Evaluation and Comparison of Bids", and Section H "The Selection Process", contain co-ordinated examples based on a tender for vaccine in 10-dose vials. These examples highlight features from several of four responsive bids in order to demonstrate various aspects of the evaluation and selection process, but do not constitute a case study.

Sample II.G.1. (Bid Summary) demonstrates the evaluation of Bidder **A**'s offer.

Sample II.G.1.a. (Financial Adjustment Sheet) shows an attachment to the Bid Summary for Bidder **C** that was not required for Bidder A.

Sample II.G.3. is an example of a Merit Point Form that would be filled out for each bid.

Form II.G.5.b. is the Tender Review Committee Summary Cover Sheet for the entire process.

Supplement II.H.1.c. discusses adjudicating bids under a Merit Point System.

Sample II.H.1.c. demonstrates an Adjudication Worksheet that includes a calculated cost per vaccination in addition to cost per dose and the total CIP price.

The Adjudication Worksheet form is discussed in Section G but appears as a sample in Section H only in order to avoid repetition.

Sample II.G.1., page 109

1. **In consultation with the procurement team and using detailed evaluation criteria developed prior to solicitation of bids, prepare a Bid Summary for each substantially responsive bid. (See discussion of substantially responsive bid under Part II.F.5., page 98.)**

Sample II.G.1.a., page 111

   a. Financial. Record price converted to a common currency. If necessary, develop a Financial Adjustment Sheet and adjust prices to common shipping basis[16] (for example, CIP Port of Entry) and / or correct for any computational errors. Enter those adjustments on the Bid Summary.

   b. Examine bid for technical merit based on evaluation criteria (see discussion in Part II.E.3.a., page 94). Record findings and pertinent details. List and cross-reference any deviations from bidding documents. Material deviation from technical specifications and / or quality criteria will disqualify the bid.

   c. Examine bid for contractual merit based on evaluation criteria (see discussion in Part II.E.3.a., page 95). Record findings and pertinent

[16] For bids tendered without shipping and insurance costs, add a reasonable estimated amount that would need to be paid in addition to the cost of the goods as packed for shipment.

details. Examine all pages of bid for modifications, exceptions or inter-lineations. List and cross-reference any deviations from bidding documents. Address any other evaluation criteria announced in Instructions to Bidders.

d. Examine bid for commercial merit based on evaluation criteria (see discussion in Part II.E.3.a., page 95); evaluate bidder for commercial viability. Record findings and pertinent details. If bidder has not previously pre-qualified for supply of vaccines to immunization programmes, facilitate review process. Do not submit bid to Tender Review Committee until financial and business references have been checked and approved by relevant authority.

2. Attach detailed Bid Summary and Record of Bid Examination for each substantially responsive bid.

Sample II.G.1., page 109
Sample II.F.7., page 103

3. If using a Merit Point System, prepare a Merit Point Form for each bid. List technical, contractual and commercial criteria with corresponding points available.

Sample II.G.3., page 112
Supplement II.H.1.c., page 119

4. Prepare an Adjudication Worksheet form for comparison of the bids.

a. List bidders across the top and detailed criteria for analysis down the left margin by defined category (i.e., financial merit, technical merit, etc.).

b. Allow space for comments / deviations from bidding documents.

c. If a Merit Point System will be used to select a winning bid, provide appropriate spaces for available points and awarded points.

Sample II.H.1.c., page 121

5. Prepare documents for Tender Review Committee.

a. Enter data from each Bid Summary (Sample II.G.1.) and Merit Point Form (Sample II.G.3.), as applicable, onto the Adjudication Worksheet (Sample II.H.1.c.), summarising technical, contractual, commercial and financial findings. Procurement personnel may propose appropriate merit points for technical, contractual and commercial attributes of each bid, but final assessment and award of merit points is the responsibility of the Tender Review Committee.

b. Prepare Summary Cover Sheet for the Tender Review Committee with space for their authorisation signatures.

Form II.G.5.b., page 113

c. Assemble all pertinent documents; if practical, prepare one set for each Tender Review Committee member and a master copy for procurement records.

- Summary cover sheet
- Adjudication worksheet
- Bid summaries and merit point forms
- Substantially responsive bids
- Written opinions and observations of procurement staff
- Record of bid opening
- Record of bid examination

d. Prepare one full set of supporting documents such as annual reports and company literature (or copies of same) for reference by Tender Review Committee.

Tender Review Committee Summary Cover Sheet

Tender Review
Submission No.: _____ Invitation to Bid No.:_____

Funder: _____ Date:_____

Project No.: _____

The Tender Review Committee is requested to review bids received in response to the above Invitation to Bid and select a supplier for the goods described below.

I. Type and Quantity of Commodity:

_____

_____

II. Bidding Information and Details

Number of Bids Received: _____

Number of Substantially Responsive Bids: _____

Evaluated Value of Lowest Acceptable Bid: _____

Evaluated Value of Highest Acceptable Bid: _____

III. Supplier Selection

Supplier Name: _____

Supplier Address: _____

Manufacturer Name: _____

Country of Manufacture: _____

Adjusted Bid Amount: _____

Payment Terms: _____

Delivery Terms: _____

Expiration Date of Bid: _____

Date for First Shipment: _____

IV. Additional Comments or Recommendations:

_____

_____

V. Approvals[17]:

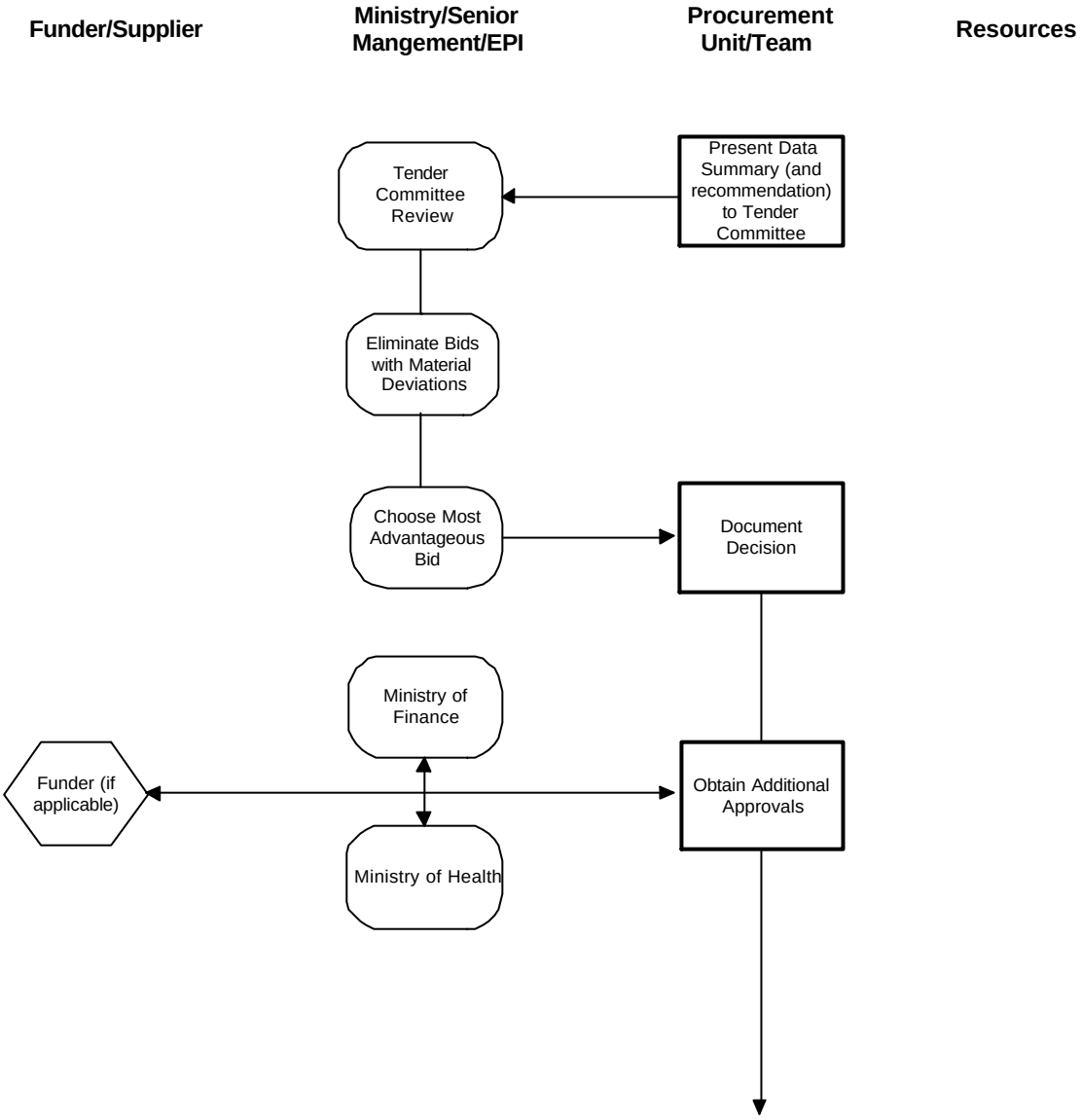
_____ Date: _____

_____ Date: _____

_____ Date: _____

[17] A representative of the National Control Authority should be a member of the Tender Review Committee and a signatory in the approval process.

H. The Selection Process



H. The Selection Process

1. Procurement Unit or Team schedules presentation to Tender Review Committee.

a. Participants in the Tender Review Committee usually include senior programme managers / EPI, Ministry of Health representatives, senior procurement unit representative(s) and technical experts, such as representatives of the National Control Authority, in addition to representatives from the Ministry of Finance. Many governments legislate the precise makeup of tender committees. In some cases, a Tender Board is appointed by the President.

b. Distribute prepared documents for study at least 24 hours before Tender Review Committee meeting.

c. Responsibilities of Tender Review Committee:

- To officially eliminate bids having *material deviations* from contractual terms and conditions or technical specifications. A material deviation is one that affects in any way the price, quantity, quality or delivery of the goods as required in the bid documents, or limits in any way the responsibilities, duties or liabilities of the bidder or any rights of the Purchaser. A bid offering unlicensed vaccine would be an example of one having a material deviation (provided the requirement was clearly stated in the bidding documents). A minor informality or irregularity in a bid that is not a *material deviation* may be waived if such waiver does not affect the relative ranking of any bidder.
- To judge supplier reliability and commercial viability using prior knowledge and the documentation provided with the bid. If the vaccine has been licensed for use in the country and the prospective supplier has been carefully pre-qualified for commercial and technical suitability by the national health authority for the supply of vaccine to the country, supplier reliability has been established and the purchasing entity may elect to forego any further commercial assessment. If there is doubt on this point, references should be checked (at least).
- To review and compare prices and calculated costs.
- To determine and direct any further action or investigation necessary before decision to award such as:
  - ◊ Bid clarifications
  - ◊ Legal advice
  - ◊ Additional reference checks
  - ◊ Testing
  - ◊ Additional technical input
  - ◊ Site visits
- To compare bids, using stated evaluation criteria, and make a determination as to which is the most advantageous bid, taking into

consideration the price or cost, technical, contractual and commercial elements of the offers.

Part II.E., page 93

- ◊ Bids may be evaluated by a combination of objective and subjective observations or by converting subjective elements into objective values by a process such as the Merit Point System.

Supplement II.H.1.c., page 119
Sample II.H.1.c., page 121

- ◊ If a Merit Point System is used, the Tender Review Committee considers each evaluation criterion and agrees on a value (up to the total points available for that criterion) that represents the degree to which each offer meets the requirements and intent of the bidding documents. Individual merit point ratings are summarised on an Adjudication Worksheet for comparison and calculation of relative rank. In some cases, the Procurement Unit or Procurement Team will propose appropriate ratings in order to facilitate the work of the Tender Review Committee.

Supplement II.H.1.c., page 119
Sample II.H.1.c., page 121

- Duties of the Tender Committee after award and during the contract period may include:
  - ◊ Monitoring correction of deficiencies
  - ◊ Authorising shipment
  - ◊ Rejecting commodities not in compliance with specifications and quality criteria

2. Procurement Unit or Team representative assists Tender Review Committee with evaluation and selection of winning bid.

a. Reviews and summarises main financial, contractual, commercial and technical review.

b. Summarises individual bids.

c. Makes tentative recommendation if there is a clearly superior offer.

d. If Merit Point System is used, assists Tender Review Committee in assigning values and calculating relative ranking.

3. Procurement Unit or Team representative records decision of Tender Committee.

a. Records minutes of meeting.

b. Enters name of selected supplier and cost information on Summary Cover Sheet.

c. Obtains appropriate signatures on Summary Cover Sheet.

4. **Procurement Unit or Team secures additional award approvals (as necessary).**

   Approvals may be required from:

   - Ministry of Finance
   - Ministry of Health
   - Funder

5. **Procurement Unit or Team monitors expiration of bid securities.**

Adjudicating Bids

One method of selecting a winning bid is to use a merit point system. This method combines both *objective* and *subjective* criteria in rating and comparing bids. Sample II.H.1.c. is an example of applying a merit point rating system to a set of offers received and documented.

Each category (i.e., financial, contractual, technical, commercial) is assigned a maximum number of points based on the perceived importance of that category as a factor in the bid selection. Further, the most important elements within each category are identified and assigned points based on their perceived importance. This determination is critical and should be undertaken by those responsible for selecting the winning bid at the time the bidding documents are developed (i.e., before Invitations to Bid are transmitted). The total points for all categories should equal 100.

The responses in each category and/or sub-category are then examined and awarded values within the limits of their assigned weights. Total merit points are calculated by adding all awarded values.

Bid price is an objective factor that requires no analysis. The following example illustrates how a merit point value for bid prices can be determined by assigning the low bid a value of 100 percent and then rating the other bid prices relative to it:

Bidder	Low Bid	÷÷	Supplier's Bid	=	Percentage[1]	x	Category Points	=	Merit Points
A:	104,250	÷÷	107,250	=	97%	x	35	=	33.95
B:	104,250	÷÷	117,000	=	89%	x	35	=	31.15
C:	104,250	÷÷	104,250	=	100%	x	35	=	35
D:	104,250	÷÷	135,000	=	77%	x	35	=	26.95

This example bases its calculations on total price, while the worksheet offered as Sample II.H.1.c. demonstrates the calculation of cost per vaccination. This variation is useful in cases where vial size and wastage rates are not consistent among bids.

Contractual, commercial and technical criteria may be either objective or subjective factors in evaluating a bid. If a bid does not fully meet a bid requirement but has not been disqualified for material deviation, a subjective judgement must be made in allocating the correct number of merit points. Commercial information that has been solicited with no stated bid requirement is always rated on an entirely subjective basis.

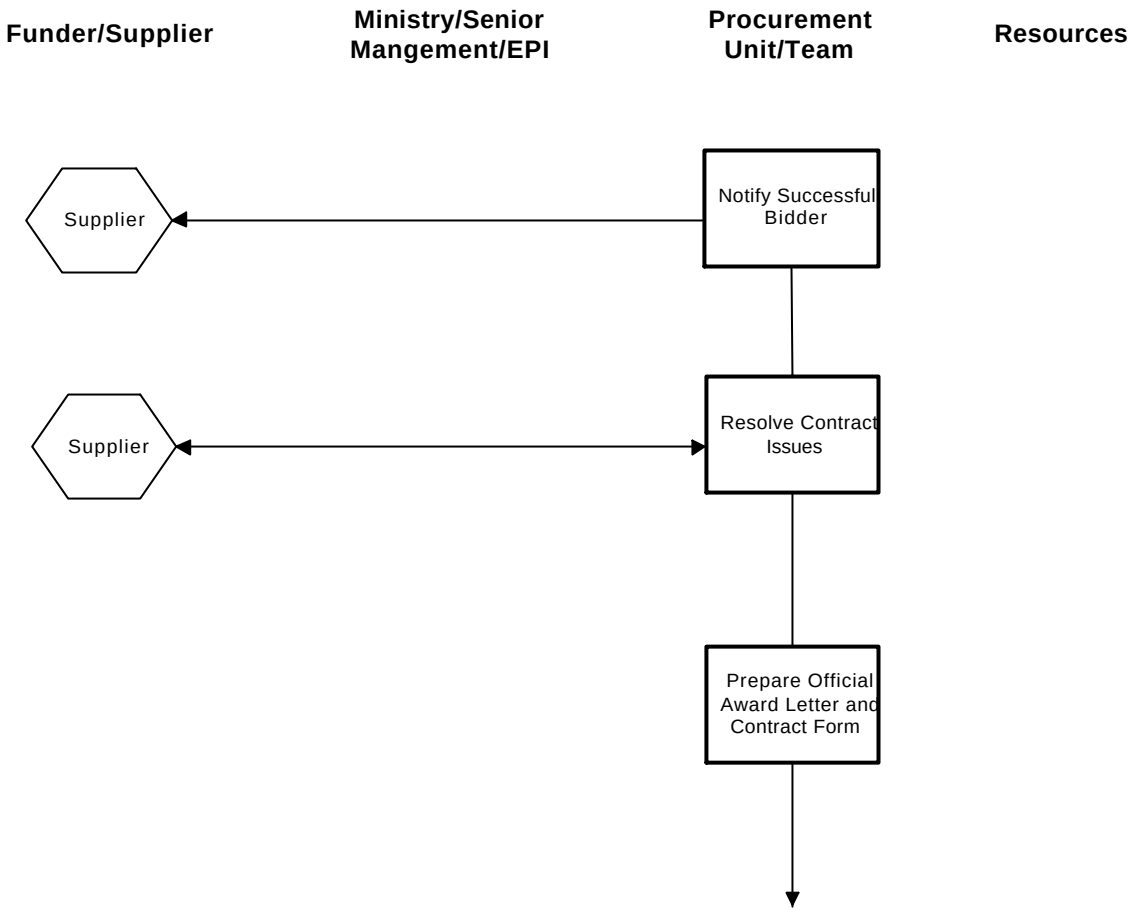
The difficulty in using a merit or weighted point plan is assigning the relative weights to each category in a correct manner and then allocating the correct number of merit points to each bid during the evaluation.[2]

[1] Low bid divided by supplier's bid.

[2] Westring, Gösta, *International Procurement: A Training Manual*. International Trade Centre, United Nations Institute for Training and Research, and the World Bank, 1985.

The strength of a merit point or weighted point plan is that it tends to establish an environment of transparency in the procurement process, since it requires that all aspects of a set of bids be thoroughly examined and assessed against the same set of pre-established criteria and the results clearly documented in an official record.

I. The Award Process



I. The Award Process

1. Notify successful bidder.

a. If bid contains unacceptable minor deviations, draft letter stating offer is being conditionally accepted pending resolution. List outstanding issues and / or unacceptable conditions; indicate next step.

Form II.I.1.a., page 125

b. Request response / acknowledgement.

2. Negotiate resolution of any minor deviations (such as acceptable changes to delivery date) and secure appropriate approvals for any modification to original draft of contract or specification.

Approvals may be required from

- Programme Management
- Ministry of Health
- Tender Committee
- Contracts / Legal Unit
- Funder

3. If required, prepare addendum reflecting acceptable deviations.

4. Prepare award letter and contract form for signature by authorised official(s), providing bidding documents incorporating all agreements between the parties.

Form II.I.4.a., page 126
Form II.I.4.a. (Enclosure), page 127

a. When no issues need to be resolved.

b. When issues have been resolved.

Form II.I.4.b., page 129
Form II.I.4.b. (Enclosure), page 130

5. Transmit official award letter, final contract with general shipping instructions and contract form signed by Buyer, for Seller's signature.

Form II.I.5., page 132

Conditional Bid Acceptance Letter

(Use this letter when outstanding issues need to be resolved between Purchaser and Supplier)

[*Date*]

[*Bidder's name*]
[*Bidder's address*]

Reference: [*Invitation to Bid No.*]
[*Conditional Bid Acceptance*]

Dear [*Supplier contact*]:

The [*appropriate ministry/agency*] is please to inform you that your bid [*reference date or supplier bid number*] to furnish [*items to be supplied*] identified in the referenced Invitation to Bid has been conditionally accepted pending resolution of the following outstanding issues:

[*Examples:*
delivery schedule
packaging requirements
warranty]

Please acknowledge receipt of this letter in writing and contact the undersigned by [*date*] to discuss amending your bid response on these issues to comply with the bid requirements.

Sincerely,

[*Signature of authorised personnel*]

Unconditional Bid Acceptance/Award Letter

(Use this letter when no outstanding issues need to be resolved between Purchaser and Supplier.)

[*Date*]

[*Bidder's name*]
[*Bidder's address*]

Reference: [*Invitation to Bid No.*]
[*Notification of Award*]
[*Contract No.*]

Dear [*Supplier contact*]:

The [*appropriate ministry/agency*] is pleased to inform you that your bid [*reference date or supplier bid number*] to furnish [*items to be supplied*] identified in the referenced Invitation to Bid has been accepted. Enclosed please find the contract form to be duly executed and returned to [*appropriate ministry/agency*] by [*designated date*]. A properly executed performance security should be submitted with the contract form. Upon our receipt of the properly executed contract and performance security, the contract will become effective per the contract date and your bid security will be returned.

As stipulated in the Invitation to Bid, failure to execute the enclosed contract form or provide the performance security in accordance with the conditions of the contract shall be sufficient grounds for the annulment of the award and forfeiture of the bid security.

If you should have any questions regarding the award, please feel free to contact the undersigned [*or other appropriate personnel*]. We look forward to working with [*supplier organisation*] in supplying [*product*] to [*name of immunization programme*].

Sincerely,

[*Signature of authorised personnel*]
Enclosure: Contract Form

Contract Form for Enclosure with
Unconditional Bid Acceptance/Award Letter
(Use when no Addendum for resolution of outstanding issues between Purchaser and Supplier is needed.)

This agreement made the _____ day of _____, 19_____, between [*Purchasing agency*] (hereinafter "the Purchaser") of one part and [*name of Supplier*] of [*city and country of Supplier*] (hereinafter "the Supplier") of the other part.

Whereas the Purchaser is desirous that certain Goods should be provided by the Supplier, [*brief description of Goods including quantity*], and has accepted a bid by the Supplier for the supply of those Goods in the sum of [*Contract Price in words and figures*] (hereinafter "the Contract Price").

NOW THIS AGREEMENT WITNESSETH AS FOLLOWS:

1. In this agreement words and expressions shall have the same meanings as are respectively assigned to them in the Conditions of Contract referred to.

2. The following documents shall be deemed to form and be read and construed as part of this agreement:

   a. the Bid Form and Price Schedule submitted by the Bidder;
   b. the Schedule of Requirements;
   c. the Technical Specifications;
   d. the General Conditions of Contract;
   e. the Special Conditions of Contract;
   f. the Purchaser's Notification of Award.

3. In consideration of the payments to be made by the Purchaser to the Supplier as hereinafter mentioned, the Supplier hereby convenants with the Purchaser to provide the Goods and Services and to remedy defects therein in conformity in all respects with the provisions of the Contract.

4. The Purchaser hereby covenants to pay the Supplier in consideration of the provision of the Goods and Services and the remedying of defects therein the Contract Price or such other sum as may become payable under the provisions of the Contract at the time and in the manner prescribed by the Contract.

IN WITNESS whereof the parties hereto have caused this Agreement to be executed in accordance with their respective laws the day and year first above written

Signed, Sealed, and Delivered by the said
_____ (for the Purchaser)

in the presence of _____

_____

_____

[*signature, name, and address of witness*]

Signed, Sealed, and Delivered by the said
_____ (for the Supplier)

in the presence of _____

_____

_____

[*signature, name, and address of witness*]

Bid Award Letter

(Use after outstanding issues mentioned in conditional bid acceptance letter have been resolved.)

[*Date*]

[*Bidder's name*]
[*Bidder's address*]

Reference: [*Invitation to Bid No.*]
[*Notification of Award*]
[*Contract No.*]

Dear [*Supplier contact*]:

The [*appropriate ministry/agency*] is pleased to inform you that with the inclusion of the terms and conditions identified in Addendum A enclosed, your bid [*reference date or supplier bid number*] to furnish [*items to be supplied*] identified in the referenced Invitation to Bid schedule has been accepted. Enclosed please find the contract form to be duly executed and returned to [*appropriate ministry/ agency*] by [*designated date*]. A properly executed performance security should be submitted with the contract form. Upon our receipt of the properly executed contract form and performance security, the contract will become effective per the contract date and your bid security will be returned.

As stipulated in the Invitation to Bid, failure to execute the enclosed contract form or provide the performance security in accordance with the conditions of the contract shall be sufficient grounds for the annulment of the award and forfeiture of the bid security.

If you should have any questions regarding the award, please feel free to contact the undersigned [*or other appropriate personnel*]. We look forward to working with [*supplier organisation*] in supplying [*product*] to [*name of immunization programme*].

Sincerely,

[*Signature of authorised personnel*]
Enclosure: Contract Form

Contract Form for Enclosure with Bid Award Letter

(Use when documents referenced include Addendum resolving outstanding issues between Purchaser and Supplier.)

This agreement made the _____ day of _____, 19_____, between [*Purchasing agency*] (hereinafter "the Purchaser") of one part and [*name of Supplier*] of [*city and country of Supplier*] (hereinafter "the Supplier") of the other part.

Whereas the Purchaser is desirous that certain Goods should be provided by the Supplier, [*brief description of Goods including quantity*], and has accepted a bid by the Supplier for the supply of those Goods in the sum of [*Contract Price in words and figures*] (hereinafter "the Contract Price").

NOW THIS AGREEMENT WITNESSETH AS FOLLOWS:

1. In this agreement words and expressions shall have the same meanings as are respectively assigned to them in the Conditions of Contract referred to.

2. The following documents shall be deemed to form, and be read, and construed as part of this agreement:

   a. the Technical Specifications;
   b. the Schedule of Requirements;
   c. the Bid Form and Price Schedule submitted by the Bidder;
   d. the Special Conditions of Contract;
   e. the General Conditions of Contract;
   f. the Purchaser's Notification of Award;
   g. Addendum[18].

3. In consideration of the payments to be made by the Purchaser to the Supplier as hereinafter mentioned, the Supplier hereby convenants with the Purchaser to provide the Goods and Services and to remedy defects therein in conformity in all respects with the provisions of the Contract.

4. The Purchaser hereby covenants to pay the Supplier in consideration of the provision of the Goods and Services and the remedying of defects therein the Contract Price or such other sum as may become payable under the provisions of the Contract at the time and in the manner prescribed by the Contract.

[18] The addition of an addendum is the only difference between this and a letter with no outstanding issues.

IN WITNESS whereof the parties hereto have caused this Agreement to be executed in accordance with their respective laws the day and year first above written.

Signed, Sealed, and Delivered by the said
_____ (for the Purchaser)

in the presence of _____

_____

_____
[*signature, name, and address of witness*]

Signed, Sealed, and Delivered by the said
_____
(for the Supplier)

in the presence of _____

_____

_____

[*signature, name, and address of witness*]

General Shipping Instructions

To: _____

Ref: Contract Number_____

1. Quality of vaccine

   The vaccines supplied to the Government of [*country*] for [*purpose*] will meet internationally recognised standards for safety, efficacy and quality as substantiated by supporting documentation outlined in Section 2 below.

2. Vaccine documents

   The following documents must be included with each lot (numbered batch) of vaccine shipped to [*country*]:

   a. For vaccine of U.S. origin:

      - USFDA lot release letter
      - Manufacturer's batch / lot Certificate of Analysis
      - Certificate of Origin
      - Certificate of Registration or Licensing in U.S.
      - Certificate of Free Sale or equivalent from country of manufacture
      - Copy of package insert
      - USFDA master file documentation (to be provided one time only, prior to first shipment of vaccine—usually this is part of the licensing process)

   b. For vaccine of non-U.S. origin:

      - Certification for the release of vaccine by the National Control Authority of the producing country
      - Manufacturer's batch / lot information, including protocols, certificate of analysis, test summary sheets and approval and release records (protocols) signed by the regulatory affairs manager for the manufacturing facility
      - Certificate of Origin
      - Certificate of Registration or Licensing in country of manufacture
      - Certificate of Free Sale or equivalent from country of manufacture
      - Copy of package insert

3. Normal and customary international shipping documents, i.e.,

   a. Air waybill
   b. Commercial invoice
   c. Packing lists
   d. Insurance certificate

4. A complete set of the documents identified in Sections 2 and 3 above must accompany each shipment and an identical set must be sent in advance of each shipment by [*international courier*] or faxed to the following parties:

   - Ministry of Health of [*country*]
     [*address and fax number*]
     Attention: [*responsible official*]

   - [*Agent, if applicable*]
     [*address and fax number*]
     Attention: [*responsible party*]

5. Packing

   The vaccine shall be packed in cartons / containers suitable for export shipment and with adequate cold chain packing to ensure the vaccine is neither frozen nor exposed to temperatures above 8 degrees C during transit to the initial delivery point in [*country*]. Shipping containers should contain appropriate temperature monitors in accordance with WHO / EPI Guidelines on the International Packaging and Shipping of Vaccines, EPI / CCIS 81.4 rev. 6.

6. Marking

   The exterior of all outer shipping cartons / containers shall note the quantity and description of contents in the language of the recipient country. All outer shipping containers must bear:

   a. Lot number
   b. Expiry date
   c. Appropriate cautionary wording, for example: “Perishable Material, Keep Refrigerated (2-8 degrees centigrade)”. Depending upon the vaccine, it may be necessary to include “Keep from freezing” in the cautionary wording.
   d. [*Special labelling, if required*]

7. Shipping instructions

   Purchaser will provide specific instructions shortly before shipment. These will include details such as routing requirements, exact packing

configuration (including volume) and specific directions for cold chain maintenance. Following are general instructions:

a. Each vaccine shipment should be consigned as follows:
[*full name, address, telephone and fax numbers of receiving party in country of destination*]

b. All invoices and shipping documents must carry the markings as stated in 6.a. above.

8. Delivery information

a. Prior to shipment, the manufacturer will fax the following information to the parties identified in 8.b. below:

- Airline flight number and departure time
- Estimated time of arrival (ETA) [*at designated airport*]
- Total quantity and description of commodity
- Dimensions of each piece
- Accurate net and gross weight per piece
- Total gross weight and cube of entire shipment

b. The manufacturer will fax the above delivery information to:

[*full names, addresses, fax numbers of receiving party and any agents or others designated by the purchaser*]

9. Inspection

Prior to shipment, [*purchaser*] reserves the right for its designated representative to inspect the shipment for conformance to contract requirements.

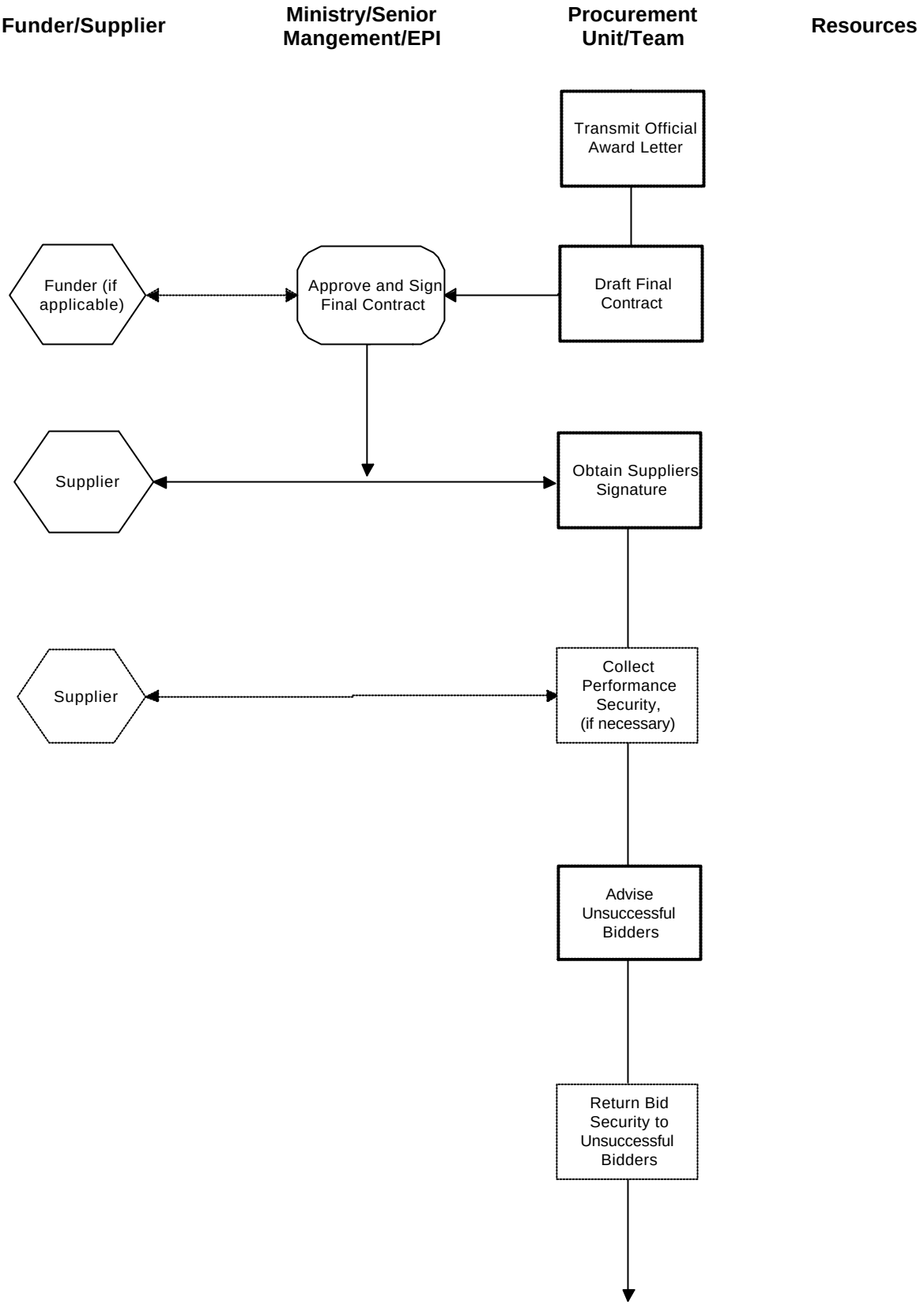
10. Shelf life

The vaccine ordered on this contract must have a minimum of [*number*] months of remaining shelf life from the date of its arrival in [*country*].

11. Shipping charges

[*Will be paid by Seller in accordance with the contract*.] or [*Purchaser will reimburse the manufacturer for actual cost of insurance and air freight to ship vaccine to (country)*]

J. The Contract



J. The Contract

1. Seller should sign and date the contract within 30 days of receipt of contract form.

2. Obtain performance guarantee from Seller if required by funder or contract (within 30 days of notification of award).

- Ideally the performance guarantee is obtained at same time the signed contract form is returned.
- Requirements may be limited to new or previously unreliable suppliers.

Appropriate performance guarantee vehicles are:

- Performance bond
- Bank guarantee
- Stand-by Letter of Credit

3. Notify unsuccessful bidders and return bid securities to them.

Form II.J.3., page 137

a. Depending on expiry date of bid security, return may be delayed until contract is signed and performance security is in place; this allows for secured switch to next lowest bidder if necessary.

b. Hold bid security of successful bidder until contract performance security is in place.

Unsuccessful Bidder Notification

[*Date*]

[*Bidder's name*]
[*Bidder's address*]

Reference: [*Invitation to Bid No.* _____]

Dear [_____]:

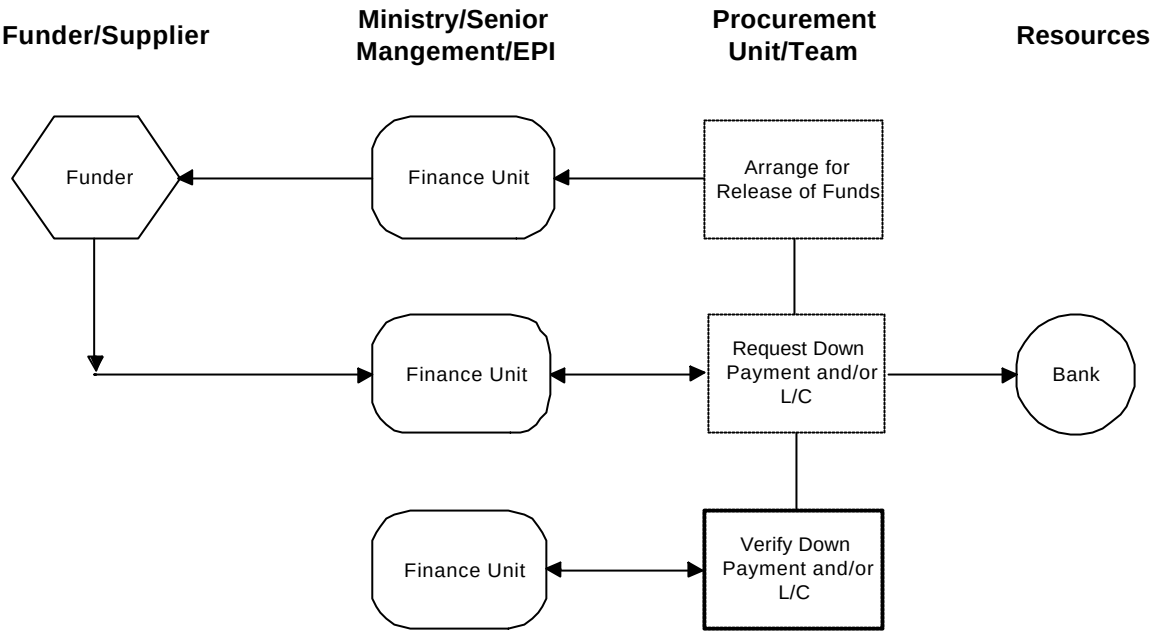
Thank you very much for your response to the above-mentioned Invitation to Bid. While your bid was of merit, we have accepted a more advantageous price and other factors considered.

Enclosed please find your unexecuted bid security in the amount of [_____].

Sincerely,

[*Signature of uthorised personnel*]

K. Financial Arrangements



K. Financial Arrangements

1. Arrange for release of funds from donor or lender if applicable.

a. World Bank has a standard withdrawal procedure using Special Commitment form 1931.

b. Asian Development Bank uses procedures similar to World Bank.

2. Request down payment, if applicable, from appropriate ministry or financial unit.

Direct bank transfer of funds is recommended.

a. Include seller's name, address, bank, account number, address of bank, etc.

b. Include reference to procurement contract number.

3. Request Letter of Credit (L/C), if applicable.

International banking practices for Letters of Credit are described in Part VI. C., Payment Options, and Supplement II.K.3.

Part VI.C.5., page 707
Supplement II.K.3., page 142

a. Opening a commercial L / C may be accomplished by a finance ministry or finance unit rather than EPI or programme managers or the procurement team. If so, a separate L / C instruction sheet should be developed from the relevant sections of the contract and submitted to the appropriate ministry or financial unit to ensure that all intended controls are included in the L / C.

Form II.K.3.a., page 143

b. Application forms are provided by issuing banks. When properly executed, they become the L / C Agreement document.

Sample II.K.3.b., page 145

c. An L / C can provide control and enforcement of contract conditions by withholding payment until documentation indicates conditions have been met.

- Quality requirements can be enforced by requiring presentation of compliance documents or authorisation for shipment based on inspection and test documentation
- Contractual requirements for shipping date, documentation and markings can be enforced through the L / C

4. Obtain verification that down payment has been made and/or Letter of Credit has been issued.

a. Record down payment and L / C dates and other pertinent data such as L / C number, issuing bank and expiration date.

- Shipping dates may be affected by the date supplier receives funds and / or secures commitment of funds (L / C)
- This is the point at which supplier normally commits resources (time and materials) to filling the order

b. Obtain a copy of the issued L / C and confirm the terms and conditions match the request made to the finance unit or ministry responsible for obtaining the L / C.

- Mistakes by issuing banks are possible; usually only a few days are allowed to make corrections without incurring amendment costs; Accountee (usually the Purchaser) notifies bank

5. Once the L/C is issued, any changes desired by Seller (Beneficiary) must be agreed upon and initiated by the Accountee.

Letter of Credit

Uniform Customs and Practice for Documentary Credits

The commercial Letter of Credit is a banking tool used extensively in international trade because it serves the interest of both parties independently:

a) It guarantees payment to the Seller (Beneficiary) when he complies with its terms.

b) It allows the Buyer (Applicant) to retain funds in his own bank until the Seller (Beneficiary) complies with its terms.

The standards and practices for Letters of Credit are published by the International Chamber of Commerce (ICC) Banking Commission under the title "Uniform Customs and Practice for Documentary Credits". These customs and practices change from time to time and each new published version is given a separate number. The current issue was published in 1993 and is known as UCP 500.

Documents for Payment:

- Proof of Performance
- Commercial Invoice*
- Certificate of Origin
- Insurance Certificate*
- Inspection Certificate
- Transport Documents* (for example, the Bill of Lading or Air Waybill)
- Authorisation for Shipment
- Other Certificates and Certifications (for example, Certificate of Analysis)

The items with an asterisk are always required. Other items are optional. The Letter of Credit can function as a tool to enforce quality assurance provisions in the contract by linking proof of compliance to payment. This proof may take the form of Inspection and / or Testing Certificates, or an Authorisation for Shipment signed by the Buyer's representative based on approval of inspection or testing results.

Buyers (Purchasers) should become familiar with the sets of rules governing documentary credits because they specify the extent of the bank's obligations and limitations with regard to monitoring compliance with the terms of the Letter of Credit.

Seller (Suppliers) should also become familiar with the sets of rules governing documentary credits as they explain the criteria the banks must use for acceptance or refusal of the documentation the Seller (Supplier) presents to the bank as a prerequisite for payment.

See Part VI.C.5., page 707, for additional discussion of Letters of Credit.

Instructions for Letter of Credit (L/C)

Date: _____

Attention: _____ (Finance unit or department that handles L / C requests)

Reference: _____ (Contract or Purchase Order number)

Please instruct our bank to open an irrevocable, confirmed, documentary Letter of Credit as follows:

1. Beneficiary: _____ (Seller's name and address)
   _____
   _____
   _____
   _____

2. Advising Bank: _____ (Bank's name and address)
   _____
   _____
   _____
   _____

3. L / C Amount: _____

4. Shipping Terms: _____

5. Shipment Via: _____

6. Shipping Date: _____

7. L / C Expiration Date: _____

8. Shipment From: _____

9. Shipment To: _____
   (Port or airport, city, and country of final destination)

10. Merchandise Description: _____

11. Merchandise Value: _____
    (Include any down payments not contained in the L / C amount.)

12. Partial Shipments: _____ not allowed _____ allowed

13. Trans-shipments: _____ not allowed _____ allowed

14. Documents Required:

This requirement varies from country to country. Please review your country's import requirements as well as your own agency's requirements. Typical shipping document for *airfreight* shipments are included below:

- Commercial Invoice (state number of each required)

  _____ originals _____ copies

- Packing List (state number of each required)

  _____ originals _____ copies

- Original Air Waybill (state number of each required) consigned to:

  _____

  _____ originals _____ copies

- Insurance Certificate for 110% of CIP value (state number of each required)

  (Insure for total cost of commodities, transportation and insurance plus minimum 10 percent. Total cost of commodities includes down payment to supplier not included in L/C amount.)

  _____ originals _____ copies

  Insurance payable to: _____

- Certificate of Conformity with contract specifications issued _____(third party inspection agent).
- Authorisation for Shipment signed by the Accountee.
- Beneficiary's signed certification that the following documents have been sent with the shipment (list required documentation).

  _____

  _____

- Beneficiary's signed certification that all the shipping boxes have been labelled with the following Shipping Marks:

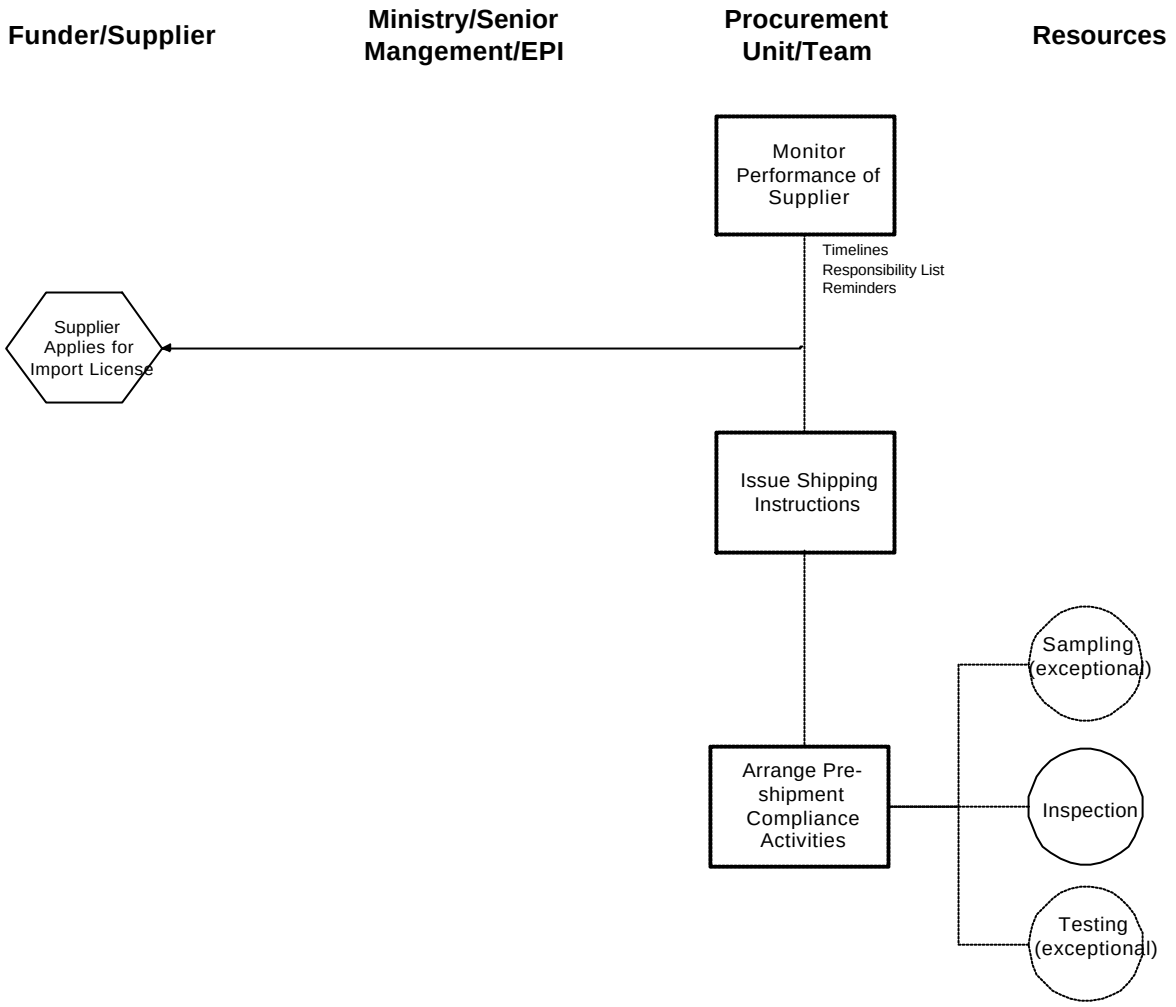
  _____

  _____

15. Special L / C Conditions:

16. L / C Transmittal Method: _____ Airmail _____ Full Text Cable

L. Contract Performance



L. Contract Performance

The Purchaser's responsibilities are to ensure that the Seller fulfils all the terms of the contract and that the vaccines arrive safely at the point of destination. This may include inspection by an appointed agent at the manufacturer's premises before shipment. The inspection agent should confirm that the physical characteristics of the shipment match the contract specifications, in particular, vial size, labelling, packing and temperature maintenance requirements have been met.

1. Set up and maintain a contract monitoring system.

a. List responsibilities of Purchaser and of Seller.

Sample II.L.1.a., page 150

- Responsibilities tied to normal execution of contract such as arrangements for inspection, provision of shipping documents, etc.
- Responsibilities tied to exceptional conditions such as notification of force majeure

b. Establish a probable shipping date based on date of down payment or issuance of L / C.

c. Develop a timeline for execution of the responsibilities based on probable shipping date and normal execution of contract.

Sample II.L.1.c., page 152

d. Systematically evaluate status of outstanding orders.

- Update timeline with actual dates as tasks are accomplished
- Remind supplier of pending deadlines; ask for status

2. Issue final shipping and marking instructions.

a. Develop a separate document covering all of the shipping information and shipping requirements mentioned in the contract. This prevents oversights and mistakes on the part of the Supplier and thus avoids delays and customs clearance problems.

b. Provide final instructions to Supplier at least 30 days but not more than 60 days before shipment. The form provided in II.I.5. represents preliminary shipping instructions that constitute part of the contract between the Buyer and the Seller.

Form II.I.5., page 132

3. Facilitate arrangements for pre-shipment inspection, sampling and testing per compliance programme requirements. Normally, only visual inspection will be required. Any sampling and testing required should be carried out under the direction of the Purchaser's NCA.

a. Excerpt requirements for inspection, (sampling and testing) from contract draft.

Part IV. D., page 573

b. Arrange for services. Independent international inspection (and testing) organisations are recommended in order to eliminate charges and countercharges of prejudice.

- Inspection (and sampling) can be arranged through Societe Generale de Surveillance SA or similar international inspection services. See Part IV.D. for a list and contact information.

Part IV.D., page 574

4. Any testing that is required due to exceptional circumstances is best done directly through a specialised independent laboratory on samples drawn and submitted by an independent international inspection service. The NCA of the purchaser's country has oversight responsibility for all testing and acceptance matters.

Part IV.D.4., page 575

Responsibilities for Contract Performance

Supplier (Seller)

Provides performance security if applicable.

Notifies purchaser in writing of all subcontracts awarded under contract if not stated in bid.

Provides reasonable facilities and assistance to inspection agents, including access to production data and quality control records, for inspection purposes.

Provides packing sufficient to prevent damage or deterioration of goods during transit.

Includes appropriate temperature monitoring devices with packing.

Complies with requested routing.

Arranges and pays for shipping and insurance (CIP terms).

Notifies Purchaser by fax, telex, cable or e-mail of full details of shipment.

Forwards shipping documents and quality assurance documents to Purchaser.

Delivers goods in accordance with time schedule of the contract.

Requests payment in writing from Purchaser (or Purchaser's bank).

Pays taxes, stamp duties, license fees and any other levies imposed outside of the destination country (foreign supplier).

Pays taxes, duties and license fees incurred or imposed locally prior to delivery (local supplier).

Replaces rejected goods.

Notifies Purchaser in writing of any impending delay in delivery, its likely duration and its causes.

Claims any adjustment in price within 30 days after receipt of change order.

Notifies Purchaser in writing of force majeure situation.

Purchaser (Buyer)

Opens Letter of Credit in favour of Supplier.

Applies for import license (if required).

Arranges and prepares for pre- and post-shipment inspections and tests.

Pays for pre-shipment inspections and tests.

Notifies Supplier in writing of identity of any representatives retained for inspections and tests.

Authorises (in writing) shipment of goods based on pre-shipment inspection and, in exceptional circumstances, test results.

Provides transportation of goods after delivery.

Arranges for payment of contract price to Supplier upon receipt of invoice and documents.

Provides Acceptance Certificate for each delivery.

Discharges and returns performance security to Supplier not later than 30 days following the date of completion of the Supplier's performance obligation, including any warranty obligation, under the contract.

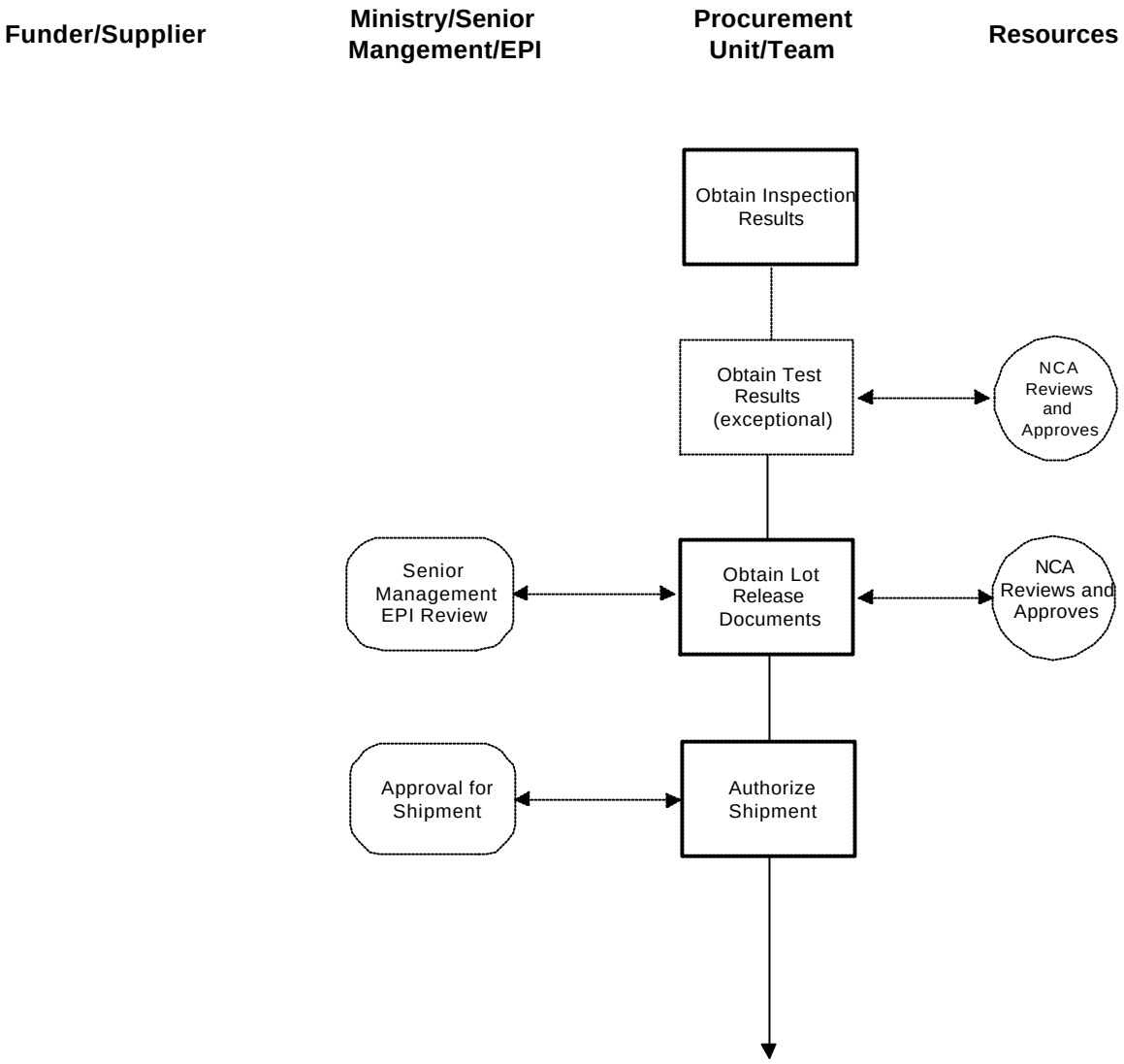
Notifies Supplier in writing of any claims arising under warranty.

Issues change orders in writing to Supplier for any modification to specifications, method of shipment, place of delivery or services.

Notifies Supplier in writing of default(s).

Notifies Supplier in writing of intention to terminate contract for any reason.

M. Shipment


Funder/Supplier
Ministry/Senior Mangement/EPI
Procurement Unit/Team
Resources
Obtain Inspection Results
Obtain Test Results (exceptional)
NCA Reviews and Approves
Senior Management EPI Review
Obtain Lot Release Documents
NCA Reviews and Approves
Approval for Shipment
Authorize Shipment

M. Shipment

1. **Compare pre-shipment inspection (and test) results against contract requirements.**[19]

   This is a cursory review by procurement personnel.

2. **Secure technical input on results of any pre-shipment compliance testing.**

   Normally, this will be the NCA of the Purchaser's country.

3. **Present pre-shipment inspection(/test) results to Tender Review Committee or quality committee.**

   a. If there are no discrepancies between specifications and inspection / test results, this is a formality.

   b. If there are deficiencies, this Committee should be responsible for decisions regarding resolution. In matters of vaccine quality, the NCA of the Purchaser's country has legal responsibility.

4. **Monitor correction of deficiencies if necessary.**

   The cost of repeating any pre-shipment compliance activities should be borne by the Seller, but activities should be controlled by the Purchaser.

5. **Obtain approval from Tender Review Committee to authorise shipment or to reject commodities.**

6. **Issue written authorisation to Supplier for shipment.**

Form II.M.6., page 155

[19] Testing will be carried out only in exceptional circumstances.

Authorisation for Shipment

Attn: _____

Ref: Contract Number _____

L/C Number _____

Authorisation for Shipment

[*Your organisation's name and address*]

Re: _____

Pre-shipment inspection [and test data] results have been received and approved by _____

Signature

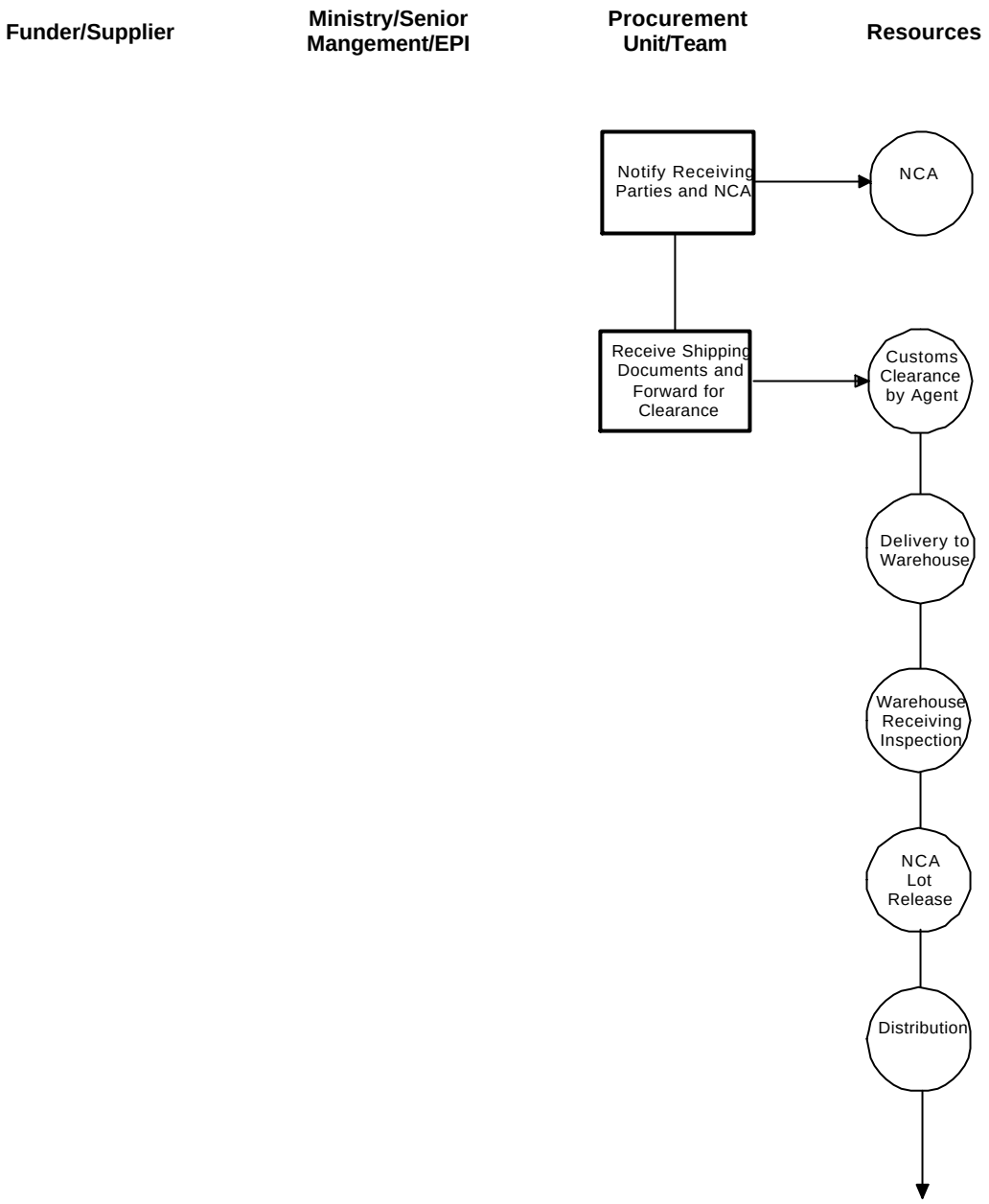
Signature of this document by the [*authorised representative*] indicates that the commodity under Contract Number _____ is released for shipment.

This certificate does not release Supplier from compliance with warranties and other conditions included in this contract.

_____
[*authorised representative*]

_____
Date

N. Customs Clearance and Delivery



N. Customs Clearance and Delivery

1. Notify receiving warehouse of scheduled delivery.

a. Allow time to plan adequate warehouse space and inland transportation.

b. Alert warehouse and logistics staff to pending arrival of documents.

2. Notify programme management and NCA.

3. Receive shipping documents and forward for clearance procedures.

a. Full set of shipping documents must be placed in hands of customs clearance staff as soon as possible. Late transmittal of documents causes delays in customs clearance.

- Original Air Waybill does not convey title to goods and is normally sent with the shipment; identification is required in order to gain possession of the goods. (Ocean Bill of Lading does convey title to goods and is sent to the Buyer's bank for release when funds have been transferred to the Seller. This procedure does not apply to vaccines which are always air shipped.)

b. Upon arrival of flight, documents are passed on to a customs broker for execution of clearance procedures.

- Vaccines must be held in cold storage facility at airport if they are not cleared immediately
- Storage charges will normally be incurred unless the freight is cleared immediately

4. Clear goods from customs.

a. Airport clearance and receiving staff should work closely with a customs broker and NCA.

b. Any pre-release inspection should be attended by consignee's airport clearance staff representative.

5. Arrange for delivery of goods to receiving warehouse, observing all appropriate cold chain procedures.

a. Customs broker may provide transportation.

b. Make sure commodities are protected from damage due to adverse weather conditions.

c. Try to arrange for insurance to cover vaccines from unloading at port through delivery to warehouse.

- Appropriate coverage through to consignee's warehouse by international air or ocean cargo insurer is often very expensive

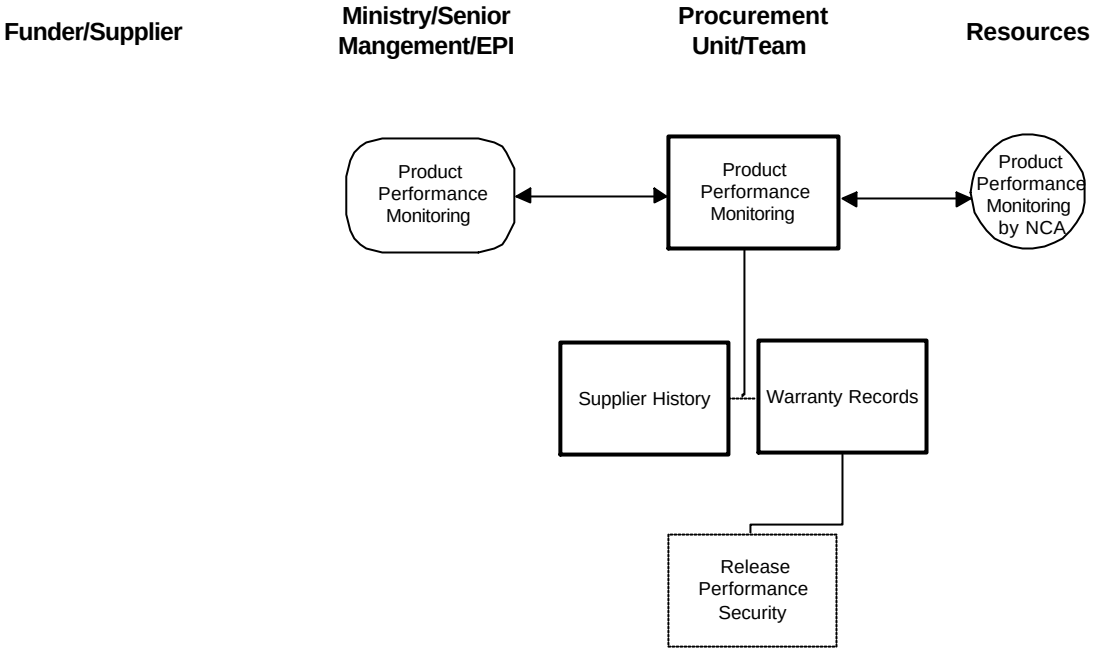
- Coverage may be available locally at minimal cost

6. Receive and inspect goods for the following details:

- Correct item
- Status of cold chain monitors
- Adherence to cold chain requirements
- Full quantities delivered
- Packing slip present and correct
- Shipping damage
- Correct markings on packaging
- Further testing required
- Manufacturer's certifications included with shipment (or documents)
- Expiry dates and vial sizes

7. Report deficiencies as necessary.

O. Closeout Activities



O. Closeout Activities

1. Maintain warranty records.

a. Define warranty period (for issues such as defects in vial closures and label application)

b. Document claims against warranty and resolution of claims.

2. Establish and maintain supplier history forms.

Form II.O.2., page 163

- Purchase order or contract number
- Commodity
- Quantity
- Unit price
- Date ordered
- Date promised
- Date delivered
- Comments (including adherence to cold chain requirements, quality defects and service)

3. Product performance and monitoring is usually a function of programme management. However, it is important for the Procurement Team to be aware of performance problems in the field and provide feedback to the Supplier. NCA monitors and assesses safety during widespread use.

Part IV.H., page 620

4. Release performance security when contracted obligations have been fulfilled.

Supplier History

Supplier:

Purchase Order Number	Date or Order	Commodity	Quantity	Unit Price	Date Promised	Date Delivered	Comments

PART III - SPECIFICATIONS

INTRODUCTION 167

A. GENERAL REQUIREMENTS AND STANDARDS 168

1. Good Manufacturing Practices for Biological Products, *WHO Technical Report Series*, No. 822, 1992 169
2. General Requirements for the Sterility of Biological Substances (Requirements for Biological Substances No. 6, revised 1973), Annexes 4 and 5, WHO *Technical Report Series*, No. 530, 1973 175
3. General Requirements for the Sterility of Biological Substances (Requirements for Biological Substances No. 6, revised 1973, amendment 1995), WHO *Technical Report Series*, No. 872, 1998 202
4. Biologics, *USP 23 - NF 18*, Paragraph 1041, 1994 208
5. General Biological Products Standards, US Food and Drug Administration HHS 21 CFR, Part 610, 1995 209
6. Injection Containers for Injectables and Accessories,ISO 8362, 1989
   Part 1: Injection vials made of glass tubing (ISO 8362-1) 221
   Part 2: Closures for injection vials (ISO 8362-2) 229

B. BCG VACCINE 239

1. Sample Procurement Requirements BCG Vaccine 240
2. BCG Vaccine Model Package Insert (UNICEF) 247
3. BCG Vaccine, *USP 23 - NF 18*, 1994 248
4. Bacillus of Calmette and Guerin (BCG) Vaccine, US Food and Drug Administration HHS 21 CFR, Part 620, Subpart E 249
5. Requirements for Dried BCG Vaccine (Requirements for Biological Substances No. 11, revised 1985), WHO *Technical Report Series* No. 745, 1987 254
6. Requirements for Dried BCG Vaccine (Amendment 1987), WHO *Technical Report Series* No. 771, part 49, 1988 270

C. DTP VACCINE (DIPHTHERIA, TETANUS AND PERTUSSIS VACCINES) 273

1. Sample Procurement Requirements DTP Vaccine 274
2. DTP and DT Vaccine Model Package Inserts (UNICEF) 281
3. Diphtheria and Tetanus Toxoids and Pertussis Vaccine, *USP 23 - NF 18*, 1994 283
4. Pertussis Vaccine, US Food and Drug Administration HHS 21 CFR, Part 620, Subpart A 285

C. DTP VACCINE (continued)

5. Requirements for Diphtheria, Tetanus, Pertusis and Combined Vaccines (Requirements for Biological Substances Nos. 8 and 10, revised 1989), WHO *Technical Report Series* No. 800, 1990 289

D. MEASLES VACCINE 336

1. Sample Procurement Requirements Measles Vaccine 337
2. Measles Vaccine Model Package Insert (UNICEF) 345
3. Measles Virus Vaccines Live, *USP 23 - NF 18*, 1994 346
4. Measles Virus Vaccines Live, US Food and Drug Administration HHS 21 CFR, Part 630, Subpart D 348
5. Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live) (Requirements for Biological Substances No. 47), WHO *Technical Report Series* No. 840, Annex 3, 1994 355

E. ORAL POLIO VACCINE (OPV) 406

1. Sample Procurement Requirements OPV Vaccine 407
2. OPV Vaccine Model Package Insert (UNICEF) 415
3. Poliovirus Vaccine Live Oral, *USP 23 - NF 18*, 1994 416
4. Poliovirus Vaccine Live Oral Trivalent, US Food and Drug Administration HHS 21 CFR, Part 630, Subpart B 417
5. Requirements for Poliomyelitis Vaccine (Oral) (Requirements for Biological Substances No. 7, revised 1989), WHO *Technical Report Series* No. 800, 1990 428

F. HEPATITIS B VACCINE 457

1. Types of Hepatitis B Vaccines 458
2. Sample Procurement Requirements Hepatitis B Vaccine 459
3. HB Vaccine Model Package Insert (UNICEF) 467
4. Hepatitis B Virus Vaccine Inactivated, *USP 23 - NF 18*, 1994 468
5. Hepatitis Requirements, US Food and Drug Administration HHS 21 CFR, Part 610, Subpart E 470
6. Requirements for Hepatitis B Vaccine Prepared from Plasma (Requirements for Biological Substances No. 31, revised 1994), WHO *Technical Report Series* No. 858, Annex 3, 1995 473
7. WHO Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques (Requirements for Biological Substances No. 45), WHO *Technical Report Series* No. 786, 1989 490

G. YELLOW FEVER VACCINE 507

1. Sample Procurement Requirements Yellow Fever Vaccine 508
2. Yellow Fever Vaccine, *USP 23 - NF 18*, 1994 515
3. Laboratories Approved by WHO for the Production of Yellow Fever Vaccine, WHO *Technical Report Series* No. 872, 1998 516
4. Requirements for Yellow Fever Vaccine (Requirements for Biological Substances No. 3, revised 1995), WHO *Technical Report Series* No. 872, 1998 517

H. TETANUS TOXOID VACCINE 556

1. Sample Procurement Requirements Tetanus Toxoid Vaccine 557
2. TT Model Package Insert (UNICEF) 564
3. Tetanus Toxoid, *USP 23 - NF 18*, 1994 565

PART III - SPECIFICATIONS

Introduction

The technical specifications for each vaccine to be used in a country are established by its National Control Authority. In many cases, the EPI manager will work with the NCA to develop programmatic specifications such as vial size and packaging, since they may vary from year to year according to the strategy for control of a particular disease.

The Sample Procurement Requirements contained in this section of the manual are based on specifications for EPI vaccines that were developed by WHO to respond to the needs of the EPI in all those countries where the EPI is being undertaken. At the time they were established, the EPI was largely being implemented in countries with tropical and sub-tropical climates. The specifications correspond to the training materials prepared by WHO, but may be augmented or changed in accordance with the specific needs of a country immunization programme. They do not constitute a recommendation.

Not all manufacturers produce vaccines that correspond precisely with the Sample Procurement Requirements. Different specifications do not necessarily affect the efficacy of a vaccine, although they may change the characteristics. For example, stability may vary in certain temperature conditions. 1996 USFDA (CFR 21) requirements for the main EPI vaccines included in Part III - Specifications demonstrate this point. They are provided for reference along with corresponding *WHO Biological Requirements*. Related excerpts from *United States Pharmacopoeia* (USP 23) *Official Monographs* have also been included.

In the face of broad advances in vaccine technology and the development of new vaccines, USFDA has recently moved away from narrowly defined specifications and production requirements and is now licensing vaccines based on their unique merits. Thus, all reference to CFR 21 has been deleted in the new USFDA regulations. Concurrently, WHO is moving toward establishment of new *"Guidelines"* that will update and replace the traditional "*Biological Requirements*".

In practice, the NCA of a country may decide to license a number of vaccines that have different characteristics and respond to the disease management requirements of the country. The EPI manager should decide which licensed products will meet programme needs and are compatible with training that has been provided to EPI workers in the field.

The Sample Procurement Requirements presented in this section may be amended as necessary, but they represent the minimum information that should be provided with the Invitation to Bid and included in the subsequent purchase contract. General requirements and standards applicable to all (or most) of the EPI vaccines have been introduced at Section A.

A. GENERAL REQUIREMENTS AND STANDARDS

Documents provided in this section include:

1. Good Manufacturing Practices for Biological Products, *WHO Technical Report Series*, No. 822, 1992 169
2. General Requirements for the Sterility of Biological Substances (Requirements for Biological Substances No. 6, revised 1973), Annexes 4 and 5, WHO *Technical Report Series*, No. 530, 1973 175
3. General Requirements for the Sterility of Biological Substances (Requirements for Biological Substances No. 6, revised 1973, amendment 1995), WHO *Technical Report Series*, No. 872, 1998 202
4. Biologics, *USP 23 - NF 18*, Paragraph 1041, 1994 208
5. General Biological Products Standards, US Food and Drug Administration HHS 21 CFR, Part 610, 1995 209
6. Injection Containers for Injectables and Accessories,ISO 8362, 1989
   Part 1: Injection vials made of glass tubing (ISO 8362-1) 221
   Part 2: Closures for injection vials (ISO 8362-2) 229

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WHO/V&B/99.12

Annex 1
Good manufacturing practices for biological products

1. Scope of these guidelines 20
2. Principles 21
3. Personnel 21
4. Premises and equipment 23
5. Animal quarters and care 25
6. Production 26
7. Labelling 26
8. Lot processing records (protocols) and distribution records 27
9. Quality assurance and quality control 28
Authors 29
Acknowledgements 29
References 29

1. Scope of these guidelines

These guidelines are intended to complement those provided in "Good manufacturing practices for pharmaceutical products" (*1*).

The regulatory procedures necessary to control biological products are in large part determined by the sources of products and methods of manufacture. Manufacturing procedures within the scope of these guidelines include:

- growth of strains of microorganisms and eukaryotic cells,
- extraction of substances from biological tissues, including human, animal and plant tissues (allergens),
- recombinant DNA (rDNA) techniques,
- hybridoma techniques,
- propagation of microorganisms in embryos or animals.

Biological products manufactured by these methods include allergens, antigens, vaccines, hormones, cytokines, enzymes, human whole blood and plasma derivatives, immune sera, immunoglobulins (including monoclonal antibodies), products of fermentation (including products derived from rDNA) and diagnostic agents for *in vitro* use.

2. Principles

The manufacture of biological products shall be undertaken in accordance with the basic principles of good manufacturing practices (GMP). The points covered by these guidelines should therefore be considered supplementary to the general requirements set out in "Good manufacturing practices for pharmaceutical products" (*1*), and relate specifically to the production and control of biological products. In drawing up these guidelines, due consideration was given to the draft "Guidelines for national authorities on quality assurance for biological products", the final version of which appears as Annex 2 to the forty-second report of the WHO Expert Committee on Biological Standardization (*2*).

The way in which biological products are produced, controlled and administered makes some particular precautions necessary. Unlike conventional pharmaceutical products, which are normally produced and controlled using reproducible chemical and physical techniques, biological products are manufactured by methods involving biological processes and materials, such as cultivation of cells or extraction of material from living organisms. These processes display inherent variability, so that the range and nature of by-products are variable. For this reason, in the manufacture of biological products full adherence to GMP is necessary for all production steps, beginning with those from which the active ingredients are produced.

Control of biological products nearly always involves biological techniques that have a greater variability than physicochemical determinations. In-process controls take on a great importance in the manufacture of biological products because certain deficiencies may not be revealed by testing the finished product.

The present guidelines do not lay down detailed requirements for specific classes of biological products, and attention is therefore directed to other guidance issued by WHO, and in particular to the Requirements for Biological Substances, which include requirements for vaccines (*2*, Annex 7).

3. Personnel

3.1 The manufacturing establishment and its personnel shall be under the authority of a person who has been trained in the techniques used in manufacturing biological substances and who possesses the scientific knowledge upon which the manufacture of these products is based. The personnel shall include specialists with training appropriate to the products made in the establishment.

3.2 Personnel required to work in clean and aseptic areas should be selected with care, to ensure that they may be relied upon to observe the appropriate codes of practice and are not subject to any disease or condition that could compromise the integrity of the product

microbiologically or otherwise. High standards of personal hygiene and cleanliness are essential. Staff should be instructed to report any conditions (e.g. diarrhoea, coughs, colds, infected skin or hair, wounds, fever of unknown origin) that may cause the shedding of abnormal numbers or types of organisms into the working environment. Health checks on personnel for such conditions should be required before employment and periodically thereafter. Any changes in health status that could adversely affect the quality of the product shall preclude the person concerned from working in the production area.

3.3 Only the minimum number of personnel required should be present in clean and aseptic areas when work is in progress. Inspection and control procedures should be conducted from outside these areas as far as possible.

3.4 During the working day, personnel shall not pass from areas where live microorganisms or animals are handled to premises where other products or organisms are handled unless clearly defined decontamination measures, including a change of clothing and shoes, are followed. Persons not concerned with the production process should not enter the production area except for essential purposes, and in that case they shall be supplied with sterile protective clothing.

3.5 The staff engaged in the manufacturing process should be separate from the staff responsible for animal care.

3.6 The names and qualifications of those responsible for approving lot processing records (protocols) should be registered with the national control authority.

3.7 To ensure the manufacture of high-quality products, personnel should be trained in good manufacturing and laboratory practices in appropriate fields such as bacteriology, virology, biometry, chemistry, medicine, immunology and veterinary medicine.

3.8 Training records should be maintained and periodic assessments of the effectiveness of training programmes should be made.

3.9 All personnel engaged in production, maintenance, testing and animal care (and inspectors) should be vaccinated with appropriate vaccines and, where appropriate, be submitted to regular testing for evidence of active tuberculosis. Apart from the obvious problem of exposure of staff to infectious agents, potent toxins or allergens, it is necessary to avoid the risk of contamination of a production batch with these agents.

3.10 Where BCG vaccines are being manufactured, access to production areas shall be restricted to staff who are carefully monitored by regular health checks. In the case of manufacture of products derived from human blood or plasma, vaccination of workers against hepatitis B is recommended.

4. Premises and equipment

4.1 As a general principle, buildings must be located, designed, constructed, adapted and maintained to suit the operations to be carried out within them. Laboratories, operating rooms and all other rooms and buildings (including those for animals) that are used for the manufacture of biological products shall be designed and constructed of materials of the highest standard so that cleanliness, especially freedom from dust, insects and vermin, can be maintained.

4.2 Interior surfaces (walls, floors and ceilings) shall be smooth and free from cracks; they shall not shed matter and shall permit easy cleaning and disinfection. Drains should be avoided wherever possible and, unless essential, should be excluded from aseptic areas. Where installed they should be fitted with effective, easily cleanable traps and with breaks to prevent back-flow. The traps may contain electrically operated heating devices or other means for disinfection. Any floor channels should be open, shallow and easily cleanable and be connected to drains outside the area in a manner that prevents ingress of microbial contaminants.

4.3 Sinks shall be excluded from aseptic areas. Any sink installed in other clean areas shall be of suitable material such as stainless steel, without an overflow, and be supplied with water of potable quality. Adequate precautions shall be taken to avoid contamination of the drainage system with dangerous effluents. Airborne dissemination of pathogenic microorganisms and viruses used for production and the possibility of contamination by other types of viruses or substances during the production process, including those from personnel, shall be avoided.

4.4 Lighting, heating, ventilation and, if necessary, air-conditioning should be designed to maintain a satisfactory temperature and relative humidity, to minimize contamination and to take account of the comfort of personnel working in protective clothing. Buildings shall be in a good state of repair. The condition of the buildings should be reviewed regularly and repairs carried out when and where necessary. Special care should be exercised to ensure that building repair or maintenance operations do not compromise products. Premises should provide sufficient space to suit the operations to be carried out, allowing an efficient flow of work and effective communication and supervision. All buildings and rooms shall be clean and sanitary at all times. If rooms intended for the manufacture of biological substances are used for other purposes, they shall be cleaned thoroughly and, if necessary, sanitized before the manufacture of biological substances is resumed. Areas used for processing animal tissue materials and microorganisms not required for the current manufacturing process and for performing tests involving animals or microorganisms must be separated from premises used for manufacturing sterile biological products and have completely separate ventilation systems and separate staff.

4.5 If certain products are to be produced on a campaign basis, the layout

and design of the premises and equipment shall permit effective decontamination by fumigation, where necessary, as well as cleaning and sanitizing after the production campaign.

4.6 Seed lots and cell banks used for the production of biological products should be stored separately from other material. Access should be restricted to authorized personnel.

4.7 Live organisms shall be handled in equipment that ensures that cultures are maintained in a pure state and are not contaminated during processing.

4.8 Products such as killed vaccines, including those made by rDNA techniques, toxoids and bacterial extracts may after inactivation be dispensed into containers on the same premises as other sterile biological products, providing that adequate decontamination measures are taken after filling, including, if appropriate, sterilization and washing.

4.9 Spore-forming organisms shall be handled in facilities dedicated to this group of products until the inactivation process is accomplished. For *Bacillus anthracis*, *Clostridium botulinum* and *Clostridium tetani*, strictly dedicated facilities should be utilized for each individual product. Where campaign manufacture of spore-forming organisms occurs in a facility or suite of facilities, only one product should be processed at any one time.

4.10 Dedicated facilities and equipment shall be used for the manufacture of medicinal products derived from human blood or plasma.

4.11 All containers of biological substances, regardless of the stage of manufacture, shall be identified by securely attached labels. Cross-contamination should be prevented by adoption of some or all of the following measures:

- processing and filling in segregated areas;
- avoiding manufacture of different products at the same time, unless they are effectively segregated;
- containing material transfer by means of airlocks, air extraction, clothing change and careful washing and decontamination of equipment;
- protecting against the risks of contamination caused by recirculation of untreated air, or by accidental re-entry of extracted air;
- using "closed systems" of manufacture;
- taking care to prevent aerosol formation (especially by centrifugation and blending);
- excluding pathological specimens sent in for diagnosis from areas used for manufacturing biological substances;
- using containers that are sterilized or are of documented low "bioburden".

4.12 Positive-pressure areas should be used to process sterile products, but negative pressure is acceptable in specific areas where pathogens are processed. In general, any organisms considered to be pathogenic should be handled within specifically designed areas under negative pressures, in accordance with containment requirements for the product concerned.

4.13 Air-handling units should be dedicated to the processing area concerned. Air from operations involving pathogens shall not be recirculated and, in the cases of organisms in a group above Risk Group 2 (*3*), shall be exhausted through sterilizing filters that are regularly checked for performance.

4.14 Specific decontamination systems should be considered for effluent when infectious and potentially infectious materials are used for production.

4.15 Pipework, valves and vent filters shall be properly designed to facilitate cleaning and sterilization. Valves on fermentation vessels shall be completely steam-sterilizable. Air-vent filters shall be hydrophobic and shall be validated for their designated use.

4.16 Small stocks of substances that have to be measured or weighed during the production process (e.g. buffers) may be kept in the production area, provided that they are not returned to the general stocks. Otherwise, dry materials used to formulate buffers, culture media, etc. should be weighed and put into solution in a contained area outside the purification and aseptic areas in order to minimize particulate contamination of the product.

5. Animal quarters and care[1]

5.1 Animals are used for the manufacture and control of a number of biological products. Animals shall be accommodated in separate buildings with self-contained ventilation systems. The buildings' design and construction materials shall permit maintenance in a clean and sanitary condition free from insects and vermin. Facilities for animal care shall include isolation units for quarantine of incoming animals and provision for vermin-free food storage. Provision shall also be made for animal inoculation rooms, which shall be separate from the postmortem rooms. There shall be facilities for the disinfection of cages, if possible by steam, and an incinerator for disposing of waste and of dead animals.

5.2 The health status of animals from which starting materials are derived and of those used for quality control and safety testing should be monitored and recorded. Staff employed in animal quarters must be provided with special clothing, changing facilities and showers. Where monkeys are used for the production or quality control of biological products, special consideration is required, as laid down in the revised Requirements for Biological Substances No. 7 (Requirements for Poliomyelitis Vaccine (Oral)) (*5*).

[1] General requirements for animal quarters, care and quarantine are given in reference *4*.

6. Production

6.1 Standard operating procedures shall be available and maintained up to date for all manufacturing operations.

6.2 Specifications for starting materials should include details of their source, origin and method of manufacture and of the controls applied, in particular microbiological controls, to ensure their suitability for use. Release of a finished product is conditional on satisfactory results being obtained in the tests on starting materials.

6.3 Media and cultures shall be added to fermenters and other vessels under carefully controlled conditions to avoid contamination. Care shall be taken to ensure that vessels are correctly connected when cultures are added.

6.4 If possible, media should be sterilized *in situ*. In-line sterilizing filters for routine addition of gases, media, acids, alkalis, defoaming agents, etc. to fermenters should be used where possible.

6.5 Careful consideration should be given to the validation of sterilization methods.

6.6 When an inactivation process is performed during manufacture, measures should be taken to avoid the risk of cross-contamination between treated and untreated products.

6.7 A wide variety of equipment is used for chromatography; in general such equipment should be dedicated to the purification of one product and should be sterilized or sanitized between batches. Problems of decontamination and purification may arise through repeated use of the same equipment at the same or different stages of processing. The life span of columns and the sterilization method shall be defined. Particular care should be given to monitoring microbial loads and endotoxins.

7. Labelling

7.1 All products shall be clearly identified by labels. The labels used must remain permanently attached to the containers under all storage conditions and an area of the container should be left uncovered to allow inspection of the contents. If the final container is not suitable for labelling (for example a capillary tube), it should be in a labelled package.

7.2 The information given on the label on the container and the label on the package shall be approved by the national control authority.

7.3 The label on the container shall show:

- the name of the drug product;
- a list of the active ingredients and the amount of each present, with a statement of the net contents, e.g. number of dosage units, weight or volume;

- the batch or final lot number assigned by the manufacturer;
- the expiry date;
- recommended storage conditions or handling precautions that may be necessary;
- directions for use, and warnings and precautions that may be necessary;
- the nature and amount of any substance used in the preparation of the biological product that is likely to give rise to an adverse reaction in some recipients;
- the name and address of the manufacturer or the company and/or the person responsible for placing the drug on the market.

7.4 The label on the package shall, in addition to the information shown on the label on the container, show at least the nature and amount of any preservative or additive in the product.

7.5 The leaflet in the package should provide instructions for the use of the product, and mention any contraindications or potential adverse reactions.

8. Lot processing records (protocols) and distribution records

8.1 Processing records of regular production lots must provide a complete account of the manufacturing history of each lot of a biological preparation, showing that it has been manufactured, tested, dispensed into containers and distributed in accordance with the licensed procedures.

8.2 A separate processing record should be prepared for each lot of biological product, and should include the following information:

- the name and dosage of the product;
- the date of manufacture;
- the lot identification number;
- the complete formulation of the lot, including identification of seed or starting materials;
- the batch number of each component used in the formulation;
- the yield obtained at different stages of manufacture of the lot;
- a duly signed record of each step followed, precautions taken and special observations made throughout the manufacture of the lot;
- a record of all in-process control tests and of the results obtained;
- a specimen of the label;
- identification of packaging materials, containers and closures used;
- a dated signature of the expert responsible for approving the manufacturing operations;
- an analytical report, dated and signed by the responsible expert, showing whether the lot complies with the specifications described in the standard operating procedure registered with the national control authority;
- a record of the decision regarding the release or rejection of the lot by the quality-control department and, if the lot is rejected, a record of its disposal or utilization.

8.3 The records shall be of a type approved by the national control authority. They shall be retained for at least two years after the expiry date of a lot or batch of a biological product and be available at all times for inspection by the national control authority.

8.4 Records must make it possible to trace all steps in the manufacture and testing of a lot, and should include records of sterilization of all apparatus and materials used in its manufacture. Distribution records must be kept in a manner that permits rapid recall of any particular lot, if necessary.

9. Quality assurance and quality control

9.1 The quality-assurance and/or quality-control department should have the following principal duties:

- to prepare detailed instructions for each test and analysis;
- to ensure adequate identification and segregation of test samples to avoid mix-up and cross-contamination;
- to ensure that environmental monitoring and equipment validation are conducted as appropriate for evaluating the adequacy of the manufacturing conditions;
- to release or reject raw materials and intermediate products, if necessary;
- to release or reject packaging and labelling materials and the final containers in which drugs are to be placed;
- to release or reject each lot of finished preparation;
- to evaluate the adequacy of the conditions under which raw materials, intermediate products and finished biological preparations are stored;
- to evaluate the quality and stability of finished products and, when necessary, of raw materials and intermediate products;
- to establish expiry dates on the basis of the validity period related to specified storage conditions;
- to establish and, when necessary, revise control procedures and specifications; and
- to be responsible for the examination of returned preparations to determine whether such preparations should be released, reprocessed or destroyed; adequate records of the distribution of such preparations should be maintained.

9.2 A manufacturer's quality-control laboratory shall be separated from the production area and ideally should be in a separate building. The control laboratory should be designed and equipped and of such a size as to be a self-contained entity, with adequate provision for the storage of documents and samples, preparation of records and performance of the necessary tests.

9.3 In-process controls play a specially important role in ensuring the consistent quality of biological products. Tests that are crucial for quality control but that cannot be carried out on the finished product shall be performed at an appropriate stage of production.

9.4 Performance of all qualitative and quantitative tests mentioned in the specifications for starting materials may be replaced by a system of certificates issued by the producer of the starting material, provided that:

- there is a history of reliable production,
- the producer is regularly audited, and
- at least one specific identity test is conducted by the manufacturer of the final product.

9.5 Samples of intermediate and final products shall be retained in sufficient amount and under appropriate storage conditions to allow the repetition or confirmation of a batch control. However, reference samples of certain starting materials, e.g. components of culture media, need not necessarily be retained.

9.6 Certain operations require the continuous monitoring of data during a production process, for example monitoring and recording of physical parameters during fermentation.

9.7 Special consideration needs to be given to the quality-control requirements arising from production of biological products by continuous culture.

Authors

The first draft of "Good manufacturing practices for biological products" was prepared in January 1991 by Dr V. P. Grachev, Scientist and Dr D. I. Magrath, Chief, Biologicals, WHO, Geneva, Switzerland.

Acknowledgements

Acknowledgements are due to the following experts for their comments and advice on the draft of "Good manufacturing practices for biological products": Professor I. Addae-Mensah, Chemistry Department, University of Ghana, Accra, Ghana; Professor H. Blume, German Pharmacists' Central Laboratory, Eschborn, Germany; Dr A. Fenyves, Paul Ehrlich Institute, Langen, Germany; Dr C. Guthrie, General Manager, Blood Products Division, CSL Ltd., Parkville, Australia; Dr U. Ihrig, German Pharmacists' Central Laboratory, Eschborn, Germany; Mr K. Kawamura, Takeda Chemical Industries Ltd., Nihonbashi, Chuo-ku, Tokyo, Japan; Mr L. G. Kinnander, Chief, Pharmaceutical Industries Ltd., Nihonbashi, Chuo-ku, Tokyo, Japan; Mrs S. F. Langlois, Director, Regulatory Affairs, Connaught Laboratories Ltd., A Pasteur Mérieux Company, Willowdale, Ontario, Canada; Mr P. Lemoine, Institute of Hygiene and Epidemiology, Brussels, Belgium; Mr J. Lyng, State Serum Institute, Copenhagen, Denmark; Professor N. V. Medunitsin, Director, Tarasevich State Institute for the Standardization and Control of Medical Biological Preparations, Moscow, USSR; Dr R. Netter, Paris, France; Professor A. A. Olaniyi, Pharmaceutical & Chemistry Department, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.

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Offprint from
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GENERAL REQUIREMENTS FOR THE STERILITY OF BIOLOGICAL SUBSTANCES

(Requirements for Biological Substances No. 6)
(Revised 1973)

REQUIREMENTS FOR BIOLOGICAL SUBSTANCES AND OTHER SETS OF RECOMMENDATIONS

WHO EXPERT COMMITTEE
ON BIOLOGICAL STANDARDIZATION

Twenty-fifth Report

Annexes 4 and 5

WORLD HEALTH ORGANIZATION

GENEVA

1973

WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Geneva, 24–30 April 1973

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Annex 4

GENERAL REQUIREMENTS FOR THE STERILITY OF BIOLOGICAL SUBSTANCES

(Requirements for Biological Substances No. 6) (Revised 1973) [1]

	Page
Introduction	41
General considerations	41
Part A. Manufacturing requirements	
1. Terminology	43
2. General precautions against microbial contamination in manufacture	44
3. General precautions against microbial contamination from materials used for manufacture	47
4. General precautions against microbial contamination in sterility testing	47
5. Sterility tests	48
5.1 Sampling	48
5.2 Sterility tests for bacteria and fungi	49
5.3 Sterility test for mycoplasmas	52
5.4 Sterility test for viruses	52
5.5 Tests for specific micro-organisms	52
6. Records	53
7. Additional samples	53
Part B. National control requirements	
1. General	53
2. Release and certification	53
Appendix 1. Media for the detection of aerobic and anaerobic bacteria and fungi	54
Appendix 2. Procedure for sterility test using membrane filtration	55
Appendix 3. Tests for mycoplasma	56
Appendix 4. Acknowledgements	57

[1] These revised requirements have been derived from the document entitled "Sterility and Sterility Testing of Pharmaceutical Preparations and Biological Substances" (unpublished working document WHO/BS/73.1062; WHO/PHARM/73.474) which was prepared for consideration of the WHO Expert Committee on Biological Standardization and of the WHO Expert Committee on Specifications for Pharmaceutical Preparations; additional information was obtained from a number of other sources. The names of those who prepared the original unpublished working document and the names of those who have submitted suggestions and comments to date on the document are given in Appendix 4 on p. 57 *et seq.*

Introduction

General requirements for the sterility of biological substances (Requirements for Biological Substances No. 6) were formulated by a WHO Study Group in 1959.[1] These general requirements were applicable to any biological product from which the exclusion of microbial contamination is imperative, and they have been quoted in all the sets of requirements for individual biological substances that have since been formulated. The twenty-fourth Expert Committee on Biological Standardization[2] agreed that in view of recent developments in methods of sterility testing of biological products and the improvements in control measures that were now feasible, the general requirements for sterility should be revised. This task was therefore undertaken. Since the preparation of a set of amendments or alterations would be an unsatisfactory way of revising the requirements, it was decided to provide a complete document embodying all the requirements concerned. Where provisions formulated by the earlier Study Group were retained unchanged they have been embodied as such in the present revised requirements.

In preparing these revised requirements account has been taken of the opinions of consultants, the relevant regulations and requirements that have been formulated in a number of countries, as well as information from both published and unpublished reports. In addition, opinions and data relevant to these requirements have been received from a number of experts to whom grateful acknowledgement is made (see Appendix 4, page 57).

General Considerations

The requirements in this document apply to all immunological biological substances, i.e., vaccines and sera, that must be sterile and are used for administration to man. Many of the provisions, however, may be used for the control of sterility of other biological preparations, or of blood and blood products, with suitable modifications, where necessary.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1960, No. 200. The members of the Study Group were: Dr M. Weis Bentzon, Statens Seruminstitut, Copenhagen, Denmark; Dr P. H. Bonnel, Centre de Transfusion — Réanimation de l'Armée, Seine, France (*Rapporteur*); Dr P. de Góes, Institute of Microbiology, Rio de Janeiro, Brazil; Dr M. Pittman, Division of Biologics Standards, National Institutes of Health, Bethesda, Md., USA (*Chairman*); Dr G. Penso, Laboratory of Microbiology, Istituto Superiore di Sanità, Rome, Italy; Dr R. H. Regamey, Institut d'Hygiène de l'Université, Geneva, Switzerland; Dr Sumiatno, Pasteur Institute, Bandung, Indonesia (*Vice-Chairman*); Dr J. O'H. Tobin, Biological Standards Control Laboratory, Medical Research Council, Hampstead, London, England (*Rapporteur*); Dr G. V. Vygodchikov, N. F. Gamaleja Institute of Epidemiology and Microbiology, Moscow, USSR, *Secretariat*: Dr B. K. Bhattacharya, Medical Officer, Biological Standardization, WHO; Dr N. K. Jerne, Chief Medical Officer, Biological Standardization, WHO, acted as Secretary.

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1972, No. 486, p. 19.

The Study Group on General Requirements for the Sterility of Biological Substances [1] (in 1959) pointed out that confidence in the sterility of biological preparations depended on two important considerations; first, adequate control tests for the sterility of biological preparations in their final containers, using a sufficient number of random samples, and secondly the use of suitable precautions with respect to source materials and manufacturing procedures. The Study Group considered available information on sampling procedures adopted in several countries and also discussed a suggested schedule for sampling of final containers to ensure an acceptably low probability of the release of contaminated final containers.

Theoretically, sterility may be defined as the absence of all micro-organisms capable of multiplying.[2] Sterility tests employing a reasonable number of samples are capable of detecting contamination only in a lot with a high percentage of contaminated units. Accordingly, it is not possible to detect, on the basis of acceptable confidence limits, a low percentage of contaminated units in a homogeneous lot of final product. If the finished product is a single final container of bulk material, then the control measures are applicable on the basis of an assumed homogeneity of material in such a single final container. Sterility, therefore, cannot be assured in the control laboratory, but must be built into the product during processing. Experience in many countries over the years has confirmed that greater reliance must be placed on appropriate techniques and procedures throughout the manufacture of the product (including in-process sterility testing at various stages) rather than simply depending on sterility tests made on a number of samples of the final batch as the sole basis for criteria of sterility. Notwithstanding the limitations of sterility tests, every effort should be made to use the most effective procedure and to strengthen and validate the tests in the light of the most recent knowledge and experience.

The tests for sterility mentioned in the previous general requirements have been revised in the present document, both in nature and in scope. They now include recommendations for test procedures for mycoplasmas and for tests using membrane filtration. Tests for viral contaminants are not dealt with in detail because requirements concerning such contaminants of biological products and appropriate tests for viral sterility have been included in the sets of Requirements for Biological Substances [3] that have been formulated for individual products. Similarly, information on when

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1960, No. 200, p. 4.

[2] In the case of a product consisting of living micro-organisms, e.g., certain vaccines, sterility consists of the absence of contamination by other micro-organisms. Practically, howerer, assertions regarding sterility relate to the probability that all units in the lot are sterile. For example, in some countries, a lot of a product is accepted as sterile if there be no more than one living micro-organism in one million units.

[3] See *Wld Hlth Org. techn. Rep. Ser.*, 1973, No. 530, p. 62, for a complete list.

and where sterility tests are to be made will be found in the requirements for individual products and this question is not discussed in detail in the present document.

Each of the following sections constitutes a recommendation. The parts of each section that are printed in large type have been written in the form of requirements so that, if a health administration so desires, these parts as they appear may be included in definitive national requirements. The parts of each section that are printed in small type are comments and recommendations for guidance.

Should individual countries wish to adopt these requirements as the basis for their national regulations concerning sterility of biological products, it is recommended that a clause be included that would permit modifications of manufacturing requirements on the condition that it be demonstrated, to the satisfaction of the national control authority, that such modified requirements ensure a degree of sterility of the product at least equal to that provided by the requirements formulated below. It is desirable that the World Health Organization should then be informed of the action taken.

The terms " national control authority " and " national control laboratory " as used in these requirements, always refer to the country in which the product is manufactured.

Part A. Manufacturing Requirements

1. Terminology

Contamination : The presence of live extraneous micro-organisms. The term extraneous applies not only to micro-organisms that may enter the product during processing, but also to those that may be present in the materials used for preparing the product, such as SV40 virus in tissues used to propagate certain viruses for vaccine production. Micro-organisms, as referred to in this document, include bacteria, fungi, viruses, rickettsias, mycoplasmas, and chlamydia.

Bulk material is a quantity of partly or wholly processed material, present in a single container at any stage prior to distribution into final containers.

Final bulk material is the homogeneous finished preparation present in a single container from which the final containers are filled, either directly or indirectly, through one or more intermediate containers.

In some cases a final bulk may constitute the manufacturer's final product.

Product lot : All finished material, in sealed final containers, that has been derived from the same final bulk, all of which, at the last stage of

processing capable of altering its composition, has been processed together and therefore has a uniform composition.

In the case, for example, of a vaccine such a lot may be termed a vaccine lot. The terms product batch and vaccine batch may be used as appropriate.

Final lot : A collection of sealed final containers derived from a single final bulk, which are homogeneous with respect to the risk of contamination during filling, sealing and, if applicable, during drying. A final lot must therefore have been filled from a single container, and, if applicable, dried in one continuous operation, e.g., in one working session, the processing being so arranged that each such operation yields a single homogeneous batch of the product.

A final lot may consist of the whole or part of a product lot.

In appropriate cases the terms final batch, filling lot, filling batch or drying lot may be used.

2. General precautions against microbial contamination in manufacture

Every manufacturing establishment should in addition have a separate and independent quality audit to which every batch of product would be subjected at the end of processing; the purpose of such an audit would be to ensure that the measures adopted for control of microbial contamination of the product have been carried out satisfactorily.

The general manufacturing requirements given in Part A of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [1] shall apply which the addition of the following:

The requirements concerning buildings and equipment given in Part A, section 2, of the above requirements are applicable. The most desirable situation might be the provision of separate buildings for each product. This, however, is feasible or necessary only in special circumstances or for very large-scale manufacture. The situation usually encountered is one in which more than one product is manufactured in one building or laboratory.

If the manufacturing process requires that micro-organisms be cultured and processed to yield concentrated suspensions these may become the sources of aerosols or may contaminate equipment, clothing, and personnel. The potential for cross-contamination is high and barriers should be established to prevent contamination of the environment and cross-contamination.

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 323, p. 13.

The various measures to avoid contamination are essentially barriers that may be spatial, temporal, or operational. The nature of these barriers depends on the risk of contamination and on the mechanisms by which contamination and cross-contamination may occur, and it will vary with the nature of the potential hazards involved. The barriers may include (*a*) measures to restrict certain organisms and/or operations to separate buildings or separate areas within the one building, (*b*) restriction of the movement of personnel and materials, (*c*) the treatment of air to remove or destroy aerosols containing micro-organisms and to control relative humidity, (*d*) the establishment of pressure gradients to control the direction of air movement, (*e*) the provision of special work stations such as laminar flow stations or biological safety cabinets, and (*f*) wearing of suitable clothing, including head-covering and footwear, by personnel.

If the same area is used for the successive manufacture of different products, the area as well as all equipment it contains shall be adequately cleaned and disinfected after the processing of one product has been completed and before the processing of another product is commenced.

Manufacturing procedures involving the handling of microbial spores shall be confined to a separate building or to a separate area within a building. Those involving live viruses shall be arranged so that at any time each distinct virus is confined to an area that is physically isolated by appropriate barriers. Virus product testing that requires the exclusion of adventitious agents shall also be subjected to this requirement.

The precautions against contamination given in Part A, section 3.4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [1] shall apply.

There should be careful control of the air filtration and routine microbial counts of the air in the manufacturing areas should be carried out during manufacturing operations or periodically using an appropriate method of air sampling. The results of such counts should be checked against criteria, established for the particular manufacturing area, and adequate records of the counts should be maintained. Where air filters are used in manufacturing areas, filters that remove 99.98% ± 0.01% of particles greater than 0.3 μm in diameter are suitable. The efficiency of the filters should be checked routinely.

The recommendations concerning filling and containers given in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [2] shall apply.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 323, p. 15.

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 323, p. 16.

Filling operations shall be conducted in such a way as to avoid any contamination or alteration of the product.

These operations should take place in controlled areas specially designed for the purpose, in which steps are taken to reduce to a minimum the number of micro-organisms to which the product being filled is exposed. The use of laminar flow work stations, disinfecting agents, air filters, and sterilized clothing, including head-covering and footwear for personnel conducting filling operations, is recommended. Germicidal lamps and disinfecting aerosols should not be used during filling operations.

The operations where liquid preparations are filled should be checked. This may be done, e.g., at least twice each year at the close of a working day, by filling not less than 1000 containers with a nutrient medium containing no antibiotics or bactericidal substances, and incubating the complete batch of filled containers at 30° – 36°C for at least 14 days. If containers filled and incubated in this manner show a contamination rate above 0.3%, some countries do not consider the procedure acceptable for the filling of sterile preparations with or without an antimicrobial agent.

The presence of micro-organisms in the air at the filling point should be monitored, for example, by means of settle plates or slit-sampling devices.

All material from an area in which virus is being processed shall be autoclaved before being sent to the washing and sterilizing departments, irrespective of whether each area has its own facilities or common facilities are employed.

A common washing and sterilization area for glassware and culture media may be used where two or more virus vaccines are being produced, provided that only heat-sterilized materials are issued for use in the production areas and that each production area has its own equipment suitably marked. All media that are sterilized by filtration alone should be prepared in a separate area with its own equipment for the preparation of such media.

In controlled areas, apart from specific organisms or cells being processed, all material introduced from the main washing and sterilization area should preferably be sterilized by heat. It is preferable that autoclaves or chambers used for the sterilization of materials passing in or out of an area be of the " double-ended " type, built into the wall in a suitable manner and subjected to control procedures such that the only passage way for materials between areas containing unsterilized and sterilized materials is through the autoclave or sterilization chamber itself.

Cell cultures for vaccine production shall be prepared in a special area separated from any area in which virus is being handled. Where different

cell lines are handled in an area measures shall be taken to prevent cross-contamination of one cell line with another.

The room and equipment used for filling a live virus vaccine into containers shall be used solely for this particular vaccine, unless it is adequately disinfected when it may be used later for the filling of other products.

When animals are used for obtaining tissues for cell culture, appropriate recommendations should apply.[1]

3. General precautions against microbial contamination from materials used for manufacture

Precautions shall be taken to avoid contamination from source or other materials used for manufacture that may affect the sterility of the final product.

All strains of micro-organisms used as seed materials for biological preparations shall be maintained in a manner that will assure freedom from contaminating micro-organisms.

As contamination of a seed lot introduces the possibility of subsequent contamination of a number of production lots, special precautions are necessary when preparing or manipulating seed lots.

All blood and blood-derived source materials shall be obtained under conditions that ensure a safe and effective final product. In the case of human blood material the requirements for source material given in Part A, section 3.1, of the Requirements for Human Immunoglobulin (Requirements for Biological Substances No. 14)[2] shall apply.

Particular attention should be paid to any regulations that are applicable to the manufacturer and/or organization that collects such human material.

4. General precautions against microbial contamination in sterility testing

The appropriate requirements for manufacture given in Part A, section 2, shall apply to those areas in which, and those procedures by which, sterility testing is done.

The risk of accidental contamination from the environment should be kept to a minimum by the use of disinfecting agents, germicidal lamps, and air filters. Germicidal lamps and disinfecting aerosols should not be used during actual testing opera-

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[1] See, for example, *Health aspects of the supply and use of non-human primates for biomedical purposes, report of a WHO Scientific Group* (*Wld Hlth Org. techn. Rep. Ser.*, 1971, No. 470).

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 361, p. 49.

tions. The test manipulations should be carried out in a filtered air environment or under a laminar flow hood, and the operators should be dressed in sterilized, electrically neutral clothing, including head-covering and footwear. The air pressure in the testing room should be greater than the exterior area. The performance of the laminar flow hood should be monitored by settle plates or slit-sampling devices and the efficiency of the filters and germicidal lamps checked routinely.

Sterility testing shall not be carried out in production areas.

5. Sterility tests

Sterility tests shall be performed whenever specified in the requirements for individual products.

Tests for sterility are often performed advantageously at various stages of manufacture, in addition to those given in requirements. The tests described in these requirements may be used for this purpose.

Detailed rules and precautions for the sterility test procedures should be written and carefully followed.

The staff performing the sterility tests should have experience in the duties assigned. Supervision of the work and interpretation of sterility tests should be undertaken by an individual with training in scientific subjects, who should also have been trained in microbiology or be given training in that subject.

Where sterility tests for bacteria and fungi are required, such tests shall be made as described in Part A, section 5.2. Appropriate tests for viral and rickettsial sterility, when required, shall be made as specified in the requirements for the individual products. Tests for mycoplasmas, when required, shall be made as described in Part A, section 5.3.

5.1 *Sampling*

The specified number of suitable samples shall be taken from the product. Such samples shall be taken at least from each final bulk, as well as from each final lot.

5.1.1 *Sampling from bulk*

A sample shall be taken from each bulk to be tested in such a manner as to be representative of the material to be tested. The amount taken shall be sufficient to perform the tests and any repeat tests that may be required. Such samples shall be taken so as to maintain intact the level of sterility of the material, or, if this is not possible, the samples shall be taken at the stage of further processing.

Since any microbial contaminants in a liquid may settle out, thorough mixing is required before the sample is taken.

5.1.2 *Sampling from final lot*

Samples of final containers from each final lot to be tested shall be taken in such a manner as to be representative of the lot to be tested. Appropriate periodic samples shall be taken, including samples at the beginning and the end of the filling operation.

If a product lot is filled through several outlets from a single bulk, samples should be taken from each outlet (filling lot) so as to be representative of the filling assembly.

The number of samples taken shall be at least that approved by the national control authority, provided that for final lots containing 500 or more containers, at least 20 samples shall be taken, including samples at the beginning and the end of the filling operation.

For final lots containing fewer than 500 containers, not less than 10 containers from the lot should be taken for a sample, except that for lots of less that 100 containers, only 10% of the lot need be tested.

A suggested rule[1] for calculating the number of samples is to take $0.4\sqrt{N}$ samples, where N is the number of final containers in the lot.

5.2 *Sterility tests for bacteria and fungi*

5.2.1 *Culture media*

The culture media used for sterility tests for bacteria and fungi shall be those approved by the national control authority. Such media shall have been shown to be capable of supporting the growth of a wide variety of micro-organisms, with both aerobic and anaerobic growth characteristics, including the types found in the environment of the manufacturing operations.

More than one culture medium, generally, will be needed to fulfil these criteria. The media used in many countries for sterility tests are fluid thioglycolate medium and soybean–casein digest medium.[2] Any other media that are used, however, should have been demonstrated to have at least equivalent growth supporting properties.

For testing the growth supporting qualities of each culture medium, strains of micro-organisms should be used with exacting nutritive and aerobic–anaerobic requirements, in an inoculum of only a small number of the organisms (less than 100).

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1960, No. 200, p. 31.

[2] The formulae of these culture media are given in Appendix 1.

The media should be incubated at the temperatures at which they will be used in the sterility test.

Each lot of dehydrated medium or each lot of medium prepared from basic ingredients should be tested for its growth supporting properties, since every lot may not support the growth of micro-organisms as well as desirable. It is desirable that lots so tested should be kept separate and reserved for use in sterility testing. This is a safeguard against occasional unsatisfactory components in a particular lot, or destruction of certain components by over-heating or over-sterilization, which may cause differences in growth response.

In some countries an additional test is made to verify the growth supporting properties of the medium, after the completion of a sterility test in which no growth has occurred in the medium, by inoculation with a small number of suitable organisms (less than 100).

5.2.2 *Performance of the test*

Prior to conducting a sterility test on any product, it shall have been determined wheter or not the material to be tested itself has the property of killing or inhibiting the growth of micro-organisms, or contains preservatives or other substances that have this effect. If such an effect is shown, the sterility test shall be made using a suitable procedure to counteract the effect.

The inhibitory effect in a preparation to be tested for sterility may be overcome by increasing the volume of culture medium used, so that the inhibitors are rendered ineffective, or if the preparation can be filtered, the inhibitors removed by membrane filtration.[1] The volume of medium required to overcome the inhibitory effect should be determined. Once established, these quantities may be used for subsequent sterility tests unless a change is made in the composition of the product.

5.2.2.1 *Inoculum*

For sterility testing of bulk material, at least 5 ml must be used for inoculation into each culture medium and for each temperature of incubation.

For sterility testing of a final lot from each of the final containers that contain not more than 20 ml, an amount of at least 1.0 ml shall be inoculated into each culture medium and for each temperature of incubation. If the volume in each final container is less than 1.0 ml, the amount to be inoculated shall be the entire content of the container for each culture medium and for each temperature of incubation. If the volume in each final container is greater than 20 ml, but not more than 100 ml, the amount to be inoculated shall be at least 5 ml for each culture medium and for each temperature of incubation.

[1] See Appendix 2 for a description of the procedure using membrane filtration.

5.2.2.2 *Medium*

The quantity of medium put into each vessel for conducting the sterility test shall be sufficiently large to ensure that the volume of material inoculated does not impair the growth supporting properties of the medium by dilution.

All vessels of inoculated media shall be clearly identified by labelling that is adequate to identify the product being tested, each medium used, and each temperature of incubation.

5.2.2.3 *Incubation*

All vessels shall be incubated at the appropriate temperatures for the media. The temperatures selected shall be those approved by the national control authority and shall include 20°–25°C and 30°–36°C.

If only one temperature is selected for a particular medium, generally fluid thioglycolate medium is incubated at 30°–36°C and casein digest medium at 20°–25°C.

In some countries additional temperatures are used for psychrophilic and thermophilic organisms.

It is desirable that the incubation apparatus should have a continuous temperature recording device.

All vessels shall be incubated for a period of at least 14 days and shall be examined at regular intervals and on the last day of incubation for evidence of microbial growth.

If the preparation inoculated into the test vessels has clouded the medium to such an extent that it is difficult to recognize whether or not growth has taken place, subcultures of not less than 1.0 ml should be made from the cloudy medium between the third and seventh days after the start of the test. The original and transfer vessels should each be incubated and observed for a total of not less than 14 days.

5.2.3 *Membrane filtration of test samples*

The performance of sterility tests with the aid of membrane filtration of the test samples is a valuable method of improving results of such testing in certain situations.[1]

The extra manipulations involved in these procedures additional to those for the performance of the test described in section 5.2.2 above may, however, be a source of extraneous contamination. Hence the routine use of positive and negative controls is advisable for validating the results obtained. A suitable positive control is the occasional use of known con-

[1] See Appendix 2 for a description of a procedure for sterility tests using membrane filtration.

taminated solutions containing a few micro-organisms of differing types; if the product does not contain a preservative, approximately 10 viable microbial cells may be used in the total volume employed. If the product contains a preservative, the positive control may be added to the wash fluid.

5.2.4 *Interpretation of test results*

If no evidence of growth is found in any of the vessels inoculated for the test for sterility in Part A, section 5.2, the bulk material or final lot, whichever is applicable, meets the requirements for this test. If evidence of growth is found, the preparation tested fails to meet the requirements for the test for sterility, unless it can be demonstrated to the satisfaction of the national control authority, by retests or by other means, that the test was invalid.

The decision between failure of the product to pass the test and invalidity of the test procedure requires competent judgement of a trained individual.

5.3 *Sterility test for mycoplasmas*

For viral vaccines made by growing the virus in animal tissues or cell cultures, sterility tests for mycoplasmas of virus culture fluid and control fluid specified for the particular product shall be made by a method approved by the national control authority.[1]

The samples taken may include material prior to clarification or filtration in the case of live vaccines produced from *in vitro* living cell cultures or animal tissues. In the case of inactivated virus vaccines produced from such cell cultures or tissues, the samples may be taken prior to inactivation, from each virus harvest pool and from control pooled fluid.

Control cultures should be included in each test.

5.4 *Sterility test for viruses*

The sterility tests for the detection of extraneous viruses in products where their presence is unacceptable shall be performed as specified in the requirements for the individual products.

5.5 *Tests for specific micro-organisms*

The tests to ensure the non-survival of micro-organisms in inactivated vaccines and additional tests for the absence of specific extraneous micro-organisms in such products shall be performed as specified in the requirements for the individual products.

[1] A description of tests for mycoplasmas is given in Appendix 3.

6. Records

Records shall be permanent and clearly indicate all steps in the sterility testing of the product, including temperatures and incubation times, as well as the relationship of the samples under test to the manufacturing operations followed in the production of the lot under test. The records shall be of a type approved by the national control authority. Written records shall be kept of all tests performed, irrespective of their results. They shall be retained throughout the dating period of the product, and be available for inspection by the national control authority. Records shall be maintained of all cultures kept in the establishment. Such records shall include clear labelling for the identification of the cultures and the purposes for which the cultures are used.

For each sterility test performed a record shall be kept of the name and identifying numbers of the product under test, the quantities inoculated, the batch, type and tests of culture media used, the temperatures of incubation, the dates of inoculation and the results.

7. Additional samples

For each final lot for which sterility tests are made, additional samples shall be retained as reference material throughout the dating period of the product, in a manner that ensures the identity of the lot or batch of the product.

It is desirable that, where possible, manufacturers should retain sufficient additional samples to permit repetition of the sterility tests.

Part B. National Control Requirements

1. General

The general requirements for control laboratories given in Part B of the revised General Requirements for Manufacturing Establishments and Control Laboratories (Requirements for Biological Substances No. 1)[1] shall apply.

2. Release and certification

The requirements given in Part B, section 3.3, of the revised General Requirements for Manufacturing Establishments and Control Laboratories (Requirements for Biological Substances No. 1)[2] as well as the relevant provisions in the requirements for the individual products shall apply.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 323, p. 19.

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 323, p. 22.

Appendix 1

MEDIA FOR THE DETECTION OF AEROBIC AND ANAEROBIC BACTERIA AND FUNGI *

1. Fluid thioglycolate medium

L-cystine	0.5	g
sodium chloride	2.5	g
glucose ($C_6H_{12}O_6.H_2O$)	5.5	g
agar	0.75	g
yeast extract, water soluble	5.0	g
pancreatic digest of casein	15.0	g
distilled water	1000	ml
thioglycolic acid	0.3	ml
(or sodium thioglycolate [1]	0.5	g)
resazurin sodium (0.10% fresh solution)	1.0	ml

Final pH=7.0–7.2

Preparation:

Thoroughly grind the first six ingredients, in the order given above, in a mortar. Stir in some heated water, transfer to a suitable container, add the remainder of the water and complete the solution by heating in a boiling water bath, taking special care to ensure complete solution of the L-cystine. Add the thioglycolic compound, then 1 N sodium hydroxide so that the pH of the completed and sterilized medium will be 7.0–7.2. Reheat the solution, but do not boil, filter (if necessary) through a moistened filter paper and add the resazurin solution. Distribute into suitable vessels and sterilize by autoclaving for 18–20 minutes at 121°C. Cool promptly to 25°C and store at 20°–30°C, avoiding excessive light. If the uppermost portion of the medium has changed to a pink colour and this exceeds one-third of the depth of the medium, it is unsuitable for use, but may be restored once by heating in steam. Medium more than 3 weeks old should not be used.

2. Soybean-casein digest medium

pancreatic digest of casein	17.0	g
papaic digest of soybean meal	3.0	g
sodium chloride	5.0	g
dipotassium hydrogen phosphate	2.5	g
glucose ($C_6H_{12}O_6.H_2O$)	2.5	g
distilled water	1000	ml

Final pH=7.1–7.5

Preparation

Dissolve the solids in the water, warming slightly, then cool the solution to room temperature. Adjust the reaction with 1 N sodium hydroxide if necessary, so that the pH of the completed and sterilized medium will be 7.1–7.5. Filter, if necessary, to clarify, distribute into suitable vessels and sterilize in an autoclave for 18–20 minutes at 121°C.

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* The quality of the components should be in accordance with *Specifications for Reagents mentioned in the International Pharmacopoeia*, Geneva, World Health Organization, 1963, unless otherwise stated.

[1] Reagent quality sodium thioglycolate is more stable than thioglycolic acid.

Appendix 2

PROCEDURE FOR STERILITY TEST USING MEMBRANE FILTRATION

Apparatus

A suitable unit consists of a closed reservoir and a receptacle, separated from one another by a properly supported membrane of appropriate porosity. Membranes generally suitable for sterility testing have a nominal porosity of 0.22 µm or 0.45 µm, a diameter of approximately 47 mm, and a flow rate of 55–75 ml of water per minute at a pressure of 70 cm of mercury. Preferably assemble and sterilize the entire unit with the membrane in place, prior to use. Where the sample to be tested is an oil, it may be necessary to sterilize the membrane separately and, after thorough drying, assemble the unit, using aseptic precautions. If each entire membrane is to be cultured (see below), at least two filter units are set up.

Diluting fluids

Fluid A : Dissolve 1 g of peptic digest of animal tissue [1] or the equivalent in distilled water to make one litre, filter or centrifuge to clarify, adjust to pH 7.1 ± 0.2, dispense into flasks in 100-ml quantities, and sterilize at 121°C for 18–20 minutes.

Fluid B : If the test sample contains lecithin or oil, use Fluid A to each litre of which has been added 1 ml of (*p-tert*-octylphenoxy)polyethoxyethanol, adjust to pH 7.1 ± 0.2, dispense into flasks, and sterilize at 121°C for 18–20 minutes.

Note : Any sterile diluent that does not manifest antimicrobial activity and does not affect the porosity of the membrane may be suitable for dissolving a preparation under test for sterility.

Test procedure

Liquids

The number of sample containers or the volume of bulk sample that is specified for conducting the sterility test is taken. Aseptically transfer the required volumes from each container directly to a membrane filter previously moistened with sterile water or the diluting fluid used or to two sterile vessels for pooling prior to transfer to a moist membrane. Immediately draw each sample through the filter with the aid of vacuum.

If the substance is a viscous liquid or suspension not adaptable to rapid filtration, aseptically add a sufficient quantity of Fluid A to the pooled sample to increase the flow rate. Sterile enzyme preparations such as penicillinase or cellulase may be added to the diluting fluid to aid in dissolving insoluble substances. If the substance under test has inherent antimicrobial properties or contains a preservative, wash the filter with sufficient 100-ml portions of Fluid A. If the substance under test contains lecithin or oil, replace Fluid A by Fluid B. The number of portions of fluid used should be sufficient to allow growth of a small inoculum of organisms (approximately 50) sensitive to the antimicrobial substance in the presence of the residual inhibitory material on the membrane.

Upon completion of filtration, transfer each entire membrane to 100 ml of the culture medium (or approximately one-half of each membrane to 100 ml of each of two culture media) selected for the sterility test. The samples are then incubated at the selected

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[1] Example : Peptone, dried, R, as described in *Specifications for Reagents mentioned in the International Pharmacopoeia*, Geneva, World Health Organization, 1963, p. 137. An autoclaved solution (2 in 100) is clear and is neutral or nearly so in its reaction.

temperatures and for the appropriate periods and examined as described in Part A, section 5.2 of these requirements. The period of incubation of membrane-filtered test samples used by some workers is shorter than the 14-day period prescribed in this section, since growth occurs more rapidly when micro-organisms concentrated on filters are inoculated.

Appendix 3

TESTS FOR MYCOPLASMAS

All varieties of mycoplasma are not capable of growth in the same medium. Some strains require special culture media. Suitable media should therefore be selected having regard to particular conditions, e.g., the tissue in which a virus is grown or source of animal serum, and the growth requirements of the potential contaminants, e.g., the need for yeast extract. Solid and semi-solid or liquid media containing serum fractions rich in certain lipids (sterols [1] and phospholipids) are used to provide the nutritive requirements. Control cultures on solid and semi-solid or liquid media of known fastidious strains of mycoplasma, both sterol-requiring and non-sterol-requiring, are included in each test to show that the media are capable of supporting the growth of mycoplasmas.

Samples of the material of not less than 6 ml for a single test (i.e., using one solid and one semi-solid or liquid medium) are stored either between 2° and 8°C for no longer than 24 hours or at –20°C or lower if stored for longer than 24 hours.

Not less than 2.0 ml of each sample are inoculated and evenly distributed over the surface of at least 10 plates of each solid medium used, and not less than 1.0 ml of each sample is inoculated into each of at least 4 tubes of each semi-solid or liquid medium used. Each tube should contain 10 ml of medium. Larger volumes of inoculum, e.g., making a total of 50 ml of material in 400 ml of medium, have been used advantageously. Ultracentrifugation before testing such samples has also been used.

The plates and tubes are incubated at 36°C ± 1.0°C for not less than 14 days. One half of each set is incubated aerobically in an environment of adequate humidity, and the other half is incubated anaerobically in an environment of 5–10% carbon dioxide in nitrogen, also of adequate humidity. At the end of 3 days and again at 14 days, 0.5 ml from each of 2 tubes being incubated aerobically are combined and inoculated on to not less than 4 plates. The 2 tubes being incubated anaerobically are treated likewise. Since some mycoplasmas may not grow on media in Petri dishes, such subcultures on solid media may also be made in tubes.[2]

All material seeded originally on solid media is subcultured on the day after the primary inoculation, on the third day and on the sixth day, on other suitable solid and liquid media, and these subcultures are incubated similarly, half aerobically and the other half anaerobically as described, and examined after 10 days, while the original cultures are examined at the end of the 14-day period. All plates (and tubes) after incubation for not less than 14 days are observed for the growth of mycoplasmas by appropriate procedures.

If no evidence is found of the presence of mycoplasmas the material, e.g., virus pool, meets the requirements of these tests. If evidence of growth of mycoplasmas is found, the mycoplasmas should be identified and the source traced.

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[1] Non-sterol-requiring mycoplasmas will grow on media containing sterols.

[2] In order to induce mycoplasmas to grow in liquid media, similar subculturing could be done, but three or four passages may be needed.

For detection and identification of mycoplasmas the following procedures are suggested but other methods are available.

1. For detection of mycoplasmas

(*a*) typical colonies or specific coloration (blue) by Dienes's stain of colonies spread on blocks of agar in Petri dishes, and viewed under the microscope ;

(*b*) phase contrast microscopy of colonies (strips or plaques) for globular bodies, filaments and granules ;

(*c*) examination under the electron microscope of the pellet centrifuged from a semisolid culture ;

(*d*) examination under the light microscope (× 1000) of a film stained by Giemsa.

2. For separation of mycoplasmas into clasess

(*a*) enzymatic effects ; fermentation of glucose, breakdown of arginine and reduction of triphenyltetrazole, using decoloration of phenol red as indicator for the first two effects and red colour of the tetrazole derivative for the last ;

(*b*) haemolysis in a 4% suspension of washed guineapig cells, added and incubated for 18–48 hours aerobically at 37°C ;

(*c*) haemadsorption of a 4% suspension of guineapig cells poured over the growth surface or fixation of red blood corpuscles by the colonies after incubation at 37°C for 30 minutes.

3. For specific identification

Growth inhibition by specific anti-mycoplasma serum (prepared in the rabbit) ; such inhibition might be demonstrated by depositing paper discs impregnated with the serum on 2–4 hour growth cultures and observing them from the third day.

Immunofluorescence techniques for this purpose are being developed.

Appendix 4

ACKNOWLEDGEMENTS

The unpublished working document WHO/BS/73.1062 ; WHO/PHARM/73.474 on which these requirements were based was prepared by the following experts and members of the WHO Secretariat :

Dr E. A. Christensen, Control Department, Statens Seruminstitut, Copenhagen, Denmark

Dr H. Cohen, Director, National Institute of Public Health, Bilthoven, Netherlands (*Consultant*)

Dr J. Desbordes, Director, Microbiology Section, National Public Health Laboratory, Paris, France (*Consultant*)

Dr L. F. Dodson, Director, National Biological Standards Laboratory, Commonwealth Department of Health, Canberra, Australia (*Consultant*)

Professor G. Edsall, Head, Department of Microbiology, London School of Hygiene and Tropical Medicine, London, England (*Consultant*)

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Miss A. Wehrli, Pharmaceutical Officer, Pharmaceuticals, WHO, Geneva, Switzerland

Grateful acknowledgement is made to the experts and institutions listed below for their comments and advice and for supplying additional data :

Dr F. E. André, Research Laboratories, Philips-Duphar B.V., Amsterdam, Netherlands

Dr W. R. Ashford, Assistant Director, Connaught Laboratories Limited, Willowdale, Ontario, Canada

Professor M. A. Attisso, Faculty of Pharmacy, Montpellier, France

Dr Sami Bağlum, P. Director, Refik Saydam, Central Institute of Hygiene, Ankara, Turkey

Dr M. Baharsefat, Razi State Institute for Serum and Vaccine Production, Teheran, Iran

Mr A. J. M. Bailey, Executive Officer, The Association of the British Pharmaceutical Industry, London, England

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Dr Carrol Brock, Food and Drug Administration, Washington, D.C., USA

Dr A. Brunzell, Associate Professor, The Galenical Section, Division of Pharmacy, Department of Drugs, National Board of Health and Welfare, Stockholm, Sweden

Dr J. R. Buriánek, State Institute for the Control of Drugs, Prague, Czechoslovakia

Professor A. Calò, Chief, Laboratory of Chemistry, Istituto Superiore di Sanità, Rome, Italy

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Mr I. Davidson, Central Veterinary Laboratory, Weybridge, Surrey, England

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Valuable suggestions were also obtained from the report of a discussion on recommendations for WHO Requirements that took place at the Symposium on Sterility and Sterility Testing of Biological Substances held from 9 to 12 April 1973 in Madrid, Spain, by the International Association of Biological Standardization.

Annex 5

REQUIREMENTS FOR BIOLOGICAL SUBSTANCES AND OTHER SETS OF RECOMMENDATIONS

The specification of requirements to be fulfilled by preparations of biological substances is necessary in order to ensure that these products are safe, reliable, and potent prophylactic or therapeutic agents. International recommendations on requirements are intended to facilitate the exchange of biological substances between different countries and to provide guidance to workers responsible for the production of these substances as well as to others who may have to decide upon appropriate methods of assay and control.

Recommended requirements and sets of recommendations concerned with biological substances formulated by international groups of experts and published in the *Technical Report Series* of the World Health Organization are listed hereunder :

No.	Year	
178	1959	**Requirements for Biological Substances :**
		1. General Requirements for Manufacturing Establishments and Control Laboratories
		2. Requirements for Poliomyelitis Vaccine (Inactivated)
179	1959	**Requirements for Biological Substances :**
		3. Requirements for Yellow Fever Vaccine
		4. Requirements for Cholera Vaccine
180	1959	**Requirements for Biological Substances :**
		5. Requirements for Smallpox Vaccine
200	1960	**Requirements for Biological Substances :**
		6. General Requirements for the Sterility of Biological Substances
237	1962	**Requirements for Biological Substances :**
		7. Requirements for Poliomyelitis Vaccine (Oral)
274	1964	**WHO Expert Committee on Biological Standardization :**
		8. Requirements for Pertussis Vaccine
		9. Requirements for Procaine Benzylpenicillin in Oil with Aluminium Monostearate
293	1964	**WHO Expert Committee on Biological Standardization :**
		10. Requirements for Diphtheria Toxoid and Tetanus Toxoid

No.	Year	
323	1966	WHO Expert Group : Requirements for Biological Substances (Revised 1965) 1. General Requirements for Manufacturing Establishments and Control Laboratories 2. Requirements for Poliomyelitis Vaccine (Inactivated) 7. Requirements for Poliomyelitis Vaccine (Oral) 5. Requirements for Smallpox Vaccine
329	1966	WHO Expert Committee on Biological Standardization : 11. Requirements for Dried BCG Vaccine 12. Requirements for Measles Vaccine (Live) and Measles Vaccine (Inactivated)
361	1967	WHO Expert Committee on Biological Standardization : 13. Requirements for Anthrax Spore Vaccine (Live — for Veterinary Use) 14. Requirements for Human Immunoglobulin 15. Requirements for Typhoid Vaccine 9. Requirements for Procaine Benzylpenicillin in Oil with Aluminium Monostearate (Revisions adopted 1966)
384	1968	WHO Expert Committee on Biological Standardization : 16. Requirements for Tuberculins 17. Requirements for Inactivated Influenza Vaccine
413	1969	WHO Expert Committee on Biological Standardization : 4. Requirements for Cholera Vaccine (Revised 1968) 18. Requirements for Immune Sera of Animal Origin
444	1970	WHO Expert Committee on Biological Standardization : 19. Requirements for Rinderpest Cell Culture Vaccine (Live) and Rinderpest Vaccine (Live) 20. Requirements for *Brucella abortus* Strain 19 Vaccine (Live — for Veterinary Use)
444	1970	WHO Expert Committee on Biological Standardization : Development of a National Control Laboratory for Biological Substances (A guide to the provision of technical facilities)
463	1971	WHO Expert Committee on Biological Standardization : 21. Requirements for Snake Antivenins
486	1972	WHO Expert Committee on Biological Standardization : 7. Requirements for Poliomyelitis Vaccine (Oral) (Revised 1971)
530	1973	WHO Expert Committee on Biological Standardization : 4. Requirements for Cholera Vaccine (Revised 1968) (Addendum 1973) 6. General Requirements for the Sterility of Biological Substances (Revised 1973) 17. Requirements for Inactivated Influenza Vaccine (Addendum 1973) 22. Requirements for Rabies Vaccine for Human Use

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Annex 3

General requirements for the sterility of biological substances

(Requirements for Biological Substances No. 6, revised 1973, amendment 1995)

Introduction

Since the General Requirements for the Sterility of Biological Substances were revised in 1973 (*1*), several developments have occurred. It is thus appropriate to modify as follows the existing General Requirements for the Sterility of Biological Substances.

Sterility test for mycoplasmas (section 5.3, page 52)

Replace the text of section 5.3 by the following:

"The test for mycoplasmas is carried out by culture in agar and broth or by means of an indicator cell culture and DNA staining.

Where a test for mycoplasmas is prescribed for a master cell bank, for a working cell bank, for a virus seed lot (or bank) or for cell cultures used as controls, the standard culture or the indicator cell-culture method can be used. Where a test for mycoplasmas is prescribed for a virus harvest, bulk vaccine or final lot, the standard culture method is used. The indicator cell-culture method can be used, where necessary, for the screening of media. Both the standard culture and indicator cell-culture methods are given in Appendix 3. Alternative methods may be used provided that they have been validated against the methods described here."

Tests for mycoplasmas (Appendix 3, p. 56)

Replace the text of Appendix 3 by the following:

"Standard culture method

Choice of culture media

The test is carried out with a sufficient number of both solid and liquid media to ensure the growth, under the chosen incubation conditions, of small numbers of organisms that may be present in the product being examined. Liquid media must contain phenol red dye. The

ability of the media to support the growth of at least the following organisms shall be demonstrated:

Mycoplasma orale (vaccines for human use);

Mycoplasma pneumoniae (vaccines for human use);

Mycoplasma hyorhinis (non-avian veterinary vaccines, e.g. strain DBS 1050);

Mycoplasma hyopneumoniae (non-avian veterinary vaccines);

Mycoplasma gallisepticum and *Mycoplasma synoviae* (where avian material has been used during vaccine production or where the vaccine is intended for use in poultry).

Low-passage test strains are used and are stored frozen or freeze-dried. After cloning, the strains are identified as being of the required species by a suitable method.

In some countries, specific tests for insect mycoplasmas are performed.

Incubation conditions

Divide inoculated media into two equal parts; incubate one in aerobic conditions (an atmosphere of air containing 5–10% carbon dioxide and adequate humidity) and the other in anaerobic conditions (an atmosphere of nitrogen containing 5–10% carbon dioxide and adequate humidity).

Demonstration of nutritive properties of media

Inoculate the media with the appropriate test organisms; use no fewer than 200 and no more than 400 colony-forming units per plate of solid medium and no fewer than 20 and no more than 40 colony-forming units per container of the corresponding liquid medium; use a separate plate and container for each species. Incubate the media under the conditions that will be used for testing the product to be examined (aerobically, anaerobically or both, depending on the requirements of the test organism). The media comply with the test for nutritive properties if there is adequate growth of the test organisms (solid media) and an appropriate colour change (liquid media).

Test for inhibitory substances

Carry out the test for nutritive properties in the presence of the product to be examined. If growth of the test organisms is notably less than that found otherwise, the product contains inhibitory substances that must be neutralized (e.g. by dilution) before the test for mycoplasmas is carried out. The effectiveness of the neutralization process

is checked by repeating the test for inhibitory substances after neutralization.

Test for mycoplasmas

For solid media, use plates 60 mm in diameter containing 9 ml of medium. Inoculate each of at least 2 plates of each solid medium with 0.2 ml of the product to be examined and inoculate at least 2 of each 100 ml of liquid medium with 10 ml of the product to be examined. If any significant pH change occurs when the product to be examined is added to the liquid media, restore the affected media to their original pH by the addition of a solution of either sodium hydroxide or hydrochloric acid. Incubate paired media at 35–38 °C, one aerobically and one anaerobically, for 21 days and for the same length of time incubate an uninoculated 100-ml portion of each liquid medium as a control. On the first, second or third day after inoculation, make a subculture of each inoculated liquid medium by inoculating each of two fresh plates of each solid medium with 0.2 ml of the product to be examined and incubating at 35–38 °C, one aerobically and one anaerobically, for no less than 21 days. Repeat the procedure on the sixth, seventh or eighth day and again on the thirteenth or fourteenth day of the test. Inspect the liquid media every 2–3 days, and if any colour change occurs prepare a subculture as described above immediately. Inspect solid media once per week.

If any containers of liquid media show bacterial or fungal contamination, repeat the test. If at least 7 days after inoculation, no more than 1 plate of solid medium at each stage of the test has been contaminated with bacteria or fungi, or broken, that plate may be ignored provided that on immediate examination it shows no evidence of mycoplasmal growth. If, at any stage of the test, more than one plate is contaminated with bacteria or fungi, or broken, the test is invalid and must be repeated.

At the end of each incubation period, microscopically examine inoculated solid media for the presence of mycoplasmas. The product passes the test if growth of mycoplasmas has not occurred in any of the inoculated media. If growth of mycoplasmas has occurred, the test may be repeated once with twice the amount of inoculum and media; if growth of mycoplasmas does not occur when the test is repeated, the product passes the test.

"Indicator cell-culture method

For the indicator cell-culture method, cell cultures are stained with a fluorescent dye that binds specifically to DNA. Mycoplasmas are

detected by their characteristic particulate or filamentous pattern of fluorescence on cell surfaces and, if contamination is heavy, in surrounding areas.

Verification of the substrate

With a Vero-cell substrate, pretest the procedure with an inoculum of no more than 100 colony-forming units (CFU) of a strain growing readily in liquid or solid media and demonstrate its ability to detect potential mycoplasma contaminants such as *M. hyorhinis* strain DBS 1050 and *M. orale* strain 1596 or other suitable strains. A different cell substrate may be used, e.g. the vaccine-production cell line, if it has been demonstrated that it provides at least equal sensitivity for the detection of mycoplasma contamination.

Test for mycoplasmas

Take no less than 1 ml of the material to be examined and use it to inoculate 2 or more indicator cell cultures grown to 50% confluence and representing no less than 25 cm^2 of cell culture in area; make at least one passage. The product of the last passage is incubated on coverslips placed in suitable containers or on some other suitable surface for the test procedure.

Include in the test a negative (non-inoculated) control and two positive controls inoculated with species such as *M. hyorhinis* and *M. orale*. Use an inoculum of not more than 100 CFU for the positive controls.

If for viral suspensions the interpretation of results is obscured by cytopathic effects, the virus may be neutralized by means of a specific antiserum that has no inhibitory effects on mycoplasmas. A cell culture substrate that does not allow growth of the virus may also be used.

Procedure

(a) Seed the culture at a regular density (2×10^4 to 2×10^5 cells per ml, $4 \times 10^3 - 2.5 \times 10^4$ cells/cm^2) and incubate at 36 ± 1 °C until about 50% confluence is reached. Inoculate the product to be examined and incubate to confluence.
(b) Remove and discard the medium.
(c) Rinse the monolayer with phosphate-buffered saline at pH 7.4, then with a mixture of equal volumes of phosphate-buffered saline at pH 7.4 and methanol, and finally with methanol.
(d) Add methanol and allow to stand for 10 minutes.
(e) Remove the methanol and discard.

(f) If the monolayer is to be stained later, dry it completely.
(g) If the monolayer is to be stained immediately, rinse it with sterile water and discard the wash.
(h) Add working solution of bisbenzimide or some other suitable DNA staining agent and allow to stand for 10 min.
(i) Remove the stain and rinse the monolayer with sterile water.
(j) Mount each coverslip with a drop of a mixture of equal volumes of glycerol and phosphate–citrate buffer solution at pH 5.5; remove excess mountant from the edge of the coverslip by blotting.
(k) Examine by epifluorescence (330 nm/380 nm excitation filter, LP 440 nm barrier filter) at 100–400× magnification or greater.
(l) Compare the microscopic appearance of the test cultures with that of the negative and positive controls.

The product to be examined passes the test if there is no evidence of the presence of mycoplasmas in the test cultures inoculated with it.

Reagents

Bisbenzimide ($C_{25}H_{27}Cl_3N_6O \cdot 5H_2O$ (M_r 624)) 4-[5-[5-(4-methylpiperazin-1-yl)benzimidazol-2-yl]benzimidazol-2-yl]phenol trihydrochloride pentahydrate.

Bisbenzimide stock solution: To prepare stock solution, dissolve 5 mg of bisbenzimide in sterile water and dilute to 100 ml with the same solvent. Store in the dark.

Bisbenzimide working solution: To prepare working solution, dilute 10 µl of bisbenzimide stock solution to 100 ml with phosphate-buffered saline at pH 7.4. Use immediately.

Phosphate–citrate buffer solution, pH 5.5: To prepare buffer, mix 56.85 ml of a 2.84% (28.4 g/l) solution of anhydrous disodium hydrogen phosphate and 43.15 ml of a 2.1% (21 g/l) solution of citric acid."

Acknowledgements

Acknowledgements are due to the following experts for their comments and advice on the draft Requirements:

Dr A. F. Angelo, Mycoplasma Laboratory for the Control of Biological Products, National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands; Dr J. Borvendez, Deputy Director-General, National Institute of Pharmacy, Budapest, Hungary; Dr. I. R. Clement, Director, Regulatory Affairs, Connaught Laboratories, Ontario, Canada; Dr I. D. Gust, Director, Research and Development, CSL Ltd, Victoria, Australia; Dr K. J. Healy, Head, Quality Assurance, CSL Ltd, Victoria, Australia; Professor N. V. Medunitsin, Director, Tarasevich State Institute for Standardization and Control of Medical Biological Preparations, Moscow, Russian Federation; Dr R. Pandjaitan, Director, National

Quality Control Laboratory of Drug and Food, Jakarta, Indonesia; Dr L. Palkonyay, Chief, Viral Products Division, Bureau of Biologics, Drugs Directorate, Ottawa, Ontario, Canada.

Reference

General Requirements for the Sterility of Biological Substances (Requirements for Biological Substances No. 6, revised 1973). In: *WHO Expert Committee on Biological Standardization. Twenty-fifth Report.* Geneva, World Health Organization, 1973, Annex 4 (WHO Technical Report Series, No. 530).

⟨1041⟩ BIOLOGICS

Products such as antitoxins, antivenins, blood, blood derivatives, immune serums, immunologic diagnostic aids, toxoids, vaccines, and related articles that are produced under license in accordance with the terms of the federal Public Health Service Act (58 Stat. 682) approved July 1, 1944, as amended, have long been known as "biologics." However, in Table III, Part F, of the Act, the term "biological products" is applied to the group of licensed products as a whole. For Pharmacopeial purposes, the term "biologics" refers to those products that must be licensed under the Act and comply with Food and Drug Regulations—Code of Federal Regulations, Title 21 Parts 600–680, pertaining to federal control of these products (other than certain diagnostic aids), as administered by the Center for Biologics Evaluation and Research or, in the case of the relevant diagnostic aids, by the Center for Devices and Radiological Health of the federal Food and Drug Administration.

Each lot of a licensed biologic is approved for distribution when it has been determined that the lot meets the specific control requirements for that product as set forth by the Office. Licensing includes approval of a specific series of production steps and in-process control tests as well as end-product specifications that must be met on a lot-by-lot basis. These can be altered only upon approval by the Center for Biologics Evaluation and Research and with the support of appropriate data demonstrating that the change will yield a final product having equal or superior safety, purity, potency, and efficacy. No lot of any licensed biological product is to be distributed by the manufacturer prior to the completion of the specified tests. Provisions generally applicable to biologic products include tests for potency, general safety, sterility, purity, water (residual moisture), pyrogens, identity, and constituent materials (Sections 610.10 to 610.15 and see ⟨88⟩ *Safety Tests-General* under *Biological Reactivity Tests, In vivo*, ⟨71⟩ *Sterility Tests*, ⟨921⟩ *Water Determination*, and ⟨151⟩ *Pyrogen Test*, as well as ⟨157⟩ *Bacterial Endotoxins Test*). Constituent materials include ingredients, preservatives, diluents and adjuvants (which generally should meet compendial standards), extraneous protein in cell-culture produced vaccines (which, if other than serum-originating, is excluded) and antibiotics other than penicillin added to the production substrate of viral vaccines (for which compendial monographs on antibiotics and antibiotic substances are available). Additional specific safety tests are also required to be performed on live vaccines and certain other items. Where standard preparations are made available by the Center for Biologics Evaluation and Research (Section 610.20), such preparations are specified for comparison in potency or virulence testing. The U.S. Opacity Standard is used in estimating the bacterial concentration of certain bacterial vaccines and/or evaluating challenge cultures used in tests of them. (See also *Units of Potency* in the *General Notices*.)

The Pharmacopeial monographs conform to the Food and Drug Regulations in covering those aspects of identity, quality, purity, potency, and packaging and storage that are of particular interest to pharmacists and physicians responsible for the purchase, storage, and use of biologics. Revisions of the federal requirements affecting the USP monographs will be made the subjects of *USP Supplements* as promptly as practicable.

Vehicles and Added Substances—Vehicles and added substances suitable for biologics are those named in the Food and Drug Regulations.

Containers for Injections—Containers for biologics intended to be administered by injection meet the requirements for *Containers for Injections* under ⟨1⟩ *Injections*.

Volume in Container—The volumes in containers of biologics intended to be administered by injection meet the requirements for *Volume in Container* under ⟨1⟩ *Injections*.

Labeling—Biologics intended to be administered by injection comply with the requirements for *Labeling* under ⟨1⟩ *Injections*. In addition, the label on the final container for each biologic states: the title or proper name (the name under which the product is licensed under the Public Health Service Act); the name, address, and license number of the manufacturer; the lot number; the expiration date; and the recommended individual dose for multiple-dose containers. The package label includes all of the above, with the addition of: the preservative used and its amount; the number of containers, if more than one; the amount of product in the container; the recommended storage temperature; a statement, if necessary, that freezing is to be avoided; and such other information as the Food and Drug regulations may require.

Packaging and Storage—The labeling gives the recommended storage temperature (see *General Notices*). Precautions should be taken where products labeled to be stored at a temperature between 2° and 8° are stored in a refrigerator, in order to assure that they will not be frozen. Diluents packaged with biologics should not be frozen. Some products (as defined in Section 600.15) are to be maintained during shipment at specified temperatures.

Expiration Date—For compendial articles the expiration date identifies the time during which the article may be expected to meet the requirements of the Pharmacopeial monograph, provided it is kept under the prescribed storage conditions. This date limits the time during which the product may be dispensed or used (see *General Notices*, page 1). However, for biological products the stated date on each lot determines the dating period, which begins on the date of manufacture (Section 610.50) and beyond which the product cannot be expected beyond reasonable doubt to yield its specific results and to retain the required safety, purity, and potency (Section 300.3 (1) and (m)). Such a dating period may comprise an in-house storage period during which it is permitted to be held under prescribed conditions in the manufacturer's storage, followed by a period after issue therefrom. The individual monographs usually indicate both, the latter period and (in parentheses) the permissible in-house storage period. If the product is held for a longer period in the manufacturer's storage than that indicated (in parentheses), the expiration date is set so as to reduce the dating period after issue from the manufacturer's storage by a corresponding amount.

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PART 610—GENERAL BIOLOGICAL PRODUCTS STANDARDS

Subpart A—Release Requirements

Sec.
610.1 Tests prior to release required for each lot.
610.2 Requests for samples and protocols; official release.

Subpart B—General Provisions

610.9 Equivalent methods and processes.
610.10 Potency.
610.11 General safety.
610.11a Inactivated influenza vaccine, general safety test.
610.12 Sterility.
610.13 Purity.
610.14 Identity.
610.15 Constituent materials
610.16 Total solids in serums.
610.17 Permissible combinations.
610.18 Cultures.
610.19 Status of specific products; Group A streptococcus.

Subpart C—Standard Preparations and Limits of Potency

610.20 Standard preparations.
610.21 Limits of potency.

Subpart D—Mycoplasma

610.30 Test for *Mycoplasma*.

Subpart E—Hepatitis Requirements

610.40 Test for hepatitis B surface antigen.
610.41 History of hepatitis B surface antigen.
610.45 Human Immunodeficiency Virus (HIV) requirements.

Subpart F—Dating Period Limitations

610.50 Date of manufacture.
610.53 Dating periods for licensed biological products.

Subpart G—Labeling Standards

610.60 Container label.
610.61 Package label.
610.62 Proper name; package label; legible type.
610.63 Divided manufacturing responsibility to be shown.
610.64 Name of selling agent or distributor.
610.65 Products for export.

AUTHORITY: Secs. 201, 501, 502, 503, 505, 510, 701 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 351, 352, 353, 355, 360, 371); secs. 215, 351, 352, 353, 361 of the Public Health Service Act (42 U.S.C. 216, 262, 263, 263a, 264).

SOURCE: 38 FR 32056, Nov. 20, 1973, unless otherwise noted.

CROSS REFERENCES: For U.S. Customs Service regulations relating to viruses, serums, and toxins, see 19 CFR 12.21—12.23. For U.S. Postal Service regulations relating to the admissibility to the United States mails see parts 124 and 125 of the Domestic Mail Manual, that is incorporated by reference in 39 CFR part 111.

Subpart A—Release Requirements

§610.1 Tests prior to release required for each lot.

No lot of any licensed product shall be released by the manufacturer prior to the completion of tests for conformity with standards applicable to such product. Each applicable test shall be made on each lot after completion of all processes of manufacture which may affect compliance with the standard to which the test applies. The results of all tests performed shall be

considered in determining whether or not the test results meet the test objective, except that a test result may be disregarded when it is established that the test is invalid due to causes unrelated to the product.

§610.2 Requests for samples and protocols; official release.

(a) *General.* Samples of any lot of any licensed product, except for radioactive biological products, together with the protocols showing results of applicable tests, may at any time be required to be sent to the Director, Center for Biologics Evaluation and Research. Upon notification by the Director, Center for Biologics Evaluation and Research, a manufacturer shall not distribute a lot of a product until the lot is released by the Director, Center for Biologics Evaluation and Research: *Provided*, That the Director, Center for Biologics Evaluation and Research, shall not issue such notification except when deemed necessary for the safety, purity, or potency of the product.

(b) *Radioactive biological products.* Samples of any lot of a radioactive biological product, as defined in §600.3(ee) of this chapter, together with the protocols showing results of applicable tests, may at any time be required to be sent to the Food and Drug Administration for official release. Upon notification by the Director, Center for Drug Evaluation and Research, a manufacturer shall not distribute a lot of a radioactive biological product until the lot is released by the Director, Center for Drug Evaluation and Research: *Provided*, That the Director, Center for Drug Evaluation and Research shall not issue such notification except when deemed necessary for the safety, purity, or potency of the product.

(Information collection requirements approved by the Office of Management and Budget under control number 0910-0206)

[40 FR 31313, July 25, 1975, as amended by 49 FR 23834, June 8, 1984; 50 FR 10941, Mar. 19, 1985; 55 FR 11013 and 11014, Mar. 26, 1990]

Subpart B—General Provisions

§610.9 Equivalent methods and processes.

Modification of any particular test method or manufacturing process or the conditions under which it is conducted as required in this part or in the additional standards for specific biological products in parts 620 through 680 of this chapter shall be permitted only under the following conditions:

(a) The manufacturer presents evidence, in the form of a product license supplement, demonstrating that the modification will provide assurances of the safety, purity, potency, and effectiveness of the biological product equal to or greater than the assurances provided by the method or process specified in the general standards or additional standards for the biological product; and

(b) Approval of the modification is received in writing from the Director, Center for Biologics Evaluation and Research (HFB-1), 8800 Rockville Pike, Bethesda, MD 20892.

[49 FR 15187, Apr. 18, 1984; 49 FR 21317, May 21, 1984, as amended at 51 FR 15607, Apr. 25, 1986; 55 FR 11014, Mar. 26, 1990; 59 FR 49351, Sept. 28, 1994]

§610.10 Potency.

Tests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in §600.3(s) of this chapter.

§610.11 General safety.

A general safety test for the detection of extraneous toxic contaminants shall be performed on biological products intended for administration to humans. The general safety test is required in addition to other specific tests prescribed in the additional standards for individual products in this subchapter, except that, the test need not be performed on those products listed in paragraph (g) of this section. The general safety test shall be performed as specified in this section, unless: Modification is prescribed in the additional standards for specific products, or variation is approved as a supplement to the product license under §610.9.

(a) *Product to be tested.* The general safety test shall be conducted upon a representative sample of the product in

the final container from every final filling of each lot of the product. If any product is processed further after filling, such as by freeze-drying, sterilization, or heat treatment, the test shall be conducted upon a sample from each filling of each drying chamber run, sterilization chamber, or heat treatment bath.

(b) *Test animals.* Only overtly healthy guinea pigs weighing less than 400 grams each and mice weighing less than 22 grams each shall be used. The animals shall not have been used previously for any test purpose.

(c) *Procedure.* The duration of the general safety test shall be 7 days for both species, except that a longer period may be established for specific products in accordance with §610.9. Once the manufacturer has established a specific duration of the test period for a specific product, it cannot be varied subsequently, except, in accordance with §610.9. Each test animal shall be weighed and the individual weights recorded immediately prior to injection and on the last day of the test. Each animal shall be observed every working day. Any animal response including any which is not specific for or expected from the product and which may indicate a difference in its quality shall be recorded on the day such response is observed. The test product shall be administered as follows:

(1) *Liquid product or freeze-dried product which has been reconstituted as directed on the label.* Inject intraperitoneally 0.5 milliliter of the liquid product or the reconstituted product into each of at least two mice, and 5.0 milliliters of the liquid product or the reconstituted product into each of at least two guinea pigs.

(2) *Freeze-dried product for which the volume of reconstitution is not indicated on the label.* The route of administration, test dose, and diluent shall be as approved by the Director, Center for Biologics Evaluation and Research, in accordance with §610.9. Administer the test product as approved on at least two mice and at least two guinea pigs.

(3) *Nonliquid products other than freeze-dried product.* The route of administration, test dose, and diluent shall be as approved by the Director, Center for Biologics Evaluation and Research, in accordance with §610.9. Dissolve or grind and suspend the product in the approved diluent. Administer the test product as approved on at least two mice and at least two guinea pigs.

(d) *Test requirements.* A safety test is satisfactory if all animals meet all of the following requirements:

(1) They survive the test period.

(2) They do not exhibit any response which is not specific for or expected from the product and which may indicate a difference in its quality.

(3) They weigh no less at the end of the test period than at the time of injection.

(e) *Repeat tests*—(1) *First repeat test.* If a filling fails to meet the requirements of paragraph (d) of this section in the initial test, a repeat test may be conducted on the species which failed the initial test, as prescribed in paragraph (c) of this section. The filling is satisfactory only if each retest animal meets the requirements prescribed in paragraph (d) of this section.

(2) *Second repeat test.* If a filling fails to meet the requirements of the first repeat test, a second repeat test may be conducted on the species which failed the test: *Provided,* That 50 percent of the total number of animals in that species has survived the initial and first repeat tests. The second repeat test shall be conducted as prescribed in paragraph (c) of this section, except that the number of animals shall be twice that used in the first repeat test. The filling is satisfactory only if each second repeat test animal meets the requirements prescribed in paragraph (d) of this section.

(f) [Reserved]

(g) *Exceptions.* The test prescribed in this section need not be performed for Whole Blood, Red Blood Cells, Cryoprecipitated AHF, Platelets, or Plasma.

[41 FR 10891, Mar. 15, 1976, as amended at 49 FR 15187, Apr. 18, 1984; 49 FR 23834, June 8, 1984; 50 FR 4133, Jan. 29, 1985; 51 FR 15607, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990; 59 FR 49351, Sept. 28, 1994]

§610.11a Inactivated influenza vaccine, general safety test.

For inactivated influenza vaccine, the general safety test shall be conducted in the manner indicated in §610.11 of this chapter except that, with reference to guinea pigs, the test shall be satisfied if the product provides satisfactory results using either the subcutaneous or intraperitoneal injection of 5.0 milliliters of inactivated influenza vaccine into each guinea pig. The requirements for general safety for inactivated influenza vaccine shall not be considered to be satisfied unless each lot of influenza vaccine is assayed for endotoxin in comparison to a reference preparation provided by the Food and Drug Administration, and such lot is found to contain no more endotoxin than the reference preparation.

[39 FR 40016, Nov. 13, 1974]

§610.12 Sterility.

Except as provided in paragraphs (f) and (g) of this section, the sterility of each lot of each product shall be demonstrated by the performance of the tests prescribed in paragraphs (a) and (b) of this section for both bulk and final container material.

(a) *The test.* Bulk material shall be tested separately from final container material and material from each final container shall be tested in individual test vessels as follows:

(1) *Using Fluid Thioglycollate Medium*—(i) *Bulk and final container material.* The volume of product, as required by paragraph (d) of this section (hereinafter referred to also as the "inoculum"), from samples of both bulk and final container material, shall be inoculated into test vessels of Fluid Thioglycollate Medium. The inoculum and medium shall be mixed thoroughly and incubated at a temperature of 30 to 35 °C for a test period of no less than 14 days and examined visually for evidence of growth on the third, fourth, or fifth day, and on the seventh or eighth day, and on the last day of the test period. Results of each examination shall be recorded. If the inoculum renders the medium turbid so that the absence of growth cannot be determined reliably by visual examination, portions of this turbid medium in amounts of no less than 1.0 milliliter shall be transferred on the third, fourth, or fifth day of incubation, from each of the test vessels and inoculated into additional vessels of the medium. The material in the additional vessels shall be incubated at a temperature of 30 to 35 °C for no less than 14 days. Notwithstanding such transfer of material, examination of the original vessels shall be continued as prescribed above. The additional test vessels shall be examined visually for evidence of growth on the third, fourth, or fifth day of incubation, and on the seventh or eighth day, and on the last day of the incubation period. If growth appears, repeat tests may be performed as prescribed in paragraph (b) of this section and interpreted as specified in paragraph (c) of this section.

(ii) *Final container material containing a mercurial preservative.* In addition to the test prescribed in paragraph (a)(1)(i) of this section, final container material containing a mercurial preservative shall be tested using Fluid Thioglycollate Medium following the procedures prescribed in such subparagraph, except that the incubation shall be at a temperature of 20° to 25° C.

(2) *Using Soybean-Casein Digest Medium.* Except for products containing a mercurial preservative, a test shall be made on final container material, following the procedures prescribed in paragraph (a)(1)(i) of this section, except that the medium shall be Soybean-Casein Digest Medium and the incubation shall be at a temperature of 20° to 25° C.

(b) *Repeat tests.* If growth appears in any of the test media during testing of either bulk or final container material, the test may be repeated to rule out faulty test procedures as follows:

(1) *Repeat bulk test.* Only one repeat bulk test may be conducted. The volume of inoculum to be used for the repeat bulk test shall be as prescribed in paragraph (d)(1) of this section. The repeat test shall be performed using the procedure prescribed in paragraph (a)(1)(i) of this section.

(2) *First repeat final container test.* The number of test samples and the volumes of product used for the first repeat test shall be as prescribed in paragraph (d)(2) of this section. For products that do not contain a mercurial preservative, the repeat test shall be performed, using both Fluid Thioglycollate Medium and Soybean-

Casein Digest Medium, following the procedures prescribed in paragraphs (a)(1)(i) and (a)(2), respectively, of this section. If the product contains a mercurial preservative, the repeat test shall be performed using Fluid Thioglycollate Medium and the procedures prescribed in paragraphs (a)(1) (i) and (ii) of this section.

(3) *Second repeat final container test.* If growth appears in any of the first repeat final container tests, all tests of the first repeat final container test shall be repeated, provided there was no evidence of growth in any test of the bulk material. The test samples used for the second repeat final container test shall be twice the number used for the first repeat final container test.

(c) *Interpretation of test results.* The results of all tests performed on a lot shall be considered in determining whether or not the lot meets the requirements for sterility, except that tests may be excluded when demonstrated by adequate controls to be invalid. The lot meets the test requirements if no growth appears in the tests prescribed in paragraph (a) of this section. If repeat tests are performed, the lot meets the test requirements if no growth appears in the tests prescribed in paragraph (b)(2) or (3) of this section, whichever is applicable.

(d) *Test samples and volumes*—(1) *Bulk.* Each sample for the bulk sterility test shall be representative of the bulk material and the volume tested shall be no less than 10 ml. (Note exceptions in paragraph (g) of this section.)

(2) *Final containers.* The sample used for each test medium or each incubation temperature of a test medium for the final container and first repeat final container test shall be no less than 20 final containers from each filling of each lot, selected to represent all stages of filling from the bulk vessel. If the amount of material in the final container is 1.0 milliliter or less, the entire contents shall be tested. If the amount of material in the final container is more than 1.0 milliliter, the volume tested shall be the largest single dose recommended by the manufacturer or 1.0 milliliter, whichever is larger, but no more than 10 milliliters of material or the entire contents from a single final container need be tested. If more than 2 filling machines, each with either single or multiple filling stations, are used for filling one lot, no less than 10 filled containers shall be tested from each filling machine for each test medium or each incubation temperature condition, but no more than 100 containers of each lot need be tested. The items tested shall be representative of each filling assembly and shall be selected to represent all stages of the filling operation. (Note exceptions in paragraph (g) of this section.)

(e) *Culture medium*—(1) *Formulae.* (i) The formula for Fluid Thioglycollate Medium is as follows:

FLUID THIOGLYCOLLATE MEDIUM

1-cystine	0.5 Gm.
Sodium chloride	2.5 Gm.
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	5.5 Gm.
Granular agar (less than 15% moisture by weight).	0.75 Gm.
Yeast extract (water-soluble)	5.0 Gm.
Pancreatic digest of casein	15.0 Gm.
Purified water	1,000.0 ml.
Sodium thioglycollate (or thioglycolic acid—0.3 ml).	0.5 Gm.
Resazurin (0.10% solution, 1.0 ml. freshly prepared).	
pH after sterilization 7.1±0.2.	

(ii) The formula for Soybean-Casein Digest Medium is as follows:

SOYBEAN-CASEIN DIGEST MEDIUM

Pancreatic Digest of Casein	17.0 Gm.
Papaic Digest of Soybean Meal	3.0 Gm.
Sodium Chloride	5.0 Gm.
Dibasic Potassium Phosphate	2.5 Gm.
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	2.5 Gm.
Purified water	1,000.0 ml.
pH after sterilization 7.3±0.2.	

(2) *Culture media requirements*—(i) *Definition of a lot of culture medium and test requirements.* A lot of culture medium is that quantity of uniform material identified as having been thoroughly mixed in a single vessel, dispensed into a group of vessels of the same composition and design, sterilized in a single autoclave run, and identified in a manner to distinguish one lot from another. Each lot of culture medium shall be tested for its growth-promoting qualities unless it meets the exception for dehydrated culture medium described in this subpart. The growth-promoting quality test shall be performed on the smallest sized vessel used in an autoclave run. When using a

single batch of dehydrated culture medium, a manufacturer need not perform growth-promoting tests on each lot of prepared liquid medium, provided that a validation program exists for autoclaves used to sterilize the culture medium, and the manufacturer has received approval for this practice from the Director, Center for Biologics Evaluation and Research.

(ii) *Test organisms, strains, characteristics, identity, and verification.* Two or more strains of microorganisms that are exacting in their nutritive and aerobic/anaerobic requirements shall be used to test the growth-promoting qualities of each lot of test medium. When using Fluid Thioglycollate medium, both an aerobic and an anaerobic test microorganism shall be chosen. When using Soybean Casein Digest Medium, the yeast, *Candida albicans*, shall be one of the two test microorganisms chosen. Manufacturers shall choose the strains of microorganisms from the chart in this paragraph.

Medium	Test microorganisms	Incubation temperature
	Spore-formers	
Fluid Thioglycollate	1. *Bacillus subtilis* (ATCC No. 6633)	30 to 35 °C.
	2. *Clostridium sporogenes* (ATCC No. 11437)	Do.
	Non-spore-formers	
	3. *Candida albicans* (ATCC No. 10231)	Do.
	4. *Micrococcus luteus* (ATCC No. 9341)	Do.
	5. *Bacteroides vulgatus* (ATCC No. 8482)	Do.
	Spore-formers	
Soybean-Casein Digest	1. *Bacillus subtilis* (ATCC No. 6633)	20 to 25 °C.
	Non-spore-formers	
	2. *Candida albicans* (ATCC No. 10231)	Do.
	3. *Micrococcus luteus* (ATCC No. 9341)	Do.

ATCC strains of microorganisms described in this section are available from the American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852. Periodic tests shall be performed to verify the integrity of the test organisms in accordance with §610.18 (a) and (b). The results of these periodic tests shall be recorded and retained in accordance with §600.12(b) of this chapter.

(iii) *Storage and maintenance of cultures of test organisms.* Cultures of the test organisms used to determine the growth-promoting qualities of the medium shall be stored in a manner that will prevent cross contamination or loss of identity, at a temperatre and by a method that will retain the initial characteristics of the organisms and ensure freedom from contamination and deterioration. If the test organisms are stored in the freeze-dried state, or frozen, they shall be reconstituted or thawed, whichever is applicable, and plated periodically to verify the colony count of the suspension. If the test suspensions are stored in a state other than freeze-dried or frozen, they shall be plated, and a colony count shall be performed at the time of each growth-promoting quality test to assure that not more than 100 organisms are used per test vessel. The results of tests for verification of the colony count shall be recorded and retained in accordance with §600.12(b) of this chapter.

(iv) *Storage and condition of media.* A medium shall not be used if the extent of evaporation affects its fluidity, nor shall it be reused in a sterility test of the product. Fluid Thioglycollate Medium shall be stored in the dark at room temperature if the vessels are unsealed. Sealed vessels shall be stored at the manufacturer's specified storage temperature.

Fluid Thioglycollate Medium shall not be used if more than the upper one-third of the medium has acquired a pink color. The medium may be restored once by heating on a steam bath or in free-flowing steam until the pink color disappears. The design of the test vessel for Fluid Thioglycollate Medium shall provide favorable aerobic and anaerobic conditions for growth of the microorganisms throughout the test

period. Soybean-Casein Digest Medium shall be stored in the dark at 20 to 25 °C. Unsealed vessels of either medium may be stored for more than 10 days at the proper temperature, provided they are tested monthly for growth-promotion and found to be satisfactory. Sealed vessels of either medium may be stored at the proper temperature for a period of time not to exceed 1 year, provided they are tested for growth-promotion every 3 months and found to be satisfactory. The results of such testing shall be recorded and retained in accordance with §600.12(b) of this chapter.

(v) *Criteria for a satisfactory growth-promoting quality test.* (*a*) One hundred or fewer organisms of each strain tested shall be used. The test is satisfactory if evidence of growth appears within 7 days in all vessels inoculated. If a lot of medium fails to support the growth of any test organism, or if the test results show that more than 100 organisms of a strain were used or are necessary to promote growth in the lot of medium being tested, or if the growth is not a pure culture of the test organism, a second test may be performed. If it fails the second test, the lot of medium shall be rejected.

(*b*) Inoculated Fluid Thioglycollate Medium shall be incubated at 30 to 35 °C for 7 days. If the test medium is to be used in determining the sterility of a product containing a mercurial preservative, a second test shall be performed in accordance with paragraph (e)(2)(v)(*a*) of this section, except that the test shall be incubated at 20 to 25 °C for 7 days. Inoculated Soybean-Casein Digest Medium shall be incubated at 20 to 25 °C for 7 days. The sterility of each lot of medium shall be confirmed by the incubation of uninoculated control test vessels for 7 days at the temperature(s) for that particular medium. The lot of medium is satisfactory if no growth is observed in the control test vessels within the incubation period. The tests for growth-promoting qualities of culture media may be performed simultaneously with sterility testing of biological products, provided the sterility test is considered invalid if the test medium shows no growth response.

(vi) *Volume of culture medium.* The volume of each culture medium shall be determined for each bulk and final container sterility test required for each product. The ratio of the volume of inoculum to the volume of culture medium shall result in a dilution of the product that is not bacteriostatic or fungistatic, except for products to be tested by membrane filtration. The volume of inhibitors or neutralizers of preservatives added should be considered in determining the proper ratio of inoculum/medium. Vessels of the product-medium mixture(s) and control vessels of the medium shall be inoculated with dilutions of cultures of bacteria or fungi which are viable in the product being tested, and incubated at the appropriate temperature for no less than 7 days.

(f) *Membrane filtration.* Bulk and final container material or products containing oil products in water-insoluble ointments may be tested for sterility using the membrane filtration procedure set forth in the United States Pharmacopeia (21st Revision, 1985), section entitled "Test Procedures Using Membrane Filtration," p. 1159, which is incorporated by reference (copies are available from the United States Pharmacopeial Convention Inc., 12601 Twinbrook Parkway, Rockville, MD 20852, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC 20408), except that (1) the test samples shall conform with paragraph (d) of this section; and (2) in addition, for products containing a mercurial preservative, the product shall be tested in a second test using Fluid Thioglycollate Medium incubated at 20 to 25 °C in lieu of the test in Soybean-Casein Digest Medium.

(g) *Exceptions.* Bulk and final container material shall be tested for sterility as described above in this section, except as follows:

(1) *Different sterility tests prescribed.* When different sterility tests are prescribed for a product in this subchapter.

(2) *Alternate incubation temperatures.* Two tests may be performed as prescribed in paragraph (a)(1)(i) of this section, one test using an incubation temperature of 18 to 22 °C, the other test using an incubation temperature of 30 to 37 °C, in lieu of performing one test using an incubation temperature of 30 to 35 °C, provided that growth-promoting quality tests have been performed at these temperatures.

(3) [Reserved]

(4) *Test precluded or not required.* (i) The tests prescribed in this section need not be performed for Whole Blood, Cryoprecipitated AHF, Platelets, Red Blood Cells, Plasma, Source Plasma, Smallpox Vaccine, Reagent Red Blood Cells, Anti-Human Globulin, or Blood Grouping Reagent.

(ii) Where a manufacturer submits data which the Director, Center for Biologics Evaluation and Research, finds adequate to establish that the mode of administration, the method of preparation, or the special nature of the product precludes or does not require a sterility test or that the sterility of the lot is not necessary to assure the safety, purity, and potency of the product, the Director may exempt a product from the sterility requirements of this section subject to any conditions necessary to assure the safety, purity, and potency of the product.

(5) *Number of final containers more than 20, less than 200.* If the number of final containers in the filling is more than 20 or less than 200, the sample shall be no less than 10 percent of the containers.

(6) *Number of final containers—20 or less.* If the number of final containers in a filling is 20 or less, the sample shall be two final containers, or the sample need be no more than one final container, provided (i) the bulk material met the sterility test requirements and (ii) after filling, it is demonstrated by testing a simulated sample that all surfaces to which the product was exposed were free of contaminating microorganisms. The simulated sample shall be prepared by rinsing the filling equipment with sterile 1.0 percent peptone solution, pH 7.1±0.1, which shall be discharged into a final container by the same method used for filling the final containers with the product.

(7) *Samples—large volume of product in final containers.* For Albumin (Human) and Plasma Protein Fraction (Human), when the volume of product in the final container is 50 milliliters or more, the final containers selected as the test sample may contain less than the full volume of product in the final containers of the filling from which the sample is taken: *Provided*, That the containers and closures of the sample are identical with those used for the filling to which the test applies, and the sample represents all stages of that filling.

(8) *Diagnostic biological products not intended for injection.* For diagnostic biological products not intended for injection, (i) only the Fluid Thioglycollate Medium test incubated at 30 to 35 °C is required, (ii) the volume of material for the bulk test shall be no less than 2.0 milliliters, and (iii) the sample for the final container test shall be no less than three final containers if the total number filled is 100 or less, and, if greater, one additional container for each additional 50 containers or fraction thereof, but the sample need be no more than 10 containers.

(9) *Immune globulin preparations.* For immune globulin preparations, the test samples from the bulk material and from each final container need be no more than 2.0 ml.

(h) *Records.* The records related to the testing requirements of this section shall be prepared and maintained as required by §§211.167 and 211.194 of this chapter.

(Information collection requirements approved by the Office of Management and Budget under control number 0910-0139)

[38 FR 32056, Nov. 20, 1973, as amended at 41 FR 4015, Jan. 28, 1976; 41 FR 10428, Mar. 11, 1976; 44 FR 11754, Mar. 2, 1979; 49 FR 15187, Apr. 18, 1984; 49 FR 23834, June 8, 1984; 50 FR 4133, Jan. 29, 1985; 51 FR 44906, Dec. 15, 1986; 53 FR 12764, Apr. 19, 1988; 55 FR 11013, Mar. 26, 1990]

§610.13 Purity.

Products shall be free of extraneous material except that which is unavoidable in the manufacturing process described in the approved license. In addition, products shall be tested as provided in paragraphs (a) and (b) of this section.

(a)(1) *Test for residual moisture.* Each lot of dried product shall be tested for residual moisture and shall meet and not exceed established limits as specified by an approved method on file in

the product license application. The test for residual moisture may be exempted by the Director, Center for Biologics Evaluation and Research, when deemed not necessary for the continued safety, purity, and potency of the product.

(2) *Records.* Appropriate records for residual moisture under paragraph (a)(1) of this section shall be prepared and maintained as required by the applicable provisions of §§211.188 and 211.194 of this chapter.

(b) *Test for pyrogenic substances.* Each lot of final containers of any product intended for use by injection shall be tested for pyrogenic substances by intravenous injection into rabbits as provided in paragraphs (b) (1) and (2) of this section: *Provided,* That notwithstanding any other provision of Subchapter F of this chapter, the test for pyrogenic substances is not required for the following products: Products containing formed blood elements; Cryoprecipitate; Plasma; Source Plasma; Normal Horse Serum; bacterial, viral, and rickettsial vaccines and antigens; toxoids; toxins; allergenic extracts; venoms; diagnostic substances and trivalent organic arsenicals.

(1) *Test dose.* The test dose for each rabbit shall be at least 3 milliliters per kilogram of body weight of the rabbit and also shall be at least equivalent proportionately, on a body weight basis, to the maximum single human dose recommended, but need not exceed 10 milliliters per kilogram of body weight of the rabbit, except that: (i) Regardless of the human dose recommended, the test dose per kilogram of body weight of each rabbit shall be at least 1 milliliter for immune globulins derived from human blood; (ii) for Streptokinase, the test dose shall be at least equivalent proportionately, on a body weight basis, to the maximum single human dose recommended.

(2) *Test procedure, results, and interpretation; standards to be met.* The test for pyrogenic substances shall be performed according to the requirements specified in United States Pharmacopeia XX.

(3) *Retest.* If the lot fails to meet the test requirements prescribed in paragraph (b)(2) of this section, the test may be repeated once using five other rabbits. The temperature rises recorded for all eight rabbits used in testing shall be included in determining whether the requirements are met. The lot meets the requirements for absence of pyrogens if not more than three of the eight rabbits show individual rises in temperature of 0.6° C or more, and if the sum of the eight individual maximum temperature rises does not exceed 3.7° C.

(Information collection requirements were approved by the Office of Management and Budget (OMB) and assigned OMB control number 0910-0139)

[38 FR 32056, Nov. 20, 1973, as amended at 40 FR 29710, July 15, 1975; 41 FR 10429, Mar. 11, 1976; 41 FR 41424, Sept. 22, 1976; 44 FR 40289, July 10, 1979; 46 FR 62845, Dec. 29, 1981; 49 FR 15187, Apr. 18, 1984; 50 FR 4134, Jan. 29, 1985; 55 FR 28381, July 11, 1990]

§ 610.14 Identity.

The contents of a final container of each filling of each lot shall be tested for identity after all labeling operations shall have been completed. The identity test shall be specific for each product in a manner that will adequately identify it as the product designated on final container and package labels and circulars, and distinguish it from any other product being processed in the same laboratory. Identity may be established either through the physical or chemical characteristics of the product, inspection by macroscopic or microscopic methods, specific cultural tests, or in vitro or in vivo immunological tests.

§ 610.15 Constituent materials.

(a) *Ingredients, preservatives, diluents, adjuvants.* All ingredients used in a licensed product, and any diluent provided as an aid in the administration of the product, shall meet generally accepted standards of purity and quality. Any preservative used shall be sufficiently nontoxic so that the amount present in the recommended dose of the product will not be toxic to the recipient, and in the combination used it shall not denature the specific substances in the product to result in a decrease below the minimum acceptable potency within the dating period when stored at the recommended temperature. Products in multiple-dose containers shall contain a preservative, except that a preservative need not be added to Yellow Fever Vaccine; Poliovirus Vaccine Live Oral; viral vaccines labeled for use with the jet injector; dried vaccines when the accompanying diluent contains a preservative; or to an Allergenic Product in 50 percent or more volume in volume (v/v) glycerin. An adjuvant shall not be introduced into a product unless there is satisfactory evidence that it does not affect adversely the safety or potency of the product. The amount of aluminum in the recommended individual dose of a biological product shall not exceed:

(1) 0.85 milligrams if determined by assay;

(2) 1.14 milligrams if determined by calculation on the basis of the amount of aluminum compound added; or

(3) 1.25 milligrams determined by assay provided that data demonstrating that the amount of aluminum used is safe and necessary to produce the intended effect are submitted to and approved by the Director, Center for Biologics Evaluation and Research.

(b) *Extraneous protein; cell culture produced vaccines.* Extraneous protein known to be capable of producing allergenic effects in human subjects shall not be added to a final virus medium of cell culture produced vaccines intended for injection. If serum is used at any stage, its calculated concentration in the final medium shall not exceed 1:1,000,000.

(c) *Antibiotics.* A minimum concentration of antibiotics, other than penicillin, may be added to the production substrate of viral vaccines.

[38 FR 32056, Nov. 20, 1973, as amended at 46 FR 51903, Oct. 23, 1981; 48 FR 13025, Mar. 29, 1983; 48 FR 37023, Aug. 16, 1983; 49 FR 23834, June 8, 1984; 50 FR 4134, Jan. 29, 1985; 51 FR 15607, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

§ 610.16 Total solids in serums.

Except as otherwise provided by regulation, no liquid serum or antitoxin shall contain more than 20 percent total solids.

§ 610.17 Permissible combinations.

Licensed products may not be combined with other licensed products either therapeutic, prophylactic or diagnostic, except as a license is obtained for the combined product. Licensed products may not be combined with nonlicensable therapeutic, prophylactic, or diagnostic substances except as a license is obtained for such combination.

§ 610.18 Cultures.

(a) *Storage and maintenance.* Cultures used in the manufacture of products shall be stored in a secure and orderly manner, at a temperature and by a method that will retain the initial characteristics of the organisms and insure freedom from contamination and deterioration.

(b) *Identity and verification.* Each culture shall be clearly identified as to source strain. A complete identification of the strain shall be made for each new stock culture preparation. Primary and subsequent seed lots shall be identified by lot number and date of preparation. Periodic tests shall be performed as often as necessary to verify the integrity of the strain characteristics and freedom from extraneous organisms. Results of all periodic tests for verification of cultures and determination of freedom from extraneous organisms shall be recorded and retained.

(c) *Cell lines used for manufacturing biological products*—(1) *General requirements.* Cell lines used for manufacturing biological products shall be:

(i) Identified by history;

(ii) Described with respect to cytogenetic characteristics and tumorigenicity;

(iii) Characterized with respect to in vitro growth characteristics and life potential; and

(iv) Tested for the presence of detectable microbial agents.

(2) *Tests.* Tests that are necessary to assure the safety, purity, and potency of a product may be required by the Director, Center for Biologics Evaluation and Research.

(3) *Applicability.* This paragraph applies to diploid and nondiploid cell lines. Primary cell cultures that are not subcultivated and primary cell cultures that are subsequently subcultivated for only a very limited number of population doublings are not subject to the provisions of this paragraph (c).

(d) *Records.* The records appropriate for cultures under this section shall be prepared and maintained as required by the applicable provisions of §§ 211.188 and 211.194 of this chapter.

(Approved by the Office of Management and Budget under control number 0910-0139)

[38 FR 32056, Nov. 20, 1973, as amended at 51 FR 44453, Dec. 10, 1986; 55 FR 11013, Mar. 26, 1990]

§ 610.19 Status of specific products; Group A streptococcus.

The presence of Group A streptococcus organisms and derivatives of Group A streptococcus in Bacterial Vaccines and Bacterial Antigens with "No U.S. Standard of Potency" may induce dangerous tissue reactions in humans. Available data demonstrate that they are unsafe as ingredients in products for human use. Group A streptococcus organisms and derivatives of Group A streptococcus are prohibited from Bacterial Vaccines and Bacterial Antigens with "No U.S. Standard of Potency." Any Bacterial Vaccine or Bacterial Antigen with "No U.S. Standard of Potency" containing Group A streptococcus organisms or derivatives of Group A streptococcus in interstate commerce is in violation of section 351 of the Public Health Service Act (42 U.S.C. 262).

[44 FR 1549, Jan. 5, 1979]

Subpart C—Standard Preparations and Limits of Potency

§ 610.20 Standard preparations.

Standard preparations made available by the Center for Biologics Evaluation and Research shall be applied in testing, as follows:

(a) *Potency standards.* Potency standards shall be applied in testing for potency all forms of the following:

ANTIBODIES

Botulism Antitoxin, Type A.
Botulism Antitoxin, Type B.
Botulism Antitoxin, Type E.
Diphtheria Antitoxin.
Histolyticus Antitoxin.
Oedematiens Antitoxin.
Perfringens Antitoxin.
Antipertussis Serum.
Antirabies Serum.
Sordellii Antitoxin.
Staphylococcus Antitoxin.
Tetanus Antitoxin.
Vibrion Septique Antitoxin.

ANTIGENS

Cholera Vaccine, Inaba serotype.
Cholera Vaccine, Ogawa serotype.
Diphtheria Toxin for Schick Test.
Pertussis Vaccine.
Tuberculin, Old.
Tuberculin, Purified Protein Derivative.
Typhoid Vaccine.

BLOOD DERIVATIVE

Thrombin.

(b) *Opacity standard.* The U.S. Opacity Standard shall be applied in estimating the bacterial concentration of all bacterial vaccines. The assigned value of the standard when observed visually is 10 units. The assigned value of the standard when observed with a photometer is (1) 10 units when the wavelength of the filter is 530 millimicrons, (2) 10.6 units when the wavelength of the filter is 650 millimicrons, and (3) 9 units when the wavelength of the filter is 420 millimicrons.

[38 FR 32056, Nov. 20, 1973, as amended at 41 FR 10429, Mar. 11, 1976; 41 FR 18295, May 3, 1976; 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§ 610.21 Limits of potency.

The potency of the following products shall be not less than that set forth below and products dispensed in the dried state shall represent liquid products having the stated limitations.

ANTIBODIES

Diphtheria Antitoxin, 500 units per milliliter.
Tetanus Antitoxin, 400 units per milliliter.
Tetanus Immune Globulin (Human), 50 units of tetanus antitoxin per milliliter.

ANTIGENS

Cholera Vaccine, 8 units each of Inaba and Ogawa serotype antigens per milliliter.
Pertussis Vaccine, 12 units per total human immunizing dose.
Typhoid Vaccine, 8 units per milliliter.

[41 FR 10429, Mar. 11, 1976, as amended at 41 FR 18295, May 3, 1976]

Subpart D—Mycoplasma

§ 610.30 Test for *Mycoplasma*.

Except as provided otherwise in this subchapter, prior to clarification or filtration in the case of live virus vaccines produced from in vitro living cell cultures, and prior to inactivation in the case of inactivated virus vaccines produced from such living cell cultures, each virus harvest pool and control fluid pool shall be tested for the presence of *Mycoplasma*, as follows:

Samples of the virus for this test shall be stored either (1) between 2° and 8° C. for no longer than 24 hours, or (2) at −20° C. or lower if stored for longer than 24 hours. The test shall be performed on samples of the viral harvest pool and on control fluid pool obtained at the time of viral harvest, as follows: No less than 2.0 ml. of each sample shall be inoculated in evenly distributed amounts over the surface of no less than 10 plates of at least two agar media. No less than 1.0 ml. of sample shall be inoculated into each of four tubes containing 10 ml. of a semisolid broth medium. The media shall be such as have been shown to be capable of detecting known *Mycoplasma* and each test shall include control cultures of at least two known strains of *Mycoplasma*, one of which must be *M. pneumoniae*. One half of the plates and two tubes of broth shall be incubated aerobically at 36° C. ±1° C. and the remaining plates and tubes shall be incubated anaerobically at 36° C. ±1° C. in an environment of 5–10 percent CO_2 in N_2. Aerobic incubation shall be for a period of no less than 14 days and the broth in the two tubes shall be tested after 3 days and 14 days, at which times 0.5 ml. of broth from each of the two tubes shall be combined and subinoculated on to no less than 4 additional plates and incubated aerobically. Anaerobic incubation shall be for no less than 14 days and the broth in the two tubes shall be tested after 3 days and 14 days, at which times 0.5 ml. of broth from each of the two tubes shall be combined and subinoculated onto no less than four additional plates and incubated anaerobically. All inoculated plates shall be incubated for no less than 14 days, at which time observation for growth of *Mycoplasma* shall be made at a magnification of no less than 300×. If the Dienes Methylene Blue-Azure dye or an equivalent staining procedure is used, no less than a one square cm. plug of the agar shall be excised from the inoculated area and examined for the presence of *Mycoplasma*. The presence of the *Mycoplasma* shall be determined by comparison of the growth obtained from the test samples with that of the control cultures, with respect to typical colonial and microscopic morphology. The virus pool is satisfactory for vaccine manufacture if none of the tests on the samples show evidence of the presence of *Mycoplasma*.

Subpart E—Hepatitis Requirements

§ 610.40 Test for hepatitis B surface antigen.

(a) *Test sensitivity.* Each donation of blood, plasma, or serum to be used in preparing a biological product shall be tested for the presence of hepatitis B surface antigen by a method of sufficient sensitivity to detect all sera labeled A, (A), B, (B), and C in the Reference Hepatitis B Surface Antigen Panel distributed by the Center for Biologics Evaluation and Research; except that, in emergency situations, a test method of sufficient sensitivity to detect all sera labeled A, (A), and B in the Reference Hepatitis B Surface Antigen Panel may be used and, in dire emergency situations, blood and blood products may be issued without any HB_sAg testing, provided that a test otherwise required by this paragraph is performed as soon as possible after issuance of the blood and blood product.

(b) *Procedures.* Only Antibody to Hepatitis B Surface Antigen licensed under this subchapter shall be used in performing the test and the test method(s) used shall be that for which the antibody product is specifically designed to be effective as recommended by the manufacturer in the package insert. The sample of blood, plasma, or serum to be tested shall have been taken from the donor at the time of donation of that unit. The test need not be performed on the day of the withdrawal of the sample. If the radioimmunoassay method is used, it must be performed in one of the following ways:

(1) The complete test is performed at the collection facility.

(2) The test is performed at the collection facility up to the point of counting the radioactivity of the samples, which counting, thereafter, is performed at another facility by personnel from the collection facility or by personnel from the counting facility.

(3) The complete test is performed by the personnel at an establishment licensed to manufacture blood or blood derivatives under section 351(a) of the

Public Health Service Act (42 U.S.C. 262(a)), or by a clinical laboratory which meets the standards of the Clinical Laboratories Improvement Act of 1967 (CLIA) (42 U.S.C. 263a), provided the establishment or the clinical laboratory is qualified to perform radioimmunoassay testing for the presence of hepatitis B surface antigen.

(4) Except as provided in this paragraph (b)(4), a collection facility shall not ship any blood product as a biological product or ship such a blood product where it is intended for use in manufacturing a biological product until the test for hepatitis B surface antigen is completed and the written test results are received by the collection facility. Notwithstanding the provisions of §610.1 of this chapter, in the case of an emergency, or as otherwise approved in writing by the Director, Center for Biologics Evaluation and Research, a collection facility may ship a blood product before the test for hepatitis B surface antigen is completed. To obtain approval for such shipments, the collection facility shall submit a description of the control procedures to be used by both the collection facility and the manufacturing facility to the Director, Center for Biologics Evaluation and Research (HFB-1), Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892. The control procedures to be used by the collection facility and the manufacturing facility shall include, but may not be limited to, a system of communicating the test results to the manufacturing facility, use of specific labeling warnings for the product to ensure that persons handling the shipment know that it may be infectious, procedures for quarantine of the untested or incompletely tested product both at the collection facility and at the manufacturing facility, and a procedure at the manufacturing facility to identify, preclude use of, and dispose of any blood product that is received and later found to be reactive for hepatitis B surface antigen.

(c) *Materials in storage.* All blood, plasma, or serum in storage which has not been tested for the presence of the hepatitis B surface antigen shall be tested as required in paragraphs (a) and (b) of this section before use as a biological product, or before use in the manufacture of a biological product. All blood, plasma, or serum in storage which has been tested for the presence of the hepatitis B surface antigen by a method of second generation sensitivity may be used as a biological product or in manufacture of a biological product, provided it is used on or before March 15, 1976.

(d) *Restrictions on use.* Blood, plasma, or serum that is reactive when tested for hepatitis B surface antigen or that was collected from a donor known to be reactive for hepatitis B surface antigen shall not be used in manufacturing biological products except as provided in paragraphs (d) (1) and (2) of this section.

(1) *Injectable biological products and licensed in vitro diagnostic biological products.* Blood, plasma, or serum that is reactive when tested for hepatitis B surface antigen or that was collected from a donor known to be reactive for hepatitis B surface antigen may be used in manufacturing hepatitis B vaccine and licensed in vitro diagnostic biological products if all of the following conditions are met:

(i) The final product cannot be prepared from blood, plasma, or serum that is nonreactive when tested for hepatitis B surface antigen, due either to the nature or to the scarcity of the final product.

(ii) The label of the source blood, plasma, or serum conspicuously states either that it is reactive when tested for hepatitis B surface antigen and it may transmit viral hepatitis; or that the source blood, plasma, or serum was collected from a donor known to be reactive for hepatitis B surface antigen and it may transmit viral hepatitis, although confirmatory hepatitis testing has not been done.

(iii) The package label of the licensed in vitro diagnostic biological product prepared from such blood, plasma, or serum states conspicuously that either the product was prepared from source material that was reactive when tested for hepatitis B surface antigen and it may transmit viral hepatitis; or that the source material was collected from a donor known to be reactive for hepatitis B surface antigen and it may transmit viral hepatitis, although confirmatory hepatitis testing has not been done.

(iv) The package label of the licensed injectable biological product prepared from such blood, plasma, or serum states that the product has been inactivated.

(v) The Director, Center for Biologics Evaluation and Research (HFB-1), Food and Drug Administration, 8800 Rockville Pike, Bethesda MD 20892, is notified in writing at the time of the shipment, or in the case of repetitive shipments, or April 1 and October 1 of each year, of each shipment of source blood, plasma, or serum for manufacture into hepatitis B vaccine or into a licensed in vitro diagnostic biological product. Such shipments shall not be subject to the requirements of paragraph (b)(3) of this section. Each notification shall identify the kind and amount of source material shipped, the name and address of the consignee, the date of shipment, and the manner in which the source material is labeled.

(2) *Unlicensed in vitro diagnostic biological products.* Blood, plasma, or serum that is reactive when tested for hepatitis B surface antigen or that was collected from a donor known to be reactive for hepatitis B surface antigen may be used in manufacturing unlicensed in vitro diagnostic biological products including clinical chemistry control reagents if all of the following conditions are met:

(i) The final product cannot be prepared from blood, plasma, or serum that is nonreactive when tested for hepatitis B surface antigen, due either to the nature or to the scarcity of the final product.

(ii) The label of the source blood, plasma, or serum states conspicuously that either it is reactive when tested for hepatitis B surface antigen and it may transmit viral hepatitis; or that the source blood, plasma, or serum was collected from a donor known to be reactive for hepatitis B surface antigen and it may transmit viral hepatitis, although confirmatory hepatitis testing has not been done.

(iii) The manufacturer of the source blood, plasma, or serum obtains written assurance from the manufacturer(s) of the final unlicensed product that package labels of all unlicensed products will conspicuously state, as required by §809.10(a)(4) of this chapter, that the product was prepared from blood, plasma, or serum that was reactive when tested for hepatitis B surface antigen and it may transmit viral hepatitis; or that the source material was collected from a donor known to be reactive for hepatitis B surface antigen and it may transmit viral hepatitis, although confirmatory hepatitis testing has not been done.

(iv) At the time of shipment, the Director, Center for Biologics Evaluation and Research (HFB-1), Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892, is notified in writing of each shipment of source blood, plasma, or serum signifying the kind and the amount of source material shipped, the name and address of the consignee, the date of shipment, and the manner in which such source material was labeled. Such shipments shall not be subject to the requirements of paragraph (b)(3) of this section.

(e) *Manufacturing responsibility.* When the radioimmunoassay method for hepatitis B surface antigen testing is performed by personnel other than those of the facility collecting the blood, plasma, or serum, as provided in paragraph (b) of this section, it shall not be considered as divided manufacturing as described in §610.63, provided the following conditions are met:

(1) The collecting facility has obtained a written agreement that the testing laboratory will permit authorized representatives of the Food and Drug Administration to inspect its testing procedures and facilities during reasonable business hours.

(2) The testing laboratory will participate in any proficiency testing programs undertaken by the Center for Biologics Evaluation and Research, Food and Drug Administration.

(f) The information collection requirements in paragraph (d) of this section were approved by the Office of

Management and Budget and assigned OMB control number 0910-0136.

(Information collection requirements contained in paragraph (b)(4) were approved by the Office of Management and Budget under control number 0910-0168)

[40 FR 29710, July 15, 1975, as amended at 48 FR 23181, May 24, 1983; 49 FR 23834, June 8, 1984; 49 FR 26718, June 29, 1984; 51 FR 15607, Apr. 25, 1986; 55 FR 11013 and 11014, Mar. 26, 1990]

§610.41 History of hepatitis B surface antigen.

A person known to have previously tested positive for hepatitis B surface antigen, testing positive, or both, may not serve as a donor of human blood, plasma, or serum, except that under §640.120 of this chapter, such a donor may serve as a source of hepatitis B surface antigen for the manufacture of hepatitis B vaccine or the preparation of a diagnostic product for laboratory tests, or a person known to have previously tested positive for hepatitis B surface antigen may serve as a source of antibody to hepatitis B surface antigen for the preparation of a biological product or a diagnostic product for laboratory tests.

[48 FR 23182, May 24, 1983, as amended at 57 FR 10814, Mar. 31, 1992]

§610.45 Human Immunodeficiency Virus (HIV) requirements.

(a) *Testing requirements.* (1) Each donation of human blood or blood components intended for use in preparing a product shall be tested for antibody to HIV by a test approved for such use by FDA, except as otherwise approved in writing by FDA. When the test for antibody to HIV is required, blood and blood products may be issued before the results of the test for antibody to HIV are available only in dire emergency situations or as otherwise approved in writing by FDA and, provided the test required by this paragraph is performed as soon as possible after issuance of the blood or blood product.

(2) Tests approved by FDA for the screening of blood and blood components for evidence of HIV may only be used in place of a test for antibody to HIV to satisfy the requirements of this section and related sections if so specified by FDA.

(b) *Testing responsibility.* The test for antibody to HIV shall be performed by the collection facility, by personnel of an establishment licensed to manufacture blood or blood derivatives under section 351(a) of the Public Health Service Act (42 U.S.C. 262(a)), or by a clinical laboratory which meets the standards of the Clinical Laboratory Improvement Act of 1967 (CLIA) (42 U.S.C. 263a), provided the establishment or clinical laboratory is qualified to perform the test.

(c) *Restrictions on use.* (1) Blood, plasma, or other blood components that are repeatably reactive to a test for antibody to HIV or that were collected from a donor whose blood is known to be repeatably reactive to a test for antibody to HIV, shall not be shipped or used to prepare any product, including products not subject to licensure; except that such blood and blood components shall be shipped or used only for purposes and under conditions specifically approved in writing by FDA.

(2) The restrictions on use contained in this paragraph shall not apply in the following cases:

(i) Blood and blood components testing repeatably reactive or from a donor whose blood is known to be repeatably reactive that are shown to be negative for evidence of HIV infection by a method or process approved for such use by FDA;

(ii) The distribution of blood, plasma, or serum samples, except when intended for use in the manufacture of a product;

(iii) The in-house use of blood and blood components for research purposes; or

(iv) The distribution of blood and blood components for research purposes, if not distributed by sale, barter, or exchange.

[53 FR 116, Jan. 5, 1988]

Subpart F—Dating Period Limitations

§610.50 Date of manufacture.

The date of manufacture shall be determined as follows:

(a) For products for which an official standard of potency is prescribed in either §610.20 or §610.21, or which are subject to official potency tests, the date of initiation by the manufacturer of the last valid potency test.

(b) For products that are not subject to official potency tests, (1) the date of removal from animals, (2) the date of extraction, (3) the date of solution, (4) the date of cessation of growth, or (5) the date of final sterile filtration of a bulk solution, whichever is applicable.

[38 FR 32056, Nov. 20, 1973, as amended at 42 FR 27582, May 31, 1977]

§610.53 Dating periods for licensed biological products.

(a) *General.* The minimum dating periods in paragraph (c) of this section are based on data relating to usage, clinical experience, or laboratory tests that establish the reasonable period beyond which the product cannot be expected to yield its specific results and retain its safety, purity, and potency, provided the product is maintained at the recommended temperatures. The standards prescribed by the regulations in this subchapter are designed to ensure the continued safety, purity, and potency of the products and are based on the dating periods set forth in paragraph (c) of this section. Package labels for each product shall recommend storage at the stated temperatures.

(b) *When the dating period begins.* The dating period for a product shall begin on the date of manufacture, as prescribed in §610.50. The dating period for a combination of two or more products shall be no longer than the dating period of the component with the shortest dating period.

(c) *Table of dating periods.* In using the table in this paragraph, a product in column A may be stored by the manufacturer at the prescribed temperature and length of time in either column B or C, plus the length of time in column D. The dating period in column D shall be applied from the day the product leaves the manufacturer's storage, provided the product has not exceeded its maximum storage period, as prescribed in column B or C. If a product is held in the manufacturer's storage beyond the period prescribed, the dating period for the product being distributed shall be reduced by a corresponding period.

Product A	Manufacturer's storage period 1 to 5 °C (unless otherwise stated) B	Manufacturer's storage period 0 °C or colder (unless otherwise stated) C	Dating period after leaving manufacturer's storage when stored at 2 to 8 °C (unless otherwise stated) D
Adenovirus Vaccine Live Oral	6 months	Not applicable	6 months.
Albumin (Human)	3 years	do	(a) 5 years.
	do	do	(b) 3 years, provided labeling recommends storage at room temperature, no warmer than 37 °C.
	Not applicable	do	(c) 10 years, if in a hermetically sealed metal container and provided labeling recommends storage between 2 and 8 °C.
Allergenic Extracts labeled "No U.S. Standard of Potency":			
1. With 50 percent or more glycerin	3 years	do	3 years.
2. With less than 50 percent glycerin	18 months	do	18 months.
3. Products for which cold storage conditions are inappropriate.	Not applicable	do	18 months (from date of manufacture), provided labeling recommends storage at 30 °C or colder.
4. Powders and tablets	do	do	5 years (from date of manufacture), provided labeling recommends storage at 30 °C or colder.
5. Freeze-dried products:			
a. Unreconstituted	do	do	4 years (from date of manufacture).
b. Reconstituted	do	do	18 months (cannot exceed 4-year unreconstituted dating period plus an additional 12 months).
Allergenic Extracts, Alum Precipitated labeled "No U.S. Standard of Potency".	18 months	do	18 months.
Anthrax Vaccine Adsorbed	2 years	do	1 year.

Product A	Manufacturer's storage period 1 to 5 °C (unless otherwise stated) B	Manufacturer's storage period 0 °C or colder (unless otherwise stated) C	Dating period after leaving manufacturer's storage when stored at 2 to 8 °C (unless otherwise stated) D
Antibody to Hepatitis B Surface Antigen:			
1. Antibody to Hepatitis B Surface Antigen.	6 months	do	6 months.
2. Lyophilized coated red blood cells	do	do	Do.
3. Enzyme conjugated products	do	do	Do.
Iodinated (^{125}I) products	Not applicable	do	45 days (from date of manufacture).
Antihemophilic Factor (Human)	do	do	1 year (from date of manufacture).
Anti-Human Globulin Liquid	do	do	2 years.
Anti-Inhibitor Coagulant Complex	do	do	Do.
Antirabies Serum	1 year	do	Do.
Antivenin (*Crotalidae*) Polyvalent	do	do	5 years with an initial 10 percent excess of potency, provided labeling recommends storage at 37 °C or colder.
Antivenin (*Latrodectus Mactans*)	do	do	5 years with an initial 10 percent excess of potency.
Antivenin (*Micurus fulvius*)	do	do	Do.
Asparaginase	Not applicable	do	18 months from the date of the last valid potency test.
BCG Vaccine	1 year	Not applicable	6 months.
Blood Grouping Reagents			
1. Liquid	Not applicable	Not applicable	2 years.
2. Dried	1 year	2 years	5 years.
Blood Group Substance AB	do	do	2 years.
Blood Group Substance A	do	do	Do.
Blood Group Substance B	do	do	Do.
Botulism Antitoxin	do	Not applicable	5 years with an initial 20 percent excess of potency.
Cholera Vaccine	do	do	18 months.
Coccidioidin	do	do	3 years.
Collagenase	Not applicable	do	4 years (from date of manufacture), provided labeling recommends storage at 37 °C or colder.
Cryoprecipitated AFH	do	do	12 months from the date of collection of source blood, provided labeling recommends storage at −18 °C or colder.
Diphtheria Antitoxin:			
1. Liquid	1 year	do	5 years with an initial 20 percent excess of potency.
2. Dried	do	2 years	5 years with an initial 10 percent excess of potency.
Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed.	do	Not applicable	18 months.
Diphtheria and Tetanus Toxoids, Adsorbed.	do	do	2 years.
Diphtheria Toxin for Schick Test	do	do	1 year.
Diphtheria Toxoid	do	do	2 years.
Diphtheria Toxoid Adsorbed	do	2 years	Do.
Diphtheria Toxoid-Schick Test Control	Not applicable	Not applicable	1 year.
Factor IX Complex	do	do	1 year (from date of manufacture).
Fibrinolysin (Human)	1 year	2 years	2 years.
Fibrinolysin and Desoxyribonuclease Combined (Bovine).	do	do	3 years, provided labeling recommends storage at 30 °C or colder.
Fibrinolysin and Desoxyribonuclease Combined (Bovine) with Chloramphenicol.	do	do	Do.
Hepatitis B Surface Antigen:			
1. Unlyophilized coated red blood cells.	Not applicable	do	14 days (from date of manufacture).
2. Iodinated (^{125}I) product	do	do	45 days (from date of manufacture).
3. Enzyme conjugated product	6 months	do	6 months.
Histoplasmin	1 year	Not applicable	2 years.
Immunoglobulins:			
1. Hepatitis B Immune Globulin (Human).	Not applicable	do	1 year.
2. Immune Globulin (Human)	3 years	do	3 years.
3. Immune Globulin Intravenous (Human).	Not applicable	do	1 year.

Product A	Manufacturer's storage period 1 to 5 °C (unless otherwise stated) B	Manufacturer's storage period 0 °C or colder (unless otherwise stated) C	Dating period after leaving manufacturer's storage when stored at 2 to 8 °C (unless otherwise stated) D
4. Lymphocyte Immune Globulin, Anti-Thymocyte Globulin (Equine).	do	Not applicable	2 years.
5. Pertussis Immune Globulin (Human).	3 years	do	3 years from date the dried or frozen bulk product is placed in final solution.
6. Rabies Immune Globulin (Human)	1 year	do	1 year.
7. Rh_0(D) Immune Globulin (Human)	6 months	do	6 months.
8. Tetanus Immune Globulin (Human).	1 year	do	3 years with an initial 10 percent excess of potency.
9. Vaccinia Immune Globulin (Human).	3 years	do	3 years.
10. Varicella-Zoster Immune Globulin (Human).	Not applicable	do	1 year.
Hepatitis B Vaccine	2 years at 2 to 8 °C.	Not applicable	3 years.
Influenza Virus Vaccine	1 year	do	18 months.
Limulus Amebocyte Lysate	Not applicable	Not applicable	18 months (from date of manufacture).
Measles, Mumps, and Rubella Virus Vaccine Live.	do	1 year (−20 °C or colder).	1 year.
Measles and Mumps Virus Vaccine Live	do	do	1 year.
Measles and Rubella Virus Vaccine Live	do	do	Do.
Measles Live and Smallpox Vaccine	Not applicable	do	1 year (from date of manufacture).
Measles Virus Vaccine Live	do	do	1 year.
Meningococcal Polysaccharide Vaccine Group A:			
1. Final bulk powder	do	2 years (−20 °C or colder).	Not applicable.
2. Final container	Not applicable	3 years (−20 °C or colder).	2 years.
Meningococcal Polysaccharide Vaccine Group C:			
1. Final bulk powder	do	2 years (−20 °C or colder).	Not applicable.
2. Final container	do	3 years (−20 °C or colder).	2 years.
Meningococcal Polysaccharide Vaccine Groups A and C combined:			
1. Final bulk powder	do	2 years (−20 °C or colder).	Not applicable.
2. Final container	do	3 years (−20 °C or colder).	2 years.
Meningococcal Polysaccharide Vaccine Groups A, C, Y, and W135 combined:			
1. Final bulk power	do	2 years (−20 °C or colder).	Not applicable.
2. Final container	do	3 years (−20 °C or colder).	2 years.
Mumps Skin Test Antigen	6 months	Not applicable	18 months.
Mumps Virus Vaccine Live	Not applicable	1 year (−20 °C or colder).	1 year.
Normal Horse Serum	1 year	2 years	5 years.
Pertussis Vaccine	do	Not applicable	18 months.
Pertussis Vaccine Adsorbed	do	do	Do.
Plague Vaccine	do	do	Do.
Plasma products:			
1. Fresh Frozen Plasma	Not applicable	do	1 year from date of collection of source blood (−18 °C or colder).
2. Liquid Plasma	do	do	(a) 26 days from date of collection of source blood (between 1 and 6 °C). (b) 40 days from date of collection of source blood only when CPDA-1 solution is used as the anticoagulant (between 1 and 6 °C).
3. Plasma	do	do	5 years from date of collection of source blood (−18 °C or colder).

Product	Manufacturer's storage period 1 to 5 °C (unless otherwise stated)	Manufacturer's storage period 0 °C or colder (unless otherwise stated)	Dating period after leaving manufacturer's storage when stored at 2 to 8 °C (unless otherwise stated)
A	B	C	D
4. Platelet Rich Plasma	do	do	72 hours from time of collection of source blood, provided labeling recommends storage (20 to 24 °C or between 1 and 6 °C). 5 days if certain approved containers are used (20 to 24 °C).
5. Source Leukocytes	do	do	In lieu of expiration date, the collection date shall appear on the label.
6. Source Plasma	do	do	10 years (at the recommended storage temperature stated on the label).
7. Therapeutic Exchange Plasma	do	do	10 years.
Plasma Protein Fraction (Human)	1 year	do	(a) 5 years. (b) 3 years provided labeling recommends storage at room temperature, no warmer than 30 °C).
Platelets	Not applicable	do	72 hours from time of collection of source blood, provided labeling recommends storage at 20 to 24 °C or between 1 and 6 °C. 5 days if certain approved containers are used (20 to 24 °C).
Pneumococcal Vaccine Polyvalent:			
1. Final bulk powder	do	24 months after potency assay (−20 °C or colder).	Not applicable.
2. Final container	do	Not applicable	2 years (from date of manufacture).
Poliovirus Vaccine Inactivated	1 year	do	1 year.
Poliovirus Vaccine Live Oral Trivalent:			
1. Frozen	Not applicable	1 year (−10 °C or colder).	1 year, provided labeling recommends storage at a temperature which will maintain ice continuously in a solid state.
2. Liquid	do	Not applicable	30 days, provided labeling recommends storage between 2 and 8 °C and container has been unopened.
Poliovirus Vaccine Live Oral Type I:			
1. Frozen	do	1 year (−10 °C or colder).	1 year, provided labeling recommends storage at a temperature which will maintain ice continuously in a solid state.
2. Liquid	do	Not applicable	30 days, provided labeling recommends storage between 2 and 8 °C and container has been unopened.
Poliovirus Vaccine Live Oral Type II:			
1. Frozen	do	1 year (−10 °C or colder).	1 year, provided labeling recommends storage at a temperature which will maintain ice continuously in a solid state.
2. Liquid	do	Not applicable	30 days, provided labeling recommends storage between 2 and 8 °C and container has been unopened.
Poliovirus Vaccine Live Oral Type III:			
1. Frozen	do	1 year (−10 °C or colder).	1 year, provided labeling recommends storage at a temperature which will maintain ice continuously in a solid state.
2. Liquid	do	Not applicable	30 days, provided labeling recommends storage between 2 and 8 °C and container has been unopened.
Polyvalent bacterial antigens with "No U.S. Standard of Potency" liquid.	1 year	do	18 months.
Polyvalent bacterial vaccines with "No U.S. Standard of Potency" liquid.	do	do	Do.
Rabies Vaccine:			
1. Dried	do	2 years	Do.
2. Liquid	3 months	Not applicable	6 months.

Product	Manufacturer's storage period 1 to 5 °C (unless otherwise stated)	Manufacturer's storage period 0 °C or colder (unless otherwise stated)	Dating period after leaving manufacturer's storage when stored at 2 to 8 °C (unless otherwise stated)
A	B	C	D
Reagent red blood cells	Not applicable	Not applicable	Thirty-five days from earliest date of collection if kept in liquid form (indefinite storage of reagent red blood cell source material at −65 °C or colder).
ACD Red Blood Cells	do	do	(a) 21 days from date of collection of source blood, provided labeling recommends storage between 1 and 6 °C and the hermetic seal is not broken during processing. (b) 24 hours after plasma removal, provided labeling recommends storage between 1 and 6 °C and the hermetic seal is broken during processing.
CPD Red Blood Cells	do	do	(a) 21 days from date of collection of source blood, provided labeling recommends storage between 1 and 6 °C and the hermetic seal is not broken during processing. (b) 24 hours after plasma removal, provided labeling recommends storage between 1 and 6 °C and the hermetic seal is broken during processing.
CPDA–1 Red Blood Cells	do	do	(a) 35 days from date of collection of source blood, provided labeling recommends storage between 1 and 6 °C and the hermetic seal is not broken during processing. (b) 24 hours after plasma removal, provided labeling recommends storage between 1 and 6 °C and the hermetic seal is broken during processing.
Red Blood Cells Deglycerolized	do	do	24 hours after removal from storage at −65 °C or colder, provided labeling recommends storage between 1 and 6 °C.
Red Blood Cells Frozen	do	do	3 years from date of collection of source blood, provided labeling recommends storage at −65 °C or colder.
Rubella and Mumps Virus Vaccine Live	do	1 year (−20 °C or colder).	1 year.
Rubella Virus Vaccine Live	do	°C	Do.
Skin Test Antigens for Cellular Hypersensitivity.	6 months	Not applicable	Do.
Smallpox Vaccine:			
1. Liquid	Not applicable	9 months (−10 °C or colder, if product is maintained as glycerinated or equivalent vaccine in bulk or final containers).	3 months, provided labeling recommends storage at 0 °C or colder.
2. Dried	6 months	Not applicable	18 months.
Streptokinase	Not applicable	do	Do.
Tetanus and Diphtheria Toxoids Adsorbed for Adult Use.	1 year	do	2 years.
Tetanus Antitoxin:			
1. Liquid	do	do	5 years with an initial 20 percent excess or potency.
2. Dried	do	2 years	5 years with an initial 10 percent excess or potency.
Tetanus Toxoid	do	Not applicable	2 years.
Tetanus Toxoid Adsorbed	do	do	Do.
Thrombin	do	2 year	3 years.
Thrombin Impregnated Pad	Not applicable	Not applicable	1 year, or 6 months at 20 to 24 °C.

Product A	Manufacturer's storage period 1 to 5 °C (unless otherwise stated) B	Manufacturer's storage period 0 °C or colder (unless otherwise stated) C	Dating period after leaving manufacturer's storage when stored at 2 to 8 °C (unless otherwise stated) D
Tuberculin:			
1. Purified Protein Derivative, diluted	6 months	do	1 year.
2. Old or Purified Protein Derivative dried on multiple puncture device.	1 year (not to exceed 30 °C; do not refrigerate).	do	2 years, provided labeling recommends storage at a temperature not to exceed 30 °C. Do not refrigerate.
3. Old on multiple puncture device	do	do	Do.
Typhoid Vaccine	1 year	do	18 months.
ACD Whole Blood	Not applicable	do	21 days from date of collection, provided labeling recommends storage between 1 and 6 °C.
CPD Whole Blood	do	do	Do.
CPDA–1 Whole Blood	do	do	35 days from date of collection, provided labeling recommends storage between 1 and 6 °C.
Heparin Whole Blood	do	do	48 hours from date of collection, provided labeling recommends storage between 1 and 6 °C.
Yellow Fever Vaccine	do	1 year (−20 °C or colder).	1 year, provided labeling recommends storage at 5 °C or colder.

(d) *Exemptions.* Exemptions or modifications shall be made only upon written approval, in the form of a supplement of the product license, issued by the Director, Center for Biologics Evaluation and Research (HFB–1).

[50 FR 4134, Jan. 29, 1985, as amended at 51 FR 15607, Apr. 25, 1986; 51 FR 19750, June 2, 1986; 52 FR 37450, Oct. 7, 1987; 53 FR 12764, Apr. 19, 1988; 55 FR 11014, Mar. 26, 1990; 59 FR 49351, Sept. 28, 1994]

Subpart G—Labeling Standards

§610.60 Container label.

(a) *Full label.* The following items shall appear on the label affixed to each container of a product capable of bearing a full label:

(1) The proper name of the product;

(2) The name, address, and license number of manufacturer;

(3) The lot number or other lot identification;

(4) The expiration date;

(5) The recommended individual dose, for multiple dose containers.

(6) The statement: "Caution: Federal law prohibits dispensing without prescription," for prescription biologicals.

(b) *Package label information.* If the container is not enclosed in a package, all the items required for a package label shall appear on the container label.

(c) *Partial label.* If the container is capable of bearing only a partial label, the container shall show as a minimum the name (expressed either as the proper or common name), the lot number or other lot identification and the name of the manufacturer; in addition, for multiple dose containers, the recommended individual dose. Containers bearing partial labels shall be placed in a package which bears all the items required for a package label.

(d) *No container label.* If the container is incapable of bearing any label, the items required for a container label may be omitted, provided the container is placed in a package which bears all the items required for a package label.

(e) *Visual inspection.* When the label has been affixed to the container a sufficient area of the container shall remain uncovered for its full length or circumference to permit inspection of the contents.

[38 FR 32056, Nov. 20, 1973, as amended at 47 FR 22518, May 25, 1982]

§610.61 Package label.

The following items shall appear on the label affixed to each package containing a product:

(a) The proper name of the product;

(b) The name, address, and license number of manufacturer;

(c) The lot number or other lot identification;

(d) The expiration date;

(e) The preservative used and its concentration, or if no preservative is used and the absence of a preservative is a safety factor, the words "no preservative";

(f) The number of containers, if more than one;

(g) The amount of product in the container expressed as (1) the number of doses, (2) volume, (3) units of potency, (4) weight, (5) equivalent volume (for dried product to be reconstituted), or (6) such combination of the foregoing as needed for an accurate description of the contents, whichever is applicable;

(h) The recommended storage temperature;

(i) The words "Shake Well", "Do not Freeze" or the equivalent, as well as other instructions, when indicated by the character of the product;

(j) The recommended individual dose if the enclosed container(s) is a multiple-dose container;

(k) The route of administration recommended, or reference to such directions in an enclosed circular;

(l) Known sensitizing substances, or reference to an enclosed circular containing appropriate information;

(m) The type and calculated amount of antibiotics added during manufacture;

(n) The inactive ingredients when a safety factor, or reference to an enclosed circular containing appropriate information;

(o) The adjuvant, if present;

(p) The source of the product when a factor in safe administration;

(q) The identity of each microorganism used in manufacture, and, where applicable, the production medium and the method of inactivation, or reference to an enclosed circular containing appropriate information;

(r) Minimum potency of product expressed in terms of official standard of potency or, if potency is a factor and no U.S. standard of potency has been prescribed, the words "No U.S. standard of potency."

(s) The statement: "Caution: Federal law prohibits dispensing without prescription," for prescription biologicals.

[38 FR 32056, Nov. 20, 1973, as amended at 47 FR 22518, May 25, 1982; 55 FR 10423, Mar. 21, 1990]

§610.62 Proper name; package label; legible type.

(a) *Position.* The proper name of the product on the package label shall be placed above any trademark or trade name identifying the product and symmetrically arranged with respect to other printing on the label.

(b) *Prominence.* The point size and typeface of the proper name shall be at least as prominent as the point size and typeface used in designating the trademark and trade name. The contrast in color value between the proper name and the background shall be at least as great as the color value between the trademark and trade name and the background. Typography, layout, contrast, and other printing features shall not be used in a manner that will affect adversely the prominence of the proper name.

(c) *Legible type.* All items required to be on the container label and package label shall be in legible type. "Legible type" is type of a size and character which can be read with ease when held in a good light and with normal vision.

§610.63 Divided manufacturing responsibility to be shown.

If two or more establishments participate in the manufacture of a product, the name, address, and license number of each must appear on the package label, and on the label of the container if capable of bearing a full label.

§610.64 Name of selling agent or distributor.

The name and address of the selling agent or distributor of a product may appear on the label under the designation of "selling agent" or "distributor" provided that the name and address of the manufacturer is given precedence in prominence.

§610.65 Products for export.

Labels on packages or containers of products for export may be adapted to

meet specific requirements of the regulations of the country to which the product is to be exported provided that in all such cases the minimum label requirements prescribed in § 610.60 are observed.

INTERNATIONAL STANDARD

ISO 8362-1

First edition
1989-11-01

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Injection containers for injectables and accessories —

Part 1 :
Injection vials made of glass tubing

Récipients et accessoires pour produits injectables —
Partie 1 : Flacons en verre étiré

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Reference number
ISO 8362-1 : 1989 (E)

Contents

Page

Foreword iii

Introduction iv

1 Scope 1

2 Normative references 1

3 Definitions 1

4 Dimensions and designation 2

5 Material 4

6 Performance 4

7 Requirements 4

8 Marking 4

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International Organization for Standardization
Case postale 56 • CH-1211 Genève 20 • Switzerland
Printed in Switzerland

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 8362-1 was prepared by Technical Committee ISO/TC 76, *Transfusion, infusion and injection equipment for medical use.*

ISO 8362 consists of the following parts under the general title *Injection containers for injectables and accessories*:

- *Part 1: Injection vials made of glass tubing*
- *Part 2: Closures for injection vials*
- *Part 3: Aluminium caps for injection vials*
- *Part 4: Injection vials made of moulded glass*

Introduction

The purpose of this part of ISO 8362 is to specify the dimensions, capacities, form and requirements of glass vials intended for medical use. Containers made from glass tubing are considered to be suitable for the packaging and storage of injectable preparations until they are administered for medicinal purposes. Such containers may be made from different types of glass which can affect the chemical resistance properties. For example, those made from borosilicate glass will have a very high level of chemical resistance whereas others made from soda-lime-silica glass will have a lower, but adequate, chemical resistance for the purpose for which they are intended. The chemical resistance of the internal surface of containers made from soda-lime-silica glass can be improved by a treatment during production to produce a chemical resistance equal to that of those made from borosilicate glass for single use. This level of chemical resistance will be maintained as long as the interior surface is not destroyed by chemical attack, in which case it will be reduced to that of untreated soda-lime-silica glass.

Because containers may be made from different types of glass and because it is the chemical behaviour of the internal surface which is important when they are filled with injectable preparations, it is essential to specify test procedures by which this performance can be measured. The procedures recommended in this part of ISO 8362 will allow this performance, based on the hydrolytic resistance, to be measured and, from the result of measurement, it is possible to classify containers into their correct category. The procedure also allows containers to be tested and to determine, after an intermediate stage, whether the hydrolytic resistance is produced by the composition of the glass as a material or by a treatment of the internal surface.

Injection containers for injectables and accessories —

Part 1:
Injection vials made of glass tubing

1 Scope

This part of ISO 8362 specifies the form, dimensions and capacities of glass vials for injectable preparations. It also specifies the material from which such containers shall be made and the performance requirements of those containers.

This part of ISO 8362 applies to colourless or amber glass containers made from borosilicate or soda-lime-silica glass, made from glass tubing, whether internally surface-treated or not, and intended to be used in the packaging, storage or transportation of products intended for injection.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 8362. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 8362 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 719: 1985, *Glass — Hydrolytic resistance of glass grains at 98 °C — Method of test and classification.*

ISO 720: 1985, *Glass — Hydrolytic resistance of glass grains at 121 °C — Method of test and classification.*

ISO 1101: 1983, *Technical drawings — Geometrical tolerancing — Tolerancing of form, orientation, location and run-out — Generalities, definitions, symbols, indications on drawings.*

ISO 4802-1: 1988, *Glassware — Hydrolytic resistance of the interior surfaces of glass containers — Part 1: Determination by titration method and classification.*

ISO 4802-2: 1988, *Glassware — Hydrolytic resistance of the interior surfaces of glass containers — Part 2: Determination by flame spectrometry and classification.*

3 Definitions

For the purposes of this part of ISO 8362, the definitions given in ISO 4802-1 and ISO 4802-2 apply.

4 Dimensions and designation

4.1 Dimensions

The dimensions of injection vials made of glass tubing shall be as shown in figure 1 and as given in table 1; the overflow capacity and mass shall be as given in table 1.

Dimensions in millimetres

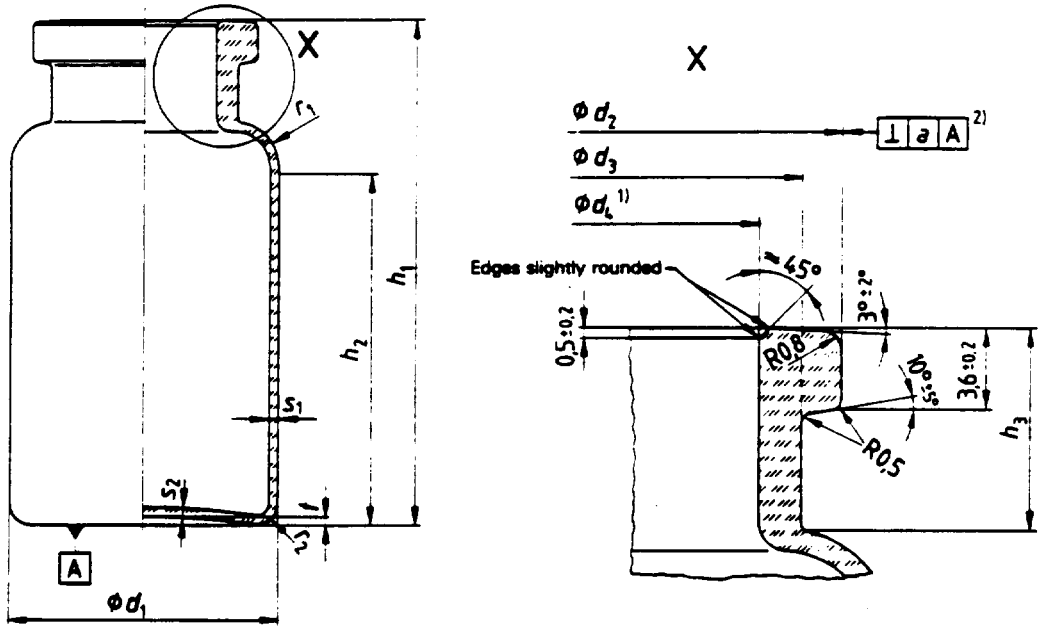

Figure 1 — Typical example of injection vial made of glass tubing

1) The opening of the vial should have a constant diameter, as specified by d_4, over the entire distance given by h_3, i.e. it should exhibit a cylindrical shape. A slightly conical shape can be accepted if the following requirements are complied with:

a) the truncated cone shall have the height as specified by h_3;

b) the larger diameter shall be located at the flange;

c) the larger diameter shall not exceed the smaller one by more than 0,3 mm.

2) The perpendicularity tolerance a (as defined in ISO 1101) is a limit for the deviation of the plumb-line through the centre of the bottom part and the axis of the vial at the upper edge of the flange; it is measured at the brim.

Table 1 — Dimensions, overflow capacity and mass

Dimensions in millimetres

Size designation of injection vial	Overflow capacity ml	tol.	a	d_1	tol.	d_2	d_3	d_4	h_1	tol.	h_2	h_3	tol.	r_1	r_2	s_1	tol.	s_2	t	Mass g
						$^{+0,2}_{-0,3}$	max.	±0,2			min.			≈	≈			min.	max.	≈
2R	4	±0,5	1	16	±0,2	13	10,5	7	35	±0,5	23	8	±0,5	2,5	1,5	1	±0,04	0,7	0,7	5
4R	6								45		33									6,1
6R	10		1,2	22		20	16	12,6	40		27	8,5		3,5	2					8,3
8R	11,5								45		32									9,4
10R	13,5	±1		24			16,5		45		31	9								10,2
15R	19								60		46									12,8
20R	25	±1,5	1,5	30	±0,3		17,5		55	±0,7	35	10	±0,75	5,5	2,5	1,2	±0,05		1	17,4
25R	30,5								65		45									20
30R	36								75		55									22,7

4.2 Designation

Designation example of an injection vial, size 10 (10R), made of amber glass (br) tubing of hydrolytic resistance container class ISO 4802 - HC 1 (1) complying with the requirements specified in this part of ISO 8362:

Vial ISO 8362-1 10R - br - 1

5 Material

Colourless (cl) or amber (br) borosilicate glass[1] or soda-lime-silica glass[1] of one of the following hydrolytic resistance grain classes:

- ISO 720 - HGA 1
- ISO 719 - HGB 3 or ISO 720 - HGA 2

shall be used.

NOTE — A change in the chemical composition of the glass material or of the colouring oxides should be notified to the user at least nine months in advance.

6 Performance

6.1 Injection vials shall not contain seed or bubbles to an extent which will interfere with the visual examination of the contents.

6.2 Injection vials shall have a sealing surface which is flat and free from ripples or undulations which would affect the sealing performance of the closure.

7 Requirements

7.1 Hydrolytic resistance

When tested in accordance with ISO 4802-1 or ISO 4802-2, the hydrolytic resistance of the internal surface of injection vials shall comply with the requirements specified for one of the following hydrolytic resistance container classes:

- ISO 4802 - HC 1
- ISO 4802 - HC 2
- ISO 4802 - HC 3.

7.2 Annealing quality

The injection vials shall be annealed so that the maximum residual stress does not produce an optical retardation exceeding 40 nm per millimetre of glass thickness, when the vials are viewed in a strain viewer.

8 Marking

The number of pieces and the designation according to 4.2 together with the name or symbol of the manufacturer shall be shown on the package.

Further information may appear subject to agreement.

1) For definitions, see ISO 4802-1 and ISO 4802-2.

UDC 666.171. 615.456.014.83

Descriptors : medical equipment, parenteral infusion equipment, containers, glassware, flasks, specifications, dimensions, designation marking.

Price based on 4 pages

INTERNATIONAL STANDARD

ISO
8362-2

First edition
1988-12-15

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION
ORGANISATION INTERNATIONALE DE NORMALISATION
МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ

Injection containers for injectables and accessories —

Part 2 :
Closures for injection vials

Récipients et accessoires pour produits injectables —

Partie 2: Bouchons pour flacons

Reference number
ISO 8362-2 : 1988 (E)

Contents

Page

Foreword iii

Introduction iv

1 Scope 1

2 Normative references 1

3 Dimensions and designation 2

4 Material 3

5 Requirements 3

6 Sample 4

7 Marking 4

Annexes

A Test method for fragmentation 5

B Test method for penetrability 7

C Test method for closure/container integrity and self-sealing 8

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Printed in Switzerland

Injection containers for injectables and accessories —

Part 2 :
Closures for injection vials

1 Scope

This part of ISO 8362 specifies the design, dimensions, material, performance, requirements and tests for closures for injection vials covered by ISO 8362-1 and ISO 8362-4.

Closures specified in this part of ISO 8362 are intended for single use only.

The closure shall be made from the formulation originally tested and approved by the end-user. The closure manufacturer will certify identity of the closure as well as conformance of the closure to previously agreed functional parameters or compendium requirements.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 8362. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 8362 are encouraged to investigate the possibility of applying the most recent editions of the standards listed below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 48 : 1979, *Vulcanized rubbers — Determination of hardness (Hardness between 30 and 85 IRHD).*

ISO 2859 : 1974, *Sampling procedures and tables for inspection by attributes.*

ISO 3302 : 1976, *Rubber — Dimensional tolerances of solid moulded and extruded products.*

ISO 3696 : 1987, *Water for analytical laboratory use — Specification and test methods.*

ISO 7864 : 1984, *Sterile hypodermic needles for single use.*

ISO 7886 : 1984, *Sterile hypodermic syringes for single use.*

ISO 8362-1 : 1988, *Injection containers for injectables and accessories — Part 1: Injection vials made of glass tubing.*

ISO 8362-4 : 1988, *Injection containers for injectables and accessories — Part 4: Injection vials made of moulded glass.*

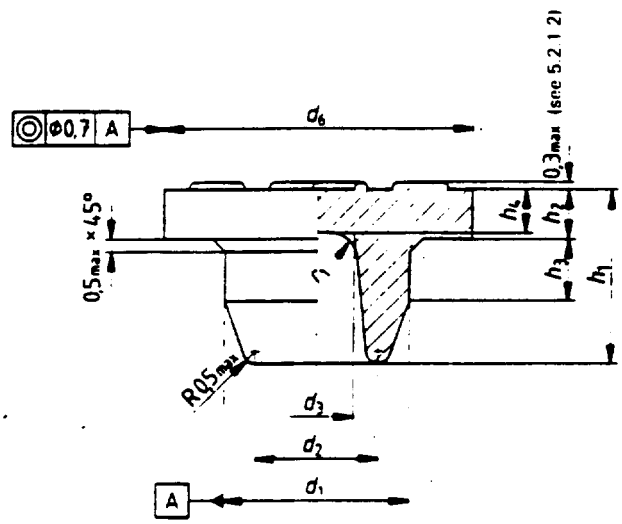
ISO 8871 : 1988, *Elastomeric parts for aqueous parenteral preparations — Identification, requirements, test methods.*

3 Dimensions and designation

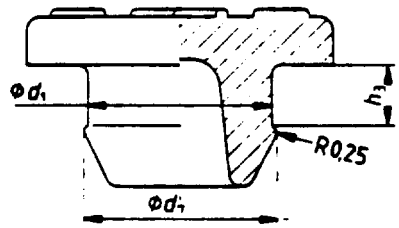
3.1 Dimensions

The dimensions of closures shall be as shown in figure 1 and as given in table 1. Figure 1 illustrates two types of closure, types A and B.

Dimensions in millimetres



Type A



NOTE — Other dimensions are as indicated for type A closures.

Type B

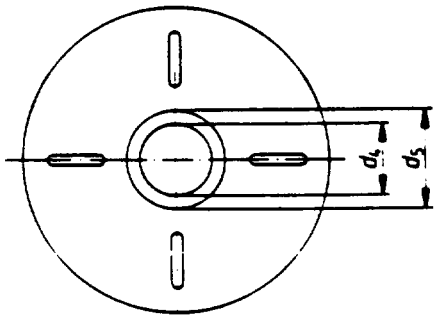


Figure 1 — Dimensions and configuration of types A and B closures

Table 1 — Dimensions of injection closures

Dimensions in millimetres

Type	Nominal size	d_1 ±0.1	d_2 max.	$d_3 = d_4$ min.	d_5 max.	d_6 ±0.2	d_7 ±0.2	h_1 ±0.3	h_2 ±0.25	h_3 ±0.25	h_4	r_1	Injection vials ISO 8362-1	Injection vials ISO 8362-4
A	13	7.5	5	3	4	12.5	–	7	2	2.5	1.75 ± 0.25	1	2 R and 4 R	–
	20	13.2	10	4.5	5.5	18.8	–	8.8	3.3	2.5	3.3 max. 1.5 min.	2	6 R to 30 R	5 H to 100 H
B	13	7.4	5	3	4	12.5	7.6	7	2	1.5	1.5 min.	1	–	2 I to 10 I
	20	13	10	4.5	5.5	18.8	13.3	8.8	3.3	1.5	3.3 max. 1.5 min.	2	–	5 H to 100 H

3.2 Designation

Closures are designated according to type: the two types A and B are illustrated in figure 1. The designation is expressed as the number of this part of ISO 8362 followed by the nominal size of the vial followed by the type letter.

For example a type A closure for injection vials of nominal size 13 complying with the requirements laid down in this part of ISO 8362 is designated:

Injection closure ISO 8362-2 - 13 - A

4 Material

The type of elastomeric material used shall meet the requirements specified in clause 5.

5 Requirements

5.1 General

The requirements specified in 5.2 to 5.4 represent minimum requirements which refer to the condition of the elastomeric closures on receipt by the user.

NOTE — The resistance to ageing depends on the actual circumstances of storage and handling. The minimum shelf life of the closure should be agreed upon between closure manufacturer and user.

The useful lifetime of the closure in contact with the pharmaceutical product is part of the compatibility tests to be carried out by the user.

5.2 Physical requirements and performance

5.2.1 Dimensions

5.2.1.1 If not otherwise specified, general dimensional tolerances shall be in accordance with ISO 3302.

5.2.1.2 If spacers are located on top of the flange, they shall not interfere with the marks for the injection site within the area of diameter d_5 (see figure 1). The height of the spacers shall not exceed 0,3 mm.

NOTE — The spacers in figure 1 for types A and B closures are shown for illustrative purposes only and do not form part of the requirements of this part of ISO 8362.

5.2.1.3 If the flange of the closure has a slightly conical shape, it shall be 0,3 mm max. in relation to the diameter in order to facilitate production. The tolerances of the trimming edge of the flange shall comply with the tolerances specified in table 1 for the diameter of the flange d_6.

5.2.1.4 All edges of the closure may be rounded.

5.2.2 Hardness

When tested by the method given in ISO 48 using test plates supplied by the manufacturer, the hardness shall not differ from the value stated by the manufacturer by more than ±5 IRHD.

5.2.3 Fragmentation (coring)

When tested for fragmentation in accordance with annex A, not more than 5 fragments per 100 piercings shall be observed.

5.2.4 Penetration

When tested for penetration in accordance with annex B, the force needed to penetrate the closure shall not exceed 10 N for any of the ten closures tested.

5.2.5 Closure/container integrity

When tested in accordance with annex C, no methylene blue solution shall leak into the interior of the vial.

5.2.6 Self-sealing

If the closure has to be pierced more than once, but not more than three times, the requirements for self-sealing shall be met. When tested in accordance with annex C, no methylene blue solution shall leak into the interior of the vial.

5.3 Chemical requirements

The material of the closure shall satisfy the limits specified in table 2.

Table 2 — Chemical limits for injection closures

Characteristics	Limit	Test method as described in ISO 8871
Reducing matter (oxidizables)	<7,0 ml of $c(KMnO_4)$ = 2 mmol/l per 20 ml	Annex C
Heavy metals (calculated as Pb^{2+})	< 10 µg Pb^{2+}/10 ml	Annex D
Ammonium (calculated as NH_4^+)	<20 µg NH_4^+/10 ml	Annex E
Acidity/ alkalinity	<1,0 ml of c(HCl) or c(NaOH) = 5 mmol/l per 20 ml	Annex G
Residue on evaporation (total solids)	< 4 mg/100 ml	Annex H
Volatile sulfides (at ≈ pH 2)	Discoloration of lead acetate paper <50 µg Na_2S/20 cm^2 of rubber surface	Annex I
Zinc (calculated as Zn^{2+})	Zn^{2+} < 30 µg/10 ml	Annex K
Conductivity	<40 µS/cm	Annex L
Turbidity	Not exceeding 3	Annex M

5.4 Biological requirements

The elastomeric closure shall not release any substances which may adversely affect the therapeutic effectiveness of the injectable products, including those substances which may exhibit toxic, pyrogenic or haemolytic reactions.

NOTE — Since biological tests are usually requested by most of the national Pharmacopoeias or related regulations of health authorities, they are mandatory for producers and users in countries where they exist.

If this is not the case, reference should be made to biological tests, e.g. as described in the United States Pharmacopoeia, European Pharmacopoeia or other Pharmacopoeias.

6 Sample

6.1 Sample size

The closures to be tested shall be taken from a sample collected as described in ISO 8871; the sample size is deduced from the rules given in ISO 2859.

The minimum sample size to produce a sufficient number of items for all physical and chemical tests is as follows:

— nominal size 13 : 570 closures;

— nominal size 20 : 395 closures.

6.2 Pre-conditioning for physical properties testing

Pre-condition the samples for fragmentation, penetrability and integrity and self-sealing as follows.

Estimate the total surface area, A, in square centimetres, of the quantity of closures to be treated. Wash the closures by placing them in a suitable glass container and covering with (2A) ml of purified water[1]. Boil the water for 5 min ± 15 s, and rinse 5 times with cold purified water. Place the washed closures in a wide-necked flask and add (2A) ml of purified water. Cover the mouth of the flask with aluminium foil or a borosilicate beaker. Heat in an autoclave so that a temperature of 121 °C ± 1 °C is reached in the flask within 30 min; maintain this temperature for 30 min.

Allow the closures to cool to room temperature within 30 min. Dry the closures in hot air at 60 °C within 60 min. Store the closures in a closed glass container.

7 Marking

The packaging closures given shall be marked with the designation given in 3.2.

1) Purified water corresponds to grades 1 and 2 of ISO 3696.

Annex A
(normative)

Test method for fragmentation

A.1 Principle

The purpose of the test is to measure the relative coring tendencies of different ISO rubber closures. The values obtained can be significantly affected by many factors, such as prior processing of the closures, type of crimping device, sealing force, design of the hypodermic needle point, its sharpness, the amount of lubrication on the needle, the gauge of the needle and the keenness of the operator's sight.

It is, therefore, necessary to control these variables in order to obtain comparable results. For this reason, the closures to be tested have to be compared to known samples.

A.2 Apparatus

A.2.1 **50 injection vials**, complying with ISO 8362-1 or ISO 8362-4.

A.2.2 **Hand-operated capping device** and **aluminium caps with a central hole** which fit the injection vials to be used in the test.

A.2.3 **Membrane filter set.**

A.2.4 **Disposable syringe for single use** (e.g. as specified in ISO 7886), of 1 ml capacity, fitted with a tip for an injection needle.

A.2.5 **10 injection needles**, having an outer diameter of 0,8 mm and complying with ISO 7864. The bevel types and dimensions shall be as given in figure A.1 and table A.1; both the long and the medium bevel type are allowed.

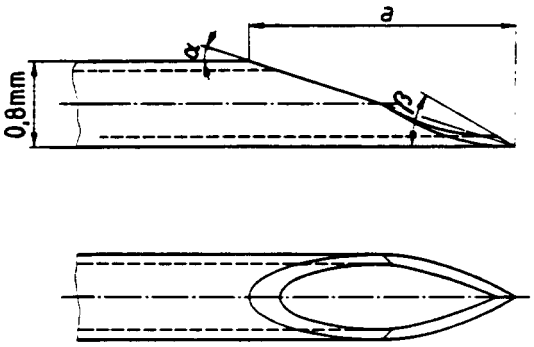


Figure A.1 — Needle point

Table A.1 — Dimensions of bevel

Bevel type	a mm min.	a mm max.	α nom.	β
L (long)	3.21	3.78	13°	22° ± 1°
M (medium)	2.70	3.09	15° 30'	26° ± 1°

A.3 Procedure

A.3.1 Degrease 10 new injection needles (A.2.5) by means of acetone or methyl-isobutylketone.

A.3.2 Select 50 vials (A.2.1) in a size matching the closure to be tested.

Place *n* ml of water into each of these vials, where *n* is 50 % of the nominal volume of the vials.

Place a closure of the type to be tested on each of 25 vials, and a closure with known fragmentation properties on each of the remaining 25 vials.

Seal all vials with an aluminium cap (A.2.2) using the hand-operated capping device.

Arrange the vials in two rows as shown in figure A.2.

A.3.3 Attach an injection needle (A.2.5) to the tip of the syringe (A.2.4) (see, however, A.3.8). Fill the syringe with water. Remove any water adhering to the needle.

A.3.4 Hold the syringe vertically by hand and pierce closure No. 1 within the marked area, leaving vial No. 1 standing firmly in a vertical position.

Withdraw the needle.

A.3.5 Repeat the procedure described in A.3.4 three more times; however, before withdrawing the needle for the last time, inject the contents of the syringe (1 ml of water) into the vial.

A.3.6 Repeat the procedures described in A.3.3 to A.3.5, using closure No. 26 fitted on vial No. 26 (i.e. the first closure/vial combination in the second row).

A.3.7 Repeat all of the procedures described in A.3.3 to A.3.6, using, alternately, vials from the two rows, until all of the closures have each been pierced four times.

A.3.8 Use a new injection needle after every 20 piercings (see figure A.2).

A.3.9 Remove the closures to be tested from the vials (first row). Pour the contents of all of them through one membrane filter. Ensure that no fragments remain in the vials. Count and record the number of fragments on the filter visible with the naked eye under normal conditions (distance between eye and filter: 25 cm).

A.3.10 Repeat the procedure described in A.3.9, using, however, the vials with closures having known fragmentation properties.

A.4 Expression of results

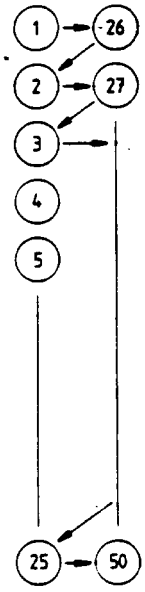
The recorded numbers of fragments per 100 piercings for the two series shall be reported.

A.5 Validity

The results obtained on the test closures shall be considered invalid if the results on the known closures lack consistency with previous results; the reason for such inconsistency shall be detected.

First row:
closures to be tested

Second row:
closures with known fragmentation properties



Needle No.[1]	Closure/vial combination No.
1	1; 26; 2; 27; 3
2	28; 4; 29; 5; 30
3	6; 31; 7; 32; 8
etc.	etc.

1) Each needle is used for 20 piercings only (see A.3.8).

Figure A.2 – Test sequence for fragmentation test

Annex B
(normative)

Test method for penetrability

Fit the closure to be tested on an injection vial and seal with an aluminium cap with a central hole. Pierce the closure with a hypodermic needle (A.2.5) degreased by means of acetone or methyl-isobutylketone, within 15 s by applying a force of 10 N; a device as shown as an example in figure B.1 may be used for the piercing operation.

NOTE — The force needed for the hypodermic needle to pierce the closure can be determined in such a way that the load on top of the piercing device is increased stepwise until the closure has been pierced. However, when testing larger series, it has proved advantageous to use a top-loading balance with immediate display; when using such a balance, the closure is pierced by the needle within 1 s. The necessary piercing force can be determined by direct reading or by means of a trailing pointer.

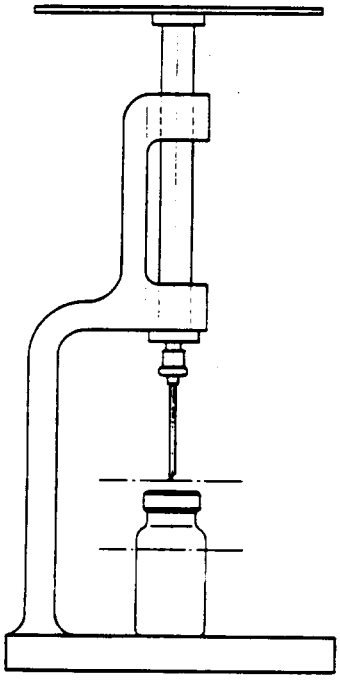


Figure B.1 — Schematic representation of device for testing penetrability

Annex C
(normative)

Test method for closure/container integrity and self-sealing

C.1 Principle

The tightness of vials sealed with rubber stoppers, both intact and after the stoppers have been perforated, is tested first by trying to extract air from bottles by a partial vacuum outside, and subsequently by observing whether a coloured liquid is sucked into the eventually sub-pressurized vials.

C.2 Apparatus

C.2.1 **20 injection vials**, complying with ISO 8362-1 and matching the closures to be tested, as follows:

Nominal size of closure	Vial
13	4 R/ 6 ml
20	6 R/10 ml

C.2.2 **Hand-operated capping device and aluminium caps with a central hole** which fit injection vials to be used in the test.

C.2.3 **Three injection needles**, having an outer diameter of 0,8 mm and complying with ISO 7864; the bevel type and dimensions shall be as specified for the long (L) type in A.2.5 and table A.1.

C.2.4 **Piercing device**, as shown in figure B.1 in annex B, possessing a shaft assembly capable of moving vertically up- and downwards.

C.2.5 **Vacuum chamber.**

C.2.6 **Beaker**, having a capacity of 1 l and containing an aqueous solution of 0,1 % methylene blue.

C.3 Procedure

C.3.1 Prepare 20 closures at least 2 h before the test acording to the procedure described below.

Place the closures in boiling water and leave them in this condition for 5 min. Separate the closure immediately from the water and dry the closures in hot air at 70 °C for 1 h.

C.3.2 Prepare the test vials (C.2.1) according to the procedure described below.

Place 3 ml of water in each of 20 vials. Close the vials with the pre-conditioned closures (see C.3.1). Seal the vials with the appropriate aluminium caps (C.2.2).

Autoclave the assemblies at 121 °C ± 1 °C for 30 min. Allow the assemblies to stand for 24 h.

C.3.3 Place an assembly prepared as described under C.3.2 in the piercing device (C.2.) fitted with a needle (C.3.3). (See, however, C.3.5.) Pierce the closure within the marked area three times at different locations.

C.3.4 Repeat the procedure described under C.3.3 on nine more assemblies.

C.3.5 Use a new needle after every 10 piercings.

C.3.6 Place all vials (with perforated and non-perforated closures) into the beaker containing the methylene blue solution so that they are completely immersed. Place the beaker in the vacuum chamber (C.3.5).

Evacuate to a residual pressure of 75 kPa ± 5 kPa and maintain this pressure for 30 min.

Restore the vacuum chamber to atmospheric pressure and maintain this pressure for 30 min.

Then remove the vials from the solution and rinse the outside of the vials with water.

Examine visually whether or not the methylene blue solution has penetrated into the interior of any of the vials.

C.4 Expression of results

The number of cases, both for the non-perforated and the perforated closures, in which the contents of the vial are seen to be coloured shall be reported. The temperature at which the evacuation and the return to normal pressure was carried out shall be reported. Any occurrence or event which is not covered by the procedure described and which might be capable of affecting the test results shall be noted.

UDC 615.014.87-036 : 666.171 : 615.456.014.83

Descriptors : medical equipment, parenteral infusion equipment, containers, flasks, rubber products, stoppers, specifications, dimensions, tests, designation, marking.

Price based on 8 pages

B. BCG VACCINE

Documents provided in this section include:

1. Sample Procurement Requirements BCG Vaccine 240
2. BCG Vaccine Model Package Insert (UNICEF) 247
3. BCG Vaccine, *USP 23 - NF 18*, 1994 248
4. Bacillus of Calmette and Guerin (BCG) Vaccine, US Food and Drug Administration HHS 21 CFR, Part 620, Subpart E 249
5. Requirements for Dried BCG Vaccine (Requirements for Biological Substances No. 11, revised 1985), WHO *Technical Report Series* No. 745, 1987 254
6. Requirements for Dried BCG Vaccine (Amendment 1987), WHO *Technical Report Series* No. 771, part 49, 1988 270

SAMPLE

PROCUREMENT REQUIREMENTS

BCG VACCINE

A. General Description of Intended Use

This vaccine is intended for use in a public-sector health programme for immunization of children (under one year of age) in _____ [*place*] beginning on _____ [*date*].

The total requirement for this solicitation is _____ [*quantity*] doses. The _____ [*name*] expects to procure additional quantities of vaccine annually beginning in _____ [*month, year*] and subsequent years as follows: _____ [*quantity*], _____ [*quantity*], and _____ [*quantity*].

B. Specific Requirements

Item: Dried BCG Vaccine with sterile diluent packed separately.

Dried living culture of the bacillus Calmette Guerin, grown in a suitable medium from a seed strain of known history that has been maintained to preserve its capacity for conferring immunity. Intended for intradermal injection.

Each dose shall contain that amount of viable bacteria such that inoculation of tuberculin-negative persons results in an acceptable tuberculin conversion rate.

The vaccine shall be free from other organisms, and contain a suitable stabiliser. It shall contain no antimicrobial agent.

	The vaccine shall meet _____ [*specify*] requirements for Dried BCG Vaccine (WHO Requirements for Biological Substances No. 11, revised 1985 or equivalent requirements of the USFDA as detailed in 21 CFR, or another fully equivalent set of biological requirements). *Editors' note: It is recognised that equivalence does not mean identical, only that both provide the same level of potency, safety and efficacy.*
	The vaccine shall be currently registered in the country of manufacture and shall meet all requirements of the licensing authority of the country of manufacture. The vaccine also shall be currently registered in the country of use and shall meet all requirements of the licensing authority of the country of use.
Quantity:	_____ doses from one production lot.
Dosage Size:	0.05 ml for infants under one year (0.1 ml for children over one year).
Dose Package:	Vials or ampoules of 20 infant doses (equivalent to 10 doses for older children) with diluent; container must protect vaccine from exposure to light.
Filling Volume:	Final reconstituted product should contain one dose in 1/20 ml + 15% overfill.
Tolerance:	Volume shall not vary by more than 5%. Weight of the antigen shall not vary by more than 5%.
Storage Temperature:	Below +8 degrees C; may be frozen. Diluent need not be refrigerated.
Shelf Life:	36 months from the beginning of the last satisfactory test for potency; at least 18 months must remain after shipment.

Stability:	Stable for a minimum of ___ years at ___ to ___ degrees C; manufacturer to specify thermal stability characteristics.
Labelling:	The label on each vial shall conform to the requirements of the country of use and shall appear in the language of _____. All labelling shall withstand immersion in water and remain intact. All labels shall state the name of the vaccine, name of the manufacturer, place of manufacture, lot number, composition, concentration, dose and mode for administration, expiry date, storage temperature and any other marking that is appropriate.
Optional Labelling:	If vaccine is destined for use in public-sector immunization programmes, the statement "Not for Sale; For Use Only in Government Approved Immunization Programmes" shall appear on each label in the language of the purchasing country.
Closures:	Vaccine vials shall be fitted with closures that conform to ISO standard 8362-2 for injection vials.
Printed Materials:	Two (2) information sheets, printed in _____ [*language*], shall be included in each box of ___ vials (see Model Package Insert, attached)[20].

C. Quality Assurance

Compliance:	The Supplier shall guarantee that the products as packed for shipment (a) comply with all provisions of the specification and related documents; (b) meet internationally recognized standards for safety,

[20] See example on page 247.

efficacy and quality; (c) are fit for the purposes made known to the Seller and (d) are free from defects in workmanship and in materials.

Evidence: The Supplier shall provide objective evidence, acceptable to the Purchaser, of the satisfaction of the requirements of this document for which no specific inspection has been mentioned.

The Supplier shall provide a copy of the manufacturing record and procedures to the Purchaser for each lot intended for shipment.

The Supplier shall provide a copy of the batch record and all quality assurance documentation to the Purchaser for each lot being supplied.

The Supplier shall provide a copy of the Certificate of Analysis to the Purchaser for each lot intended for shipment.

The Supplier shall provide to the Purchaser a copy of the approval of each source material, constituent material and component for each lot intended for shipment.

The Supplier shall retain a sample of twenty (20) vials from each lot shipped for two years beyond the printed expiration date.

Chemical, physical and microbiological test data for in-process and finished product testing must be on record for each lot shipped and must be available to Purchaser's representatives when requested.

Inspection: The Purchaser may inspect and sample, or cause to be sampled, the product at the Supplier's factory and/or warehouse at a mutually agreeable time prior to shipment of the product.

Testing: The Purchaser may cause independent laboratory testing to be performed as deemed necessary to assure that the goods conform to prescribed requirements. Said testing laboratory shall be of the Purchaser's choice and suitably equipped and qualified to conduct quality assurance tests on biological products.

D. Packing

Storage: Supplier shall state storage volume occupied per infant dose of vaccine (storage volume includes the vaccine vial, packet containing the vaccine vial and any intermediate packaging).

Inner Boxes: _____ [*number*] individual glass vials or ampoules shall be contained in sturdy white cardboard boxes outfitted with individual segments for protecting and separating each vial.

Cold Chain Monitor Cards: One cold chain monitor card (description attached)[21] in _____ language shall be packed with each ___ doses of vaccine.

Overpacking: Boxes shall be overpacked so that the vaccine remains refrigerated below +8 degrees C (however, the diluent need not be refrigerated). The containers must be suitable for export shipping in accordance with WHO/EPI Guidelines on International Packaging and Shipping of Vaccines. They must have adequate insulation and/or sufficient refrigerant to ensure that the warmest storage temperature of the vaccine does not rise above +8 degrees C in continuous outside ambient temperature of +43 degrees C during transit and for a period of at least 48 hours after arrival at the airport of destination.

[21] See example in Supplement VI.G.1.a., page 783.

Additional cushioning shall be provided, sufficient to protect the vials from breakage during transit and handling.

Exterior Shipping Cartons: Product and printed materials, packaged as specified above, shall be packed in weather-resistant, triple-wall corrugated fibreboard cartons with a bursting test strength of not less than 1,900 kPa.

The overall dimensions of the exterior shipping cartons should be such that the product does not become damaged during transportation and storage.

E. Markings

All containers and invoices must bear the name of the vaccine, expiry dates of the vaccine and appropriate storage temperature.

Inner Boxes: The inner boxes shall be marked with the following information in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Manufacturer's name and registered address
- Manufacturer's national registration number
- Lot or batch number
- Composition and concentration
- Number of vials contained in box
- Expiration date (month and year)
- Instructions for storage and handling†
- Place of manufacture (Made in _____)

Exterior Shipping Cartons: The following information shall be stencilled or labelled on the exterior shipping cartons on two opposing sides in bold letters at least __ mm high

† To be provided by Purchaser.

with waterproof ink in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Lot or batch number
- Expiration date (month and year)
- Manufacturer's name and registered address
- Manufacturer's national registration number
- Destination country license or registration number
- Consignee's address and emergency phone number
- Destination airport
- Contract number
- Number of vials contained in the carton
- Gross weight of each carton (in kg)
- Carton #___ of ___
- Instructions for storage and handling†
- Place of manufacture (Made in _____)

F. Documentation

Supplier shall provide to Purchaser a copy of the batch record, including all quality assurance documentation for the vaccine being supplied.

† To be provided by Purchaser.

BCG VACCINE

DESCRIPTION
It is a live freeze-dried vaccine made from an attenuated strain of Mycobacterium bovis. It is used for the prevention of tuberculosis.

ADMINISTRATION
For children under one year 0.05 ml and for others 0.1 ml of reconstituted vaccine is given intradermally. Reconstitute only with the diluent supplied by the manufacturer. Special syringes allow administration of the exact dose. A sterile syringe and a sterile needle should be used for each injection. The skin should not be cleaned with antiseptic. Jet injectors do not generally provide a reliable dose of BCG and should not be used. Special care is needed in opening the vials and reconstituting the vaccine so that the vaccine is not blown out of the vial. Because of sensitivity to daylight, the vaccine must be kept in the dark. When withdrawals are made from the vial, the vaccine must be exposed to the light for the minimum period of time, and never for longer than four hours. If not used immediately after reconstitution, the vaccine should be kept on ice and protected from light, and any opened container remaining at the end of a session (maximum 4 hours) must be discarded. Skin testing with tuberculin is not generally carried out before giving BCG, but when performed, those who are found to be positive reactors need not be immunized.

IMMUNIZATION SCHEDULE
BCG should be given routinely to all infants at risk of early exposure to the disease. For maximum protection, this vaccine should be given as soon after birth as possible. It can be given at the same time as DTP, DT, TT, measles, polio vaccines (OPV and IPV), Hepatitis B and Yellow Fever vaccine.

SIDE EFFECTS
A local reaction is normal after BCG. A small tender red swelling appears at the site of the injection which gradually changes to a small vesicle and then an ulcer in 2 - 4 weeks. The reaction usually resides within two to five months and in practically all children leaves a superficial scar 2-10 mm in diameter. Rarely, the nodule may persist and ulcerate.

Occasionally, enlargement of axillary lymph nodes may appear in 2 - 4 months following immunization. Inadvertent subcutaneous injection produces abscess formation and may lead to ugly retracted scars.

CONTRAINDICATIONS
The vaccine is contraindicated in those with cell-mediated immune deficiency. Keloid and lupoid reactions may occur at the site of injection and such children should not be revaccinated.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED INFANTS: HIV infected, non symptomatic infants should be immunized with BCG vaccine according to standard schedules. Infants with clinical (symptomatic) AIDS should not receive BCG vaccine (but should receive other EPI vaccines).

STORAGE
BCG vaccine should be stored and transported between 0° and +8°C. It is even more stable if stored in temperatures as low as -20°C. The diluent should not be frozen but kept cool. Protect the vaccine from the light.

PRESENTATION
The vaccine comes in boxes of ____ ampoules/vials each containing ____ doses.

BCG Vaccine

» BCG Vaccine conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a dried, living culture of the bacillus Calmette-Guérin strain of *Mycobacterium tuberculosis* var. *bovis*, grown in a suitable medium from a seed strain of known history that has been maintained to preserve its capacity for conferring immunity. It contains an amount of viable bacteria such that inoculation, in the recommended dose, of tuberculin-negative persons results in an acceptable tuberculin conversion rate. It is free from other organisms, and contains a suitable stabilizer. It contains no antimicrobial agent.

NOTE—Use the Vaccine immediately after its constitution, and discard any unused portion after 2 hours.

Packaging and storage—Preserve in hermetic containers, preferably of Type I glass, at a temperature between 2° and 8°.

Expiration date—The expiration date is not later than 6 months after date of issue, or not later than 1 year after date of issue if stored at a temperature below 5°.

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Subpart E—Bacillus of Calmette and Guerin (BCG) Vaccine

SOURCE: 44 FR 14545, Mar. 13, 1979, unless otherwise noted.

§ 620.40 BCG Vaccine.

(a) *Proper name and definition.* The proper name of this product is BCG Vaccine. The product is defined as a freeze-dried preparation containing viable bacteria of the Bacillus of Calmette and Guerin, which is an attenuated strain of *Mycobacterium bovis.*

(b) *Criteria for an acceptable strain.* The source of the BCG strain used in the manufacture of any lot of the final product must be identified by complete historical records.

(1) *Seed lot system.* The BCG strain must be maintained in the form of a primary seed lot that is to be the basic material from which all secondary seed lots are prepared. Production of BCG Vaccine may be from either primary or secondary seed lots. Each seed lot must be stored in either a freeze-dried state at −20° C or colder, or in a frozen state at −70° C or colder.

(2) *Freedom from virulence.* The BCG strain is demonstrated to be incapable of producing progressive tuberculosis in guinea pigs tested as prescribed in § 620.45, except that no fewer than 48 guinea pigs must be used to test the primary seed lot and no fewer than 12 guinea pigs must be used to test each secondary seed lot. At least two-thirds of the animals must survive the observation period of no less than 6 months.

(3) *Induction of tuberculin sensitivity in guinea pigs.* Each of at least 10 guinea pigs is to be injected with 1 human dose of BCG Vaccine and, within 4 to 6 weeks after vaccination, skin tested with tuberculin. At least 80 percent of the guinea pigs tested must develop tuberculin sensitivity, as prescribed in § 620.44(b)(3)(ii).

(4) *Clinical information.* Clinical data must establish that the BCG strain is safe and induces tuberculin sensitivity. After having passed all laboratory tests prescribed for BCG Vaccine, each primary and secondary seed lot of vaccine must be tested for its ability to induce sensitivity in tuberculin-negative persons. Only those persons tested by injection of 5 U.S. Tuberculin Units, Purified Protein Derivative, by the Mantoux technique and found negative in this test are to be selected for clinical trials. At least 100 tuberculin-negative persons must be included in the test of the primary seed lot, and at least 20 tuberculin-negative persons must be included in the test of each secondary seed lot. Within 6 to 8 weeks after BCG vaccination, the vaccinees must be tested for tuberculin reactivity by injecting not more than 10 U.S. Tuberculin Units, Purified Protein Derivative, by the Mantoux technique. The test is considered satisfactory if a least 90 percent of those persons from each group develop tuberculin reactivity as indicated by an induration reaction of at least 5 millimeters in diameter.

§ 620.41 Establishment and personnel requirements.

In addition to the applicable requirements of §§ 600.10 and 600.11 of this chapter, the following practices and procedures are required:

(a) *Isolation of BCG unit.* (1) A BCG unit is defined as the space used for storage of primary and secondary seed cultures and for vaccine preparation, including culture maintenance, media inoculation for propagation, harvesting, filling into final containers, sealing of final containers, media production, and cleaning and sterilization of glassware. For purposes of these additional standards, the space used for incubation of bulk and final container sterility tests, tests to determine the numbers of colony-forming units, animal tests, and necropsies are not part of the BCG unit.

(2) The BCG unit must be completely isolated from other production and surrounding areas and must be situated

and designed to prevent contamination of the product. It must have a separate facility for ventilation, designed to prevent contamination of the product. The facilities for water supply and sewage and trash disposal must be designed to prevent microbial contamination of the BCG unit. The equipment used in BCG Vaccine production must remain in the BCG unit at all times.

(3) Microbial controlled areas must be available for handling the BCG cultures. No cultures of microorganisms other than the BCG production strain are permitted in the BCG unit. No animals are permitted in the BCG unit. All tests necessary for the control of the vaccine, in which contaminating microorganisms may be cultured, or in which animals are used, must be conducted in space physically separated from the BCG unit.

(b) *Restrictions on personnel.* (1) A staff specially trained in maintaining the seed cultures, propagating the cultures, preparing the vaccine, and filling the vaccine into final containers shall be employed in the production of the BCG Vaccine. Such personnel shall not work with other infectious agents in any laboratory at any time and shall not be exposed to a known risk of tuberculosis. Within 30 days before employment in the BCG unit, each person working in the unit shall have had a medical examination, including a tuberculin skin test with 5 U.S. Tuberculin Units, Purified Protein Derivative, by the Mantoux procedure, and a chest X-ray. No person who has had a history of tuberculosis or mycobacterial disease is permitted in the BCG unit. There must be periodic medical examinations of BCG unit personnel, including X-ray examinations, of sufficient frequency to detect the appearance of early active tuberculosis. Repeated tuberculin skin testing of staff who are negative to tuberculin may be used as an additional diagnostic aid in isolating any potential source of tuberculosis exposure. If a person working in the BCG unit develops active tuberculosis, (i) the entire staff shall be examined for possible tuberculosis infection, (ii) all current vaccine preparations and all cultures with which the person may have come into contact since his or her last satisfactory medical examination, except cultures sealed before that examination, must be discarded, and (iii) the BCG unit and all equipment with which the person may have come in contact must be decontaminated.

(2) Personnel shall wear protective clothing and use protective devices to the extent necessary to protect the product from contamination.

(3) Any person not assigned to the BCG unit shall not be allowed into the BCG unit at any time unless a medical examination shows the person to be free from mycobacterial disease.

§ 620.42 Production.

(a) *BCG inoculum.* The inoculum of BCG used for seed lot or production of final lot in seed buildup must have been removed from the preceding seed lot in accordance with the following passage and time schedule:

(1) No more than 3 passages from primary to secondary seed lot within a 2-month period.

(2) If no secondary seed lot is used, no more than 9 passages from primary seed lot to final lot within a 6-month period.

(3) No more than 9 passages from secondary seed lot to final lot within a 6-month period.

(b) *Propagation of bacteria.* The culture medium for propagation of BCG Vaccine must not contain ingredients known to be capable of producing allergenic effects in humans or of causing the bacteria to become virulent for guinea pigs. The growth in each container must be examined visually, and only those cultures that have the typical growth pattern characteristic of BCG are to be used in a vaccine.

(c) *Colony-forming units (CFU) before and after freeze-drying.* Each lot of BCG Vaccine must be tested to determine the number of CFU per individual final container both before and after freeze-drying, by the method prescribed in § 620.44(a). The upper and lower limits of the viable count are to be established by the manufacturer of the vaccine for the particular route of administration recommended and must be specified in the license application. The loss in viability after drying must not exceed 90 percent.

§ 620.43 Reference BCG Vaccine.

A reference BCG Vaccine, for use in determining the validity of the test for colony-forming units, is to be obtained from the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892.

[44 FR 14545, Mar. 13, 1979, as amended at 49 FR 23834, June 8, 1984; 51 FR 15610, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

§ 620.44 Potency tests.

(a) *Colony-forming units (CFU).* The number of CFU must be determined on the contents of each of at least 10 individual final containers of each lot of BCG Vaccine. Of the 10 or more individual final containers, the contents of at least 5 before, and an equal number after, freeze-drying must be tested. Final containers of the freeze-dried vaccine are to be reconstituted as for human use with the diluent recommended by the manufacturer. The number of CFU to be reported for each lot of BCG Vaccine must be determined only from test tubes containing between 10 and 50 CFU. Dilutions must be made as follows:

(1) Dilutions are made from an appropriate volume of the liquid vaccine before freeze-drying or the reconstituted vaccine after freeze-drying. Appropriate dilutions are made with modified Youman's medium specified in paragraph (a)(4) of this section, up to a point where subsequent serial half-log dilutions will result in at least 1 tube containing between 10 and 50 CFU.

(2) Serial half-log dilutions are made in 16×125 millimeter screw-capped test tubes into which 4.5 milliliter aliquots of the diluent prescribed in paragraph (a)(4) of this section have been dispensed. Two milliliters of thoroughly mixed vaccine are added to the first tube of the half-log series, mixed thoroughly, and 2.0 milliliters from this tube are transferred to the next tube in the series. The process of mixing and serially transferring 2.0 milliliters is repeated through each consecutive tube and 2.0 milliliters are discarded from the last tube.

(3) After the serial half-log dilutions are completed, 0.5 milliliter of 1.5 percent agar solution that has been cooled to 42° C is quickly added, where necessary, to make a final concentration of 0.15 percent agar, and the contents of the tubes are thoroughly mixed. After mixing, all tubes are incubated at 35° to 37° C for 3 to 4 weeks.

(4) The composition of modified Youman's medium with bovine albumin is as follows:

Asparagine	5.0 grams.
Monopotassium phosphate (KH_2PO_4).	Do.
Potassium sulfate (K_2SO_4)	0.5 grams.
Magnesium citrate	1.5 grams.
Monosodium glutamate	19.0 grams.
Glycerine	20.0 milliliters.
Distillled water q.s. to	900.0 milliliters.

One hundred milliliters of 5-percent aqueous solution of bovine albumin that has been sterilized by filtration are added to the Youman's medium to produce a final concentration of 0.5 percent of bovine albumin. The pH is adjusted to 7.0 with 5*N* sodium hydroxide.

(b) *Intradermal guinea pig test.* Two or more guinea pigs, each weighing no less than 250 grams, must be injected intradermally in 4 different sites with the following amounts and dilutions of each lot of BCG Vaccine:

(1) Vaccine intended for intradermal injection is reconstituted as for human use with the diluent recommended by the manufacturer. One-tenth milliliter of reconstituted vaccine and 0.1 milliliter each of three ten-fold dilutions (1:10, 1:100, and 1:1000) of the reconstituted vaccine are injected into the guinea pigs. The diluent for the ten-fold dilutions is isotonic solution for injection.

(2) Vaccine intended for percutaneous injection into humans is reconstituted with the diluent recommended by the manufacturer so that at least one human dose (estimated to be within a range of from 1 to 33×10^5 CFU) is contained in 0.1 milliliter. A narrower range of CFU is determined for each specific vaccine by the manufacturer and specified in the license application. One-tenth milliliter of the selected dose of vaccine and 0.1 milliliter each of three ten-fold dilutions (1:10, 1:100, and 1:1000) are injected into the guinea pigs. The diluent for the ten-fold dilutions is an isotonic solution for injection.

(3) The lot of vaccine is satisfactory if:

(i) At the end of 2 to 4 weeks after BCG vaccination, nodules have developed that are graded in relation to the amount of the test dose, with the largest dose inducing a nodule of from 4 to 10 millimeters in diameter and the smallest dose inducing essentially no nodule, and all observations are read on the same day;

(ii) By the end of 4 to 6 weeks after BCG vaccination, each guinea pig shows a degree of sensitivity such that an intradermal injection of no greater than 25 U.S. Tuberculin Units, Purified Protein Derivative, in 0.1 milliliter will induce an erythematous reaction at least 10 millimeters in diameter within 18 to 24 hours; and

(iii) At the end of the test period, each guinea pig is weighed and each shows a weight increase.

(c) *Induction of tuberculin sensitivity in tuberculin-negative humans.* At least once annually, no less than one lot of BCG Vaccine that has satisfied all requirements and has been released by the Center for Biologics Evaluation and Research must be tested for its ability to induce sensitivity in 20 persons negative to Tuberculin, Purified Protein Derivative, as prescribed in § 620.40(b)(4). The results of these tests must be sent to the Director, Center for Biologics Evaluation and Research, as they are completed.

[44 FR 14545, Mar. 13, 1979, as amended at 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§ 620.45 Test for freedom from virulent mycobacteria.

(a) Each lot of BCG Vaccine must be tested to determine that it does not contain virulent mycobacteria. The test must be performed using at least 6 guinea pigs, each weighing between 250 and 300 grams. Vaccine intended for intradermal injection in humans must be tested by injecting into guinea pigs the number of bacteria contained in at least 50 human doses. Vaccine intended for percutaneous use in humans must be tested by injecting into guinea pigs 50 times the number of bacteria estimated to be introduced parenterally into humans by the recommended procedure. The vaccine for all tests must be inoculated subcutaneously or intramuscularly into the guinea pigs. All animals that die during the observation period must be examined post-mortem. All animals that survive the observation period must be sacrificed and examined post mortem. The lot passes the test if at least two-thirds of the animals on test survive an observation period of not less than 6 weeks, and if the post-mortem examination reveals no evidence of tuberculosis in any of the test animals.

(b) If any virulent mycobacteria are found in any lot of BCG Vaccine, whether or not the manufacturer intends to submit samples and protocols of this lot to the Center for Biologics Evaluation and Research for release, the following actions must be taken:

(1) In addition to the requirements of §§ 600.12 and 600.14 of this chapter, the manufacturer shall immediately report by telephone, telegraph, or cable the finding of virulent mycobacteria to the Director, Center for Biologics Evaluation and Research.

(2) All production and distribution of lots of BCG Vaccine produced from the same secondary seed lot as the contaminated lot of BCG Vaccine must be discontinued. If no secondary seed lot is used the same requirements apply to the primary seed lot.

(3) The manufacturer shall conduct a thorough and prompt investigation concerning the failure of the lot to meet the required safety and purity specifications, including retesting the suspect lot and the source secondary seed lot (or primary seed lot, if no secondary seed lot is used) and shall undertake a thorough review of all manufacturing records and procedures to determine the probable cause of the failure.

(4) A written record of the investigation, including the retest results, must be submitted to the Director, Center for Biologics Evaluation and Research.

(5) Neither production nor distribution of BCG Vaccine may be resumed until the manufacturer is notified in writing by the Director, Center for Biologics Evaluation and Research, that such activity may be resumed.

[44 FR 14545, Mar. 13, 1979, as amended at 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§ 620.46 General requirements.

(a) *Dose.* These standards are based on (1) vaccine intended for intradermal injection in a single human immunizing dose of 0.1 milliliter and (2) vaccine intended for percutaneous injection in a single skin application through which inoculation is made by a multiple puncture device.

(b) *Date of manufacture.* The date of manufacture is the date of initiation of the last valid determination for CFU after freeze-drying.

§ 620.47 Labeling.

In addition to conforming to the applicable requirements of §§ 610.60, 610.61, and 610.62 of this chapter, the package label must bear the following information:

(a) Specification of the route of administration.

(b) A statement that the vaccine contains live bacteria and should be protected against exposure to light.

(c) A statement that the vaccine must be administered within 8 hours after reconstitution, and that reconstituted vaccine not used within 8 hours must be discarded.

§ 620.48 Samples; protocols; official release.

(a) For each lot of vaccine, the following materials must be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892.

(1) Samples and diluent that will provide at least 20 milliliters when the samples are reconstituted as recommended in the package insert by the manufacturer of the vaccine.

(2) A protocol that consists of a complete summary of the manufacture of each lot, including all results of each test required by all applicable regulations. If the protocol is not included in the shipment of the samples, it must be sent promptly to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892.

(b) The BCG Vaccine must not be issued by the manufacturer until written notification of official release is received from the Director, Center for Biologics Evaluation and Research, Food and Drug Administration.

[44 FR 14545, Mar. 13, 1979, as amended at 49 FR 23834, June 8, 1984; 51 FR 15610, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

Annex 2

REQUIREMENTS FOR DRIED BCG VACCINE

(Requirements for Biological Substances No. 11)
(Revised 1985)

	Page
Introduction	60
General considerations	61
Part A. Manufacturing requirements	66
1. Definition	66
2. General manufacturing requirements	67
3. Production control	69
4. Filling and containers	74
5. Control tests on final lot	75
6. Records	79
7. Samples	80
8. Labelling	80
9. Distribution and transport	80
10. Storage and expiry date	81
Part B. National control requirements	82
1. General	82
2. Release and certification	83
3. Clinical surveillance of the vaccine in man	84
Authors	84
Acknowledgements	85
References	86
Appendix 1. Summary protocol for dried BCG vaccine production and testing	87

INTRODUCTION

The formulation of international requirements for the manufacture and control of BCG vaccine was first considered by the WHO Expert Committee on Biological Standardization in its thirteenth report (*1*). In its fourteenth report (*2*), the Committee requested WHO to make arrangements as soon as possible for the formulation of such requirements. These requirements were approved by the WHO Expert Committee on Biological

Standardization at its eighteenth meeting and appeared as Annex 1 to its report (*3*).

Subsequently, a number of developments took place in the production and control of BCG vaccine. The majority of manufacturers switched to the production of freeze-dried vaccine. Much more became known about the production of freeze-dried vaccines from seed lots, which were shown to yield satisfactory vaccines, and consideration was given to the different routes and methods of administration. Furthermore, careful consideration was also given to the dose of living organisms that causes tuberculin sensitivity without giving rise to untoward reactions.

In the light of these developments, revised requirements were drafted in 1978, taking into account the regulations and requirements for manufacture and control of dried BCG vaccine in a number of countries, as well as information from published and unpublished reports. The 1978 revision of the requirements was approved by the WHO Expert Committee on Biological Standardization and appeared as Annex 2 in its thirtieth report (*4*).

Since then, further experience in production and control has been gained, which has made the current review necessary. It should be added, however, that the main substance of these requirements is still the same as that of the 1978 revision.

GENERAL CONSIDERATIONS

BCG vaccine has been used for many years. In spite of contradictory results obtained in field trials, there is considerable evidence that immunization with BCG vaccine can give a substantial degree of protection against tuberculosis, and that the inconveniences and risks associated with vaccination are very small (*5*).

Recently, increased emphasis has been given to vaccination as early in life as possible, and BCG vaccination has been included in the WHO Expanded Programme on Immunization.

The formulation of the original international requirements for dried BCG vaccine was complicated by the following: (*a*) a number of different daughter strains derived from the original strain of the bacillus of Calmette and Guérin are used in vaccine manufacture; (*b*) a number of different manufacturing and testing procedures are employed; (*c*) there is no approved laboratory method of assaying

the protective potency of vaccines against tuberculous infection in man; (*d*) vaccines with different total bacterial content and number of culturable particles are produced; and (*e*) vaccines intended for administration by different routes are prepared. Although much is known about the characteristics of a good vaccine, several of these difficulties remain.

Scope of these requirements

These revised requirements refer to dried BCG vaccine prepared from strains derived from the bacillus of Calmette and Guérin. Where BCG vaccine is issued in liquid form, it is entirely under the responsibility of the National Control Authority. In that case, only the relevant parts of these requirements apply. These requirements have been formulated primarily to cover vaccines intended for intradermal and percutaneous administration.

The latest findings from controlled field trials have been taken into consideration in formulating these revised requirements, but there are still a number of characteristics needing further study. In particular, the maximum dose of viable organisms in the various vaccines that can be tolerated without producing an unacceptably high incidence of untoward reactions must be determined by careful studies in the various age groups. Guidance may be given by WHO on these matters, but the studies must be carried out in the communities in which the vaccines are to be used.

BCG strains

Various daughter strains, all derived from the bacillus of Calmette and Guérin, are at present being used by manufacturing establishments in the preparation of BCG vaccine. While these strains differ from one another to minor degrees when investigated in the laboratory, there is no evidence to suggest that they differ significantly in their power to protect man. There is no strain that can be said to be preferable to others or that is known to be definitely superior to the others. Only seed lots that have been shown to be acceptable by laboratory and clinical tests should be used. Several such seed lots are available through WHO. It is believed that a suitable seed lot of BCG should yield vaccines that give protection in experimental animals, produce a relatively high level of tuberculin

sensitivity in human beings, and have an acceptable (low) frequency of untoward reactions.

Most of the early trials that showed BCG to be effective in man were carried out with liquid vaccines, but there is new strong circumstantial evidence that freeze-dried vaccines are similarly effective.

In view of these considerations, a major controlled trial of some of the most frequently used strains of BCG would be of value. The aim of this trial should be to re-establish the properties of seed lots that are suitable for the preparation of a satisfactory freeze-dried BCG vaccine. National laboratories and international organizations should take up this research problem, using vaccines prepared for administration to human beings; vaccines prepared from seed lots for which there is little information concerning the safety and efficacy should be tested in man only with great caution.

Some manufacturers of dried vaccine have modified their seed lot strain to make it more suitable for their particular production procedure. The seed lots prepared in this way may not retain the same immunogenic properties, and should be used only with the approval of the national control authority.

The selection of the seed lot should be governed by practical criteria relevant to the use of a reference preparation in the laboratory as well as in field studies.

In practice, BCG seed lots may generally be investigated in man only for their properties of producing tuberculin sensitivity and vaccination lesions. The former should be measured by the distribution of tuberculin reactions according to size in persons vaccinated with a given dose of BCG vaccine. A low dose of tuberculin should be employed (e.g., 5 IU of International Standard for Purified Protein Derivative (PPD) of Mammalian Tuberculin, or 2 tuberculin units (TU) of the batch of PPD RT23 with Tween 80).

Field observations should be made in conjunction with laboratory studies in animals. The latter should include protection tests, tests of vaccination lesions, and tests for tuberculin conversion. Immunizing efficacy should be measured in terms of degree of protection afforded to the test animals against a challenge with fully virulent *Mycobacterium tuberculosis*. Sensitizing efficacy should be measured by the average dose of vaccine that will convert a negative tuberculin reaction in guinea-pigs to a positive one, as well as by the speed with which such conversion is effected. In these animal tests, the inclusion, for comparative purposes, of a reference vaccine

prepared from a seed lot known to be effective in animals and man is recommended. Such a reference vaccine is available from WHO.

Potency tests

There is some evidence that BCG seed lots that have been shown to produce vaccines with protective potency in laboratory animals and tuberculin sensitivity in man will give effective protection against tuberculosis in human beings. However, at the present time there is no established laboratory test that can be used in practice to compare the protective efficacy of different vaccines.

The laboratory tests at present in use and included in these requirements are designed to ensure that new vaccine lots do not differ appreciably from those that have already been shown to be effective in man as regards their ability to induce adequate sensitivity to tuberculin, or from a reference vaccine shown to be safe and effective in man. At present, for batch control purposes, much reliance is placed on tests for the estimation of the total bacterial content and for the number of culturable particles. It is not possible to specify single requirements for the total bacterial content and for the number of culturable particles for all vaccines (*6*), since different seed lots and methods of manufacture may yield different values for these criteria. Although the number of live bacteria in one human dose may differ depending on the strain used for vaccine production, the vaccines have been shown to be similar and of satisfactory properties as regards their ability to induce adequate sensitivity to tuberculin and their safety in man. It is therefore essential that the national control authorities carry out extensive field trials in man and decide on suitable total bacterial contents and on the number of culturable particles for a particular manufacturer's product. The allowed range in the number of culturable particles should not vary over a range wider than 1–4. In addition, it is necessary to perform animal experiments that give an indication of the safety and efficacy of the vaccines to the satisfaction of the national control authority.

Post-vaccination reactions and complications

Vaccines intended for intradermal injection should be given strictly intradermally and vaccinators should be trained accordingly.

A local reaction at the vaccination site is normal after a BCG vaccination. It may take the form of a nodule, which in many cases will break down and suppurate. The reaction developing at the vaccination site usually subsides within 2 5 months and in practically all children leaves a superficial scar of 2 10 mm in diameter. The nodule may persist and ulcerate. Swelling of regional lymph nodes may also be seen, and this may be regarded as a normal reaction. Keloid and lupoid reactions may occur at the site of the vaccination. Children with such reactions should not be revaccinated.

Inadvertent subcutaneous injections produce abscess formations and may lead to ugly retracted scars.

Among the major complications, suppurative lymphadenitis has been observed. In the case of certain vaccines, it has been revealed that there is a strong correlation between the incidence of these complications in newborns and the number of culturable particles in the vaccine. Thus, a reduction of the dose for the newborn may reduce these complications to acceptable levels. It is recommended that the dose for babies should be one-half to one-quarter of that for teenage children or adults. It is required that the concentration of the vaccine should be shown to be effective and tolerated in the age groups for which the vaccine is intended (*7*).

The national health authority should issue guidelines for the treatment of complications.

Presentation and use of the requirements

Each of the following sections constitutes a recommendation. The parts of each section that are printed in normal-size type have been written in the form of requirements, so that, if a health administration so desires, these parts as they appear may be used as definitive national requirements. The parts of each section that are printed in small type concern points on which comments seem desirable.

Should individual countries wish to adopt these requirements as the basis of their national regulations concerning BCG vaccine, it is recommended that a clause should be included permitting modifications of the manufacturing requirements on condition that such modified requirements ensure that the degree of safety and the potency of the vaccine are at least equal to those provided by the requirements formulated below. The World Health Organization should then be informed of the action taken.

The terms "national control authority" and "national control laboratory", as used in these requirements, always refer to the country in which the vaccine is manufactured.

PART A: MANUFACTURING REQUIREMENTS

1. DEFINITION

1.1 International name and proper name

The international name shall be "Vaccinum Tuberculosis (BCG) Cryodesiccatum". The proper name shall be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

1.2 Descriptive definition

Vaccinum Tuberculosis (BCG) Cryodesiccatum is a dried preparation containing live bacteria derived from a culture of the bacillus of Calmette and Guérin, known as BCG, intended for intradermal injection. The preparation shall satisfy all the requirements formulated below. The name of the dried vaccine intended for percutaneous vaccination, shall be "Vaccinum Tuberculosis (BCG) Cryodesiccatum Percutaneum".

Vaccinum Tuberculosis (BCG) Cryodesiccatum is referred to in this document as "dried BCG vaccine".

1.3 International standard and international reference preparation

An International Reference Preparation of BCG Vaccine and an International Standard for Purified Protein Derivative (PPD) of Mammalian Tuberculin have been established. These preparations are in the custody of the International Laboratory for Biological Standards, State Serum Institute, Copenhagen, and samples are distributed on request to national control laboratories. They are intended to be used for the calibration of the respective national or manufacturers' standard materials.

1.4 Terminology

Seed lot: A quantity of bacteria processed together and of uniform composition. A seed lot shall be maintained in the dried form. In each manufacturing establishment, a primary seed lot is that from which material is drawn for inoculating media for the preparation of secondary seed lots or single harvests.

Secondary seed lot: A working seed lot, prepared from the primary seed lot by as few cultural passages as possible, having the same characteristics as the primary seed lot and intended for inoculating media for the preparation of single harvests.

Single harvests: A quantity of bacteria harvested from cultures prepared in a single batch of medium and processed together. Single harvests shall be prepared from cultures originating from a seed lot by as few cultural passages as possible, and by not more than 12 passages from the primary seed lot.

Final bulk: The homogeneous finished vaccine present in a single container. It may then be filled either directly or through one or more intermediate containers.

Vaccine lot (vaccine batch): Vaccine in final containers from a single final bulk, subsequently processed together and having therefore, a uniform composition before drying.

Filling lot: A collection of filled final containers that have been filled during one working session from a single final bulk and that are homogeneous with respect to the risk of contamination during filling.

Final lot: A final lot is a collection of sealed final containers from one filling lot, all of which have been dried together in the same vacuum chamber.

2. GENERAL MANUFACTURING REQUIREMENTS

The general manufacturing requirements contained in the revised *Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)* (*8*) shall apply to establishments manufacturing BCG vaccine, with the addition of the following.

Production of BCG vaccine shall take place in completely separate areas, by means of separate equipment. Such areas shall be so situated and ventilated that the hazard of contamination is reduced to a minimum. No animals shall be permitted in the vaccine

production areas. Tests for the control of the vaccine that require cultures to be made of contaminating microorganisms shall be carried out in a completely separate area. If cultures other than for BCG production are seeded in the production area they should be removed immediately for incubation in another area. Tests in which animals are used shall also be carried out in a completely separate area.

For the purposes of these requirements, the processes of vaccine production that must take place in isolated facilities are all operations up to and including the sealing of the vaccine in the final containers. The packaging may be done in a common area.

In some countries, the production of BCG vaccine—although isolated—is carried out in a building in which other work takes place. This should be done only after consultation with, and with the approval of, the national control authority. If production takes place in part of a building, the work carried on in other parts of the building should be of such a nature that there is no possibility of hazard to the BCG vaccine.

No cultures of microorganisms other than strains of BCG approved by the national control authority for vaccine production shall be introduced into the manufacturing areas. In particular, no strains of other mycobacterial species, whether pathogenic or not, shall be permitted in the BCG vaccine production area.

BCG is susceptible to sunlight. Therefore, the procedures for the preparation of the vaccine shall be so designed that all cultures and vaccines are protected from daylight and ultraviolet light at all stages of manufacture, testing, and storage, until the vaccine is issued.

BCG vaccine shall be produced by a staff consisting of healthy persons who do not work with other infectious agents; in particular, they shall not work with virulent strains of *M. tuberculosis*, nor shall they be exposed to a known risk of tuberculous infection. Precautions shall be taken also to ensure that no worker shall be employed in the preparation of BCG vaccine unless he or she has been shown by medical examination to be free from tuberculosis. The scope and nature of the medical examination shall be at the discretion of the national control authority, but it shall include a radiological examination and shall be repeated at intervals or when there is reason to suspect illness.

The frequency with which the radiological examination should be carried out is at the discretion of the national control authority. It is advisable to keep radiation exposure to a minimum, but the examination should be of sufficient frequency to detect the appearance of early active tuberculosis. It is estimated that, if workers in BCG vaccine laboratories were given one or two conventional X-ray examinations of the chest each year, not using fluoroscopic methods, and if the best available techniques were employed to minimize the radiation dose, the doses received would be considerably lower than the maximum permissible doses for workers occupationally exposed to radiation that have been set by the International Commission on Radiological Protection (*9*, *10*).

Should an examination reveal signs of tuberculosis or suspected tuberculosis in a worker, he or she shall no longer be allowed to work in the production areas and the rest of the staff shall be examined for possible tuberculous infection. In addition, all cultures[1] shall be discarded and the production areas decontaminated. If it is confirmed that the worker has tuberculosis, all vaccine made while he or she was in the production areas shall be discarded.

Suitable clothing shall be worn in the production areas.

Persons not normally employed in the production areas shall be excluded from them unless, after a medical examination, including radiological examination, they are shown to be free from tuberculosis. In particular, persons working with mycobacteria other than the BCG seed strain shall be excluded at all times.

Written descriptions of procedures for the preparation of BCG vaccine shall be submitted for approval to the national control authority. Proposals for modification shall be submitted for approval to the national control authority before their implementation.

3. PRODUCTION CONTROL

3.1 Control of source materials

3.1.1 *Seed lot system*

The production of vaccine shall be based on the seed lot system (*11*). A seed lot prepared from a strain approved by the national control authority (see Part B, section 1.1) shall be prepared under conditions satisfying the requirements of Part A, sections 2 and 3.

[1] In this respect, the freeze-dried seed, in ampoules sealed by fusion of the glass, is not regarded as a culture.

A seed lot should have been shown in field trials in man to induce adequate sensitivity to tuberculin and to be safe; also, it should have been tested for ability to produce protection against tuberculous infection in animals.

It is recommended that a large primary seed lot should be set aside under optimum storage conditions as the basic material to which the manufacturer may return for the preparation of further seed lots. This primary seed lot may be part of a vaccine lot that has been shown in extensive field trials in man to induce adequate sensitivity to tuberculin, with only a low frequency of untoward effects, and, in laboratory experiments in animals, to protect against tuberculous infection.

If a secondary seed lot is being used, the total number of passages for a single production harvest should not exceed 12. For example, if the secondary seed lot is four culture passages removed from the primary seed lot, the number of passages from the secondary seed lot must not exceed eight.

3.1.2 *Tests on seed lot*

The seed lot used for the production of vaccine shall be tested according to the requirements of Part A, sections 5.1, 5.2, and 5.3. However, the test for absence of virulent mycobacteria, described in Part A, section 3.4.3, shall be made in at least 10 guinea-pigs. The dose of the seed lot to be used in this test shall contain a number of culturable particles equivalent to that injected when a vaccine lot is tested. The guinea-pigs shall be observed for at least 6 months. At the end of the observation period, the animals shall be sacrificed and examined post-mortem for macroscopic evidence of progressive tuberculous disease; similarly, any animals that die before the end of the observation period shall be subjected to post-mortem examination. If none of the animals shows signs of progressive tuberculosis and at least 60% of them survive the observation period, the seed lot shall be considered to be free from virulent mycobacteria.

In addition, it is advisable to test all primary seed lots for protective potency in animals. In such tests, the material, when injected into guinea-pigs and mice in a range of doses under reproducible conditions, should afford a measure of protection, against a range of challenge doses of a fully virulent human strain of *M. tuberculosis*, not less than that specified by the national control authority in terms of a national reference preparation such as the one described in Part A, section 1.3.

In some countries, an additional test is included to ensure that the first batch of vaccine produced from a new ampoule of the seed lot gives rise to tuberculin sensitivity in guinea-pigs. It is desirable also that each seed lot be tested to demonstrate that its degree of sensitivity to antituberculosis drugs has not changed.

3.1.3 *Production culture medium*

The production culture medium shall contain no substances known to cause toxic or allergic reactions in human beings. Substances used in that medium shall meet such specifications as the national control authority may prescribe.

3.2 Production precautions

The general precautions formulated in the requirements of Part A, section 3, of the revised *Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)* (*8*) shall apply to the manufacture of BCG vaccine, with the addition of the following.

Effective precautions shall be taken to prevent the accidental mixing of containers from separate lots of vaccine at all stages of production up to the stage at which final lots are labelled.

3.3 Control of single harvests

The cultures in each container shall be examined visually, and any that have grown in an uncharacteristic manner shall not be used for vaccine production.

3.4 Control of final bulk

3.4.1 *Final bulk*

The final bulk shall be prepared from a single harvest or by pooling a number of single harvests.

3.4.2 *Test for absence of contaminating microorganisms*

The final bulk shall be tested for bacterial and mycotic contamination in accordance with the requirements given in Part A,

section 5, of *Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances)* (*12*), and no vaccine lot shall be passed for use unless the final bulk has been shown to be free from such contamination.

3.4.3 *Test for absence of virulent mycobacteria*

A sample of the final bulk intended for this test shall be stored at 4 °C for not more than 72 hours after harvest at 4 °C.

At least six guinea-pigs, all of the same sex, each weighing between 250 and 400 g are used.

> Some countries prefer to use guinea-pigs that have been shown previously to be tuberculin-negative.

A dose of BCG organisms corresponding to at least 50 human doses of vaccine intended for intradermal injection shall be injected into each guinea-pig by the subcutaneous or intramuscular route.[1] The animals shall have been maintained on a diet that is free from added substances, such as antibiotics, that might interfere with the test. The guinea-pigs shall be observed for at least 6 weeks. If, during that time, they remain healthy and gain weight, the final bulk shall be considered to be free from virulent mycobacteria.

At the end of the observation period, the animals shall be sacrificed and examined post-mortem for macroscopic evidence of progressive tuberculous disease; similarly, any animals that die before the end of the observation period shall be subjected to a post-mortem examination.

The vaccine lot shall pass the test if none of the guinea-pigs shows evidence of progressive tuberculous disease and if at least two-thirds of them survive the observation period.

Should more than one-third of the guinea-pigs die during the observation period (and freedom from progressive tuberculous disease is verified), the test shall be repeated on at least 6 more guinea-pigs.

If, on the second occasion, more than one-third of the animals fail to survive the observation period, the vaccine lot shall not pass the test.

[1] When a more concentrated vaccine, intended for administration by the percutaneous route, is tested, a dilution factor approved by the national control authority shall be applied so that the mass of BCG injected corresponds to 50 human doses of intradermal vaccine.

> Should a vaccine lot fail to satisfy the requirements of this test because animals die from causes other than tuberculosis, the procedure to be followed by the manufacturer should be determined with the approval of the national control authority.

If evidence of progressive tuberculous disease is seen, the vaccine lot shall be rejected, all subsequent vaccine lots shall be withheld, and all current vaccine stocks shall be held pending further investigation. The manufacture of BCG vaccine shall be discontinued and it shall not be resumed until a thorough investigation has been made and the cause or causes of the failure determined. Production shall be allowed to resume only upon the approval of the national control authority.

3.4.4 *Test for bacterial concentration*

The bacterial concentration of the final bulk shall be estimated by a method approved by the national control authority and shall have a value within a range approved by the national control authority (see Part B, section 1.2).

> The estimation of total bacterial content may be made either directly, by determining the dry weight of the organisms, or indirectly, by an opacity method that has been calibrated in relation to the dry weight of the organisms.
>
> It is desirable that only one method of estimation be used for all the vaccine lots produced by a manufacturer.
>
> If an opacity method is used, the International Reference Preparation of Opacity,[1] or an equivalent reference preparation approved by the national control authority, may be employed in comparative tests.

3.4.5 *Test for number of culturable particles*

The number of culturable particles of each final bulk shall be determined by an appropriate method approved by the national control authority.

> The medium used in this test should be such that the number of culturable particles may be determined not more than 4 weeks after the medium has been inoculated with dilutions of the vaccine.

[1] The International Reference Preparation of Opacity is in the custody of the International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Hampstead, London NW3 6RB, England, which supplies samples on request.

There are various methods of determining the number of culturable particles in BCG vaccine, and it is desirable that only one method be used for all the vaccine lots produced by a manufacturer (6). It is also desirable that the test be carried out in parallel with a national reference preparation prepared from a strain similar to that used for the production of the vaccine.

3.4.6 *Oxygen uptake test*

Some manufacturers use a measurement of oxygen uptake rate (6) as a speedy test on the final bulk vaccine to get some indication of the viability of the batch.

Usually 30–120 mg (semi-dry weight) of the final bulk is tested in a Warburg apparatus, and the respiration rate is measured over an hour or more. Other methods of measuring oxygen uptake include polarographic methods.

3.4.7 *Substances added to the final bulk*

Substances used in preparing the final bulk, or added to it, shall meet such specifications as the national control authority may prescribe.

Substances added to improve the efficiency of the freeze-drying process or to aid the stability of the dried product shall be sterile and of high and consistent quality, and shall be used at suitable concentrations in the vaccine.

4. FILLING AND CONTAINERS

The requirements concerning filling and containers given in Part A, section 4, of the *Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)* (8) shall apply, with the addition of the following.

It shall have been demonstrated to the satisfaction of the national control authority that the containers are made from materials that have no deleterious effect on the vaccine.

The containers should be in a form that renders the process of reconstitution as simple as possible. Their packaging should be such that the reconstituted vaccine is protected from daylight. It is desirable that the capacity of the final containers be large enough for the full volume of the reconstituting fluid to be added.

Single-dose and multiple-dose containers may be used; the latter should be of a type that will not encourage storage of the reconstituted vaccine. If vials are used, special attention should be paid to the requirements of Part A, section 8.

All containers of the final vaccine shall be sterilized before filling.

Provision shall be made for adequate and continuous mixing of the final bulk during filling into the final containers.

As soon as possible after harvesting, the vaccine shall be filled into containers and freeze-dried.

Containers shall be hermetically sealed, either under vacuum or after filling with pure (especially oxygen-free), dry nitrogen or any gas not deleterious to the vaccine. It shall be shown to the satisfaction of the national control authority that the vaccines stored under a gas have satisfactory stability.

All containers sealed under vacuum shall be tested for leaks not less than 2 months after sealing, and all defective containers shall be discarded.

In some countries, the test for leaks is repeated after further storage. The most convenient time to do this is immediately before distribution.

5. CONTROL TESTS ON FINAL LOT

Tests on the final lot shall be performed after reconstitution. The fluid supplied or recommended for reconstitution shall be used, unless such fluid would interfere with any of the tests, in which case some other suitable fluid shall be used. The vaccine shall be reconstituted to the concentration at which it is to be used for injection into human beings.

An exception may be made in the case of the test for absence of virulent mycobacteria (Part A, section 5.3.1), when a higher concentration of reconstituted vaccine may be necessary.

5.1 Identity test

An identity test shall be performed on samples of the vaccine from each final lot.

The identity of each final lot of vaccine shall be verified by the morphological appearance of the bacilli in stained smears and by the characteristic appearance of the colonies grown on solid media.

5.2 Test for absence of contaminating microorganisms

Samples from each final lot shall be tested for bacterial and mycotic contamination according to the requirements given in Part A, section 5, of *Requirements for Biological Substances No. 6 (Requirements for the Sterility of Biological Substances)* (*12*).

5.3 Safety tests

5.3.1 *Test for absence of virulent mycobacteria*

If the test for the absence of virulent mycobacteria, applied to the final bulk, is unsatisfactory, it shall be repeated with a sample of a final lot (see Part A, section 3.4.3).

This test may be applied to each final lot and omitted for the final bulk.

5.3.2 *Test for skin reactions in guinea-pigs*

Some manufacturers include a test of the reactivity of each vaccine lot when injected intradermally into guinea-pigs. The injections consist of 0.1 ml of undiluted vaccine[1] and 0.1 ml each of vaccine dilutions 1:10 and 1:100. At least 4 guinea-pigs are used, all of the same sex and weighing not less than 250 g each. If female guinea-pigs are used, they should not be pregnant.

The lesions produced are observed for at least 4 weeks. The reactions caused should be compared with those caused by a reference preparation approved by the national control authority and that has been shown to induce in man adequate sensitivity to tuberculin with a low frequency of untoward effects.

The same dilutions of the reference preparation and vaccine should be injected into the same guinea-pigs at randomly selected sites.

5.4 Test for total bacterial content

The total bacterial content of the reconstituted vaccine shall be estimated for each vaccine lot by a method approved by the national control authority, and shall have a value within a range approved by the national control authority (see Part B, section 1.2).

[1] Percutaneous vaccine should be diluted (see Part A, section 3.4.3).

The estimation of total bacterial content may be made either directly, by determining the dry weight of organisms, or indirectly, by an opacity method that has been calibrated in relation to the dry weight of the organisms.

It is desirable that one method of estimation should be adhered to for all the vaccine lots produced by a manufacturer.

5.5 Test for viability

5.5.1 *Test for number of culturable particles*

The number of culturable particles of each final lot shall be determined by an appropriate method approved by the national control authority (see Part A, section 3.4.5). The viable count shall have a value within a range approved by the national control authority (see Part B, section 1.2). By comparison with the results of the test for number of culturable particles carried out on final bulk, as described in Part A, section 3.4.5, the percentage survival on freeze-drying may be calculated and this value should be not less than one approved by the national control authority.

The use of a common culture medium may be necessary in order to avoid discrepant results between laboratories.

The survival rate usually varies between 75% and 20%. Clumping occurring after reconstitution of vaccines may result in low survival rate, since it is likely that seeding of one clump of bacilli will give only one colony.

Care should be taken to avoid the presence of large aggregates in BCG vaccines.

5.5.2 *Rapid test for viability*

The bioluminescence reaction occurring in fireflies depends upon the presence of adenosine triphosphate (ATP), luciferin luciferase, oxygen, and magnesium ions. This reaction can be easily reproduced *in vitro* by mixing these components. If all components except ATP are present in excess, the amount of light emitted is proportional to the amount of ATP.

Since ATP is present in all living cells and is immediately destroyed when the cell dies, ATP is a reliable marker for living cells.

Studies (*13*) have shown that measurement of ATP using the bioluminescence reaction can be used to estimate the viable count of freeze-dried BCG vaccine within minutes, as accurately as other time-consuming methods, once the mean content of ATP per culturable particle has been estimated for a given vaccine strain.

5.6 Stability test

Each final lot shall be tested for its degree of stability by a method approved by the national control authority.

The test shall involve the determination of the number of culturable particles before and after the samples have been held at appropriate temperatures and for appropriate periods.

An accelerated degradation test shall be carried out by taking samples of the vaccine and incubating them at 37 °C for 28 days. The percentage decrease in the number of culturable particles is then compared with that of samples of the same vaccine stored at 4 °C. The number of culturable particles in the vaccine after heating shall be not less than 20% of that in the refrigerated vaccine. The absolute value shall be approved by the national control laboratory. The same test shall also be performed with a reference preparation. One method of determining the number of culturable particles shall be adhered to, as suggested in Part A, section 3.4.5.

The purpose of including a reference preparation is to have a check on the quality of the medium used for the determination of the number of culturable particles. It is not intended to adjust the count of the vaccine by comparison with the reference preparation.

All manufacturers shall keep their product for the permitted storage period and shall determine the number of culturable particles from time to time to demonstrate that the number is being maintained at an adequate level.

In some countries, the stability test is carried out only after the vaccine has been stored for 3–4 weeks after drying, since it is considered that the degree of stability during the first 3 weeks may not be related to the long-term stability of the product.

As a guide to stability, some manufacturers of dried BCG vaccine determine the residual water content of the final vaccine, since failure to achieve a certain degree of desiccation results in an unstable product. However, such a test cannot be regarded as an alternative to tests involving the determination of the number of culturable particles.

5.7 Production consistency

The manufacturer shall show to the satisfaction of the national control authority that the method of manufacture ensures a consistent product.

The degree of consistency in producing satisfactory final lots is an important factor in judging the efficacy and safety of a particular manufacturer's product.

The data that shall be considered in determining the consistency of production shall include the results obtained with consecutive vaccine lots when tested as described in Part A, section 5, for example, by the test for total bacterial content (Part A, section 5.4), the test for number of culturable particles (Part A, section 5.5), and the stability test (Part A, section 5.6).

In addition, the examination of morphological appearance of the bacteria in stained smears (Part A, section 5.1) or in unstained microscopic preparations may be used to determine the consistency of bacterial aggregation.

A specified number of consecutive vaccine lots shall have been satisfactorily prepared before any vaccine from a given manufacturer, or resulting from a new method of manufacture, is released. In subsequent routine production, if a specified proportion of vaccine lots or a specified number of consecutive vaccine lot fails to meet the requirements, the manufacture of BCG vaccine shall be discontinued and not be resumed until a thorough investigation has been made and the cause or causes of the failures determined to the satisfaction of the national control authority.

6. RECORDS

The requirements given in Part A, section 6, of the revised *Requirements for Biological Substances No. 1* (*General Requirements for Manufacturing Establishments and Control Laboratories*) (*8*) shall apply, with the addition of the following:

Written records shall be kept of all seed lots, all cultures intended for vaccine production, all single harvests, all final bulk vaccines, and all vaccine in the final containers produced by the manufacturing establishments, including all tests irrespective of their results.

The records shall be of a type approved by the national control authority. An example of a suitable protocol is given in Appendix I, on pages 87–92.

7. SAMPLES

The requirements given in Part A, section 7, of the revised *Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)* (*8*) shall apply.

It is desirable that samples should be retained for at least one year after the expiry date for all the vaccine in the final lot.

8. LABELLING

The requirements given in Part A, section 8 of the revised Requirements for *Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)* (*8*) shall apply, with the addition of the following:

The label printed on or affixed to each container shall show the volume and nature of the reconstituting fluid. Also, this label, or the label on the carton enclosing several final containers, or the leaflet accompanying the containers, shall contain the following additional information:

- the fact that the vaccine fulfils the requirements of this document;
- instructions for use of the vaccine and information concerning contraindications and the reactions that may follow vaccination;
- the conditions recommended during storage and transport, with information on the reduced stability of the vaccine if exposed to temperatures higher than that stated on the label;
- warnings that the vaccine must be protected from daylight and that, when reconstituted, the vaccine should be kept on ice; and
- a statement that, after a final container has been opened, the vaccine must be used as soon as possible, and that any opened container remaining at the end of the immunization session (maximum four hours) should be discarded.

9. DISTRIBUTION AND TRANSPORT

The requirements given in Part A, section 9, of the revised *Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)* (*8*) shall apply.

It is the responsibility of the manufacturer to use means of transport likely to maintain the potency of the vaccine.

10. STORAGE AND EXPIRY DATE

The statement concerning storage temperature and expiry dates, appearing on the label and leaflet as required in Part A, section 8, shall be based on experimental evidence obtained from the tests indicated in Part A, section 5.6, and shall be submitted for approval to the national control authority.

10.1 Storage conditions

Before being distributed by the manufacturing establishment, or before being issued from a depot for the storage of vaccine, all vaccines in their final containers shall be kept constantly at a temperature below 8 °C and shall be protected from daylight.

Precautions should also be taken to maintain the vaccine, during transport and up to the time of use, at the temperature and under the storage conditions recommended by the manufacturer.

10.2 Expiry date

The date after which dried BCG vaccine may not be used shall be determined in relation to the experimental evidence referred to in Part A, section 5.6, and with the approval of the national control authority. The date shall be not more than 12 months after the date of issue, provided that this is not more than 24 months from the date of the last satisfactory test for culturable particles, referred to in Part A, section 5.5, and provided that the vaccine has been stored continuously at the specified storage temperature and protected from daylight. In any event, the expiry date shall be not more than 4 years from the date of harvest. Each manufacturer shall test the stability of the vaccine to ensure that it satisfies these conditions.

PART B. NATIONAL CONTROL REQUIREMENTS

1. GENERAL

The general requirements for control laboratories, given in Part B of the revised *Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories)* (*8*), shall apply.

In addition, the national control authority shall provide a reference vaccine (see Part A, section 1.3) or approve one used by a manufacturer, and shall give directions concerning the use of the reference vaccine in specified tests. The national control authority shall also give directions to manufacturers concerning the BCG strain to be used in vaccine production, the total quantity of bacteria, the number of culturable particles, and the stability required of the vaccine, and shall specify the requirements to be fulfilled by the manufacturer in accordance with the provisions of Part A of this document, including those for consistency of quality in respect of the points referred to in Part A, section 5.7.

1.1 BCG strain

The strain of BCG (maintained in the form of a seed lot) used in the production of vaccine shall be derived from the original strain maintained by Calmette and Guérin and shall be identified by historical records. On the basis of cultures and biochemical and animal tests,[1] the BCG seed lot shall show characteristics that conform to those of BCG and generally differ from those of other mycobacteria. The seed lot shall show consistency in the morphological appearance of colonies on serial subculture. It shall also have been shown to yield vaccines that, upon administration by intradermal injection to children and adults, induce adequate sensitivity to tuberculin, with a low frequency of untoward effects. In addition, the seed lot shall have been shown to give adequate protection against tuberculosis in experimental animals in tests for protective potency such as are referred to in Part A, section 3.1.2.

[1] In investigating various BCG strains, it may be advisable to test their infectivity in animals, including hamsters.

1.2 Concentration of BCG vaccine

The concentration of BCG vaccine varies with different vaccines and is dependent on a number of factors, such as the strain of BCG used and the method of manufacture. It is therefore essential, for each manufacturer as well as for each different method of manufacture, for the optimum potency of vaccine to be ascertained by trials in tuberculin-negative subjects (newborn babies, older children, and adults) to determine the response to vaccination in respect of the induction of adequate sensitivity to tuberculin, the production of acceptable local skin lesions, and the occurrence of a low frequency of untoward reactions. As a result of such trials, the national control authority shall give directions to the manufacturer concerning the total bacterial content and the number of culturable particles required for the vaccine.

If a manufacturer changes his procedure of preparing BCG vaccine, and if the national control authority considers that the change might affect the final product, it may be necessary to conduct further trials in order to determine the optimum content of BCG organisms in the new product.

2. RELEASE AND CERTIFICATION[1]

A lot of dried BCG vaccine shall be released only if it fulfils Part A of the present requirements.

A statement signed by the appropriate official of the national control laboratory shall be provided at the request of the manufacturing establishment and shall certify that the lot of vaccine in question meets all national requirements as well as Part A of the present requirements. The certificate shall also state the date of the harvest, the date of the last satisfactory test for number of culturable particles, and the lot number. In addition, a copy of the official national release document shall be attached.

The purpose of the certificate is to facilitate the exchange of dried BCG vaccine between countries.

[1] Where there is no national control authority, a manufacturer may request advice and help from: Chief, Biologicals, World Health Organization, 1211 Geneva 27, Switzerland.

3. CLINICAL SURVEILLANCE OF THE VACCINE IN MAN

The national control authorities shall satisfy themselves that adequate control of BCG vaccine has been achieved, by arranging for studies in children to be made at regular intervals on some of the final lots prepared.

Such studies should be made, in at least 100 tuberculin-negative persons per year, and records should be obtained of the degree of sensitivity to tuberculin induced (distribution of tuberculin reactions by size)[1] with a defined dose of tuberculin,[2] local skin lesions (nature and size of BCG vaccine reaction), and the occurrence of untoward vaccination reactions. It is desirable that such tests should be performed in parallel on two or more vaccine lots in the same population group, one of the vaccine lots being preferably a reference vaccine.

The frequency of testing batches will depend on the number of batches of vaccine produced, but, in any case, at least one batch each year should be tested. The age groups of children in whom the vaccine is tested should be the same as those in which the vaccine will be eventually used.

If a batch of vaccine is to be exported, it must be ascertained in which age group it will be used in the importing country; the vaccine should be then tested accordingly.

AUTHORS

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[1] In some countries, the proportion of cases showing a negative reaction to tuberculin before BCG vaccination, but giving a positive result after vaccination, is called the "tuberculin conversion rate". Unless positive and negative reactions are carefully defined, however, such a rate may not include certain cases in which a weak reaction to tuberculin is changed after BCG vaccination into a strong reaction.

[2] An intradermal test with a dose of tuberculin equivalent to 5 IU of tuberculin PPD is suitable. A description of an appropriate method and a design for a study to assess BCG vaccines in man are available on application to Chief, Tuberculosis and Respiratory Infections, World Health Organization, 1211 Geneva 27, Switzerland.

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ACKNOWLEDGEMENTS

Acknowledgement is due to the following experts for their comments and advice:
Professor H. Brede, President, Paul Ehrlich Institute, Frankfurt-am-Main, Federal Republic of Germany
Professor M.P. Beumer, Ministry of Public Health and Family Affairs, Brussels, Belgium
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Appendix 1

SUMMARY PROTOCOL FOR DRIED BCG VACCINE PRODUCTION AND TESTING

Based on *Requirements for Biological Substances No. 11. Requirements for Dried BCG Vaccine* (WHO Technical Report Series, No. 745, 1987)

Identification of Final Lot

Name and address of manufacturer _____
_____
_____

Lot no. and type of vaccine Intradermal/Percutaneous/Other

Date of manufacture of final lot _____

Seed Lot

1. Information on seed lot

Reference of seed lot _____

Origin of seed lot _____

Date of preparation of seed lot _____

Date of receipt of seed lot (if applicable) _____

Date of reconstitution of seed lot ampoule _____

2. Tests on seed lot (If these data have been submitted before, completion of this paragraph is not necessary.)

(a) *Identity test*

Has the seed lot been identified as BCG? yes/no

(b) *Test for absence of contaminating microorganisms*

Date of start of test _____

Date of end of test _____

Results _____

	First test	*Repeat test if necessary*
(c) *Test for absence of virulent mycobacteria*		
Dose injected	_____	_____
No. of guinea-pigs given injection	_____	_____
Weight range of guinea-pigs	_____	_____
Observation period	_____	_____
Health of animals during test	_____	_____
Weight gains (losses)	_____	_____
Results (passed/failed)	_____	_____
Seed lot approved	yes/no	
Date of approval	_____	_____

Information on Manufacture

1. Single harvest

No. of passages from primary seed _____

Medium _____

No. and volume of containers inoculated _____

Date of inoculation _____

Date of harvest _____

Results of visual inspection _____

2. Final bulk

Date of preparation _____

No. of single harvests included _____

	First test	*Repeat test if necessary*
(a) *Absence of contamination*		
Quantity tested	_____	_____
Media	_____	_____
Duration of test	_____	_____
Results	_____	_____
(b) *Absence of virulent mycobacteria*	*First test*	*Repeat test if necessary*
No. of human doses injected per guinea-pig	_____	_____
No. of guinea-pigs given injection	_____	_____
Observation period	_____	_____
Health of animals during test	_____	_____
Weight gains (losses)	_____	_____
Results (passed/failed)	_____	_____

(c) *Nature of substances added to final bulk and final concentration* _____

3. Freeze-drying

Type and size of containers _____

No. of doses per container _____

Method used for sealing the containers vacuum-sealing/sealing under gas _____

No. of containers in the final lot _____

Information on Final Product Controls

Recommended reconstitution fluid _____

Volume of reconstitution fluid per final container _____

1. Identity test

Type of test _____

Results _____

2. Absence of contamination

	First test	*Repeat test if necessary*
No. of containers tested	_____	_____
Media	_____	_____
Duration of test	_____	_____
Results	_____	_____

3. Safety tests

(a) *Absence of virulent mycobacteria (if test not performed on final bulk)*

	First test	*Repeat test if necessary*
No. of human doses injected per guinea-pig	_____	_____
Dilution factor applied if percutaneous vaccine	_____	_____
No. of guinea-pigs	_____	_____
Observation period	_____	_____
Health of animals during test	_____	_____
Weight gains (losses)	_____	_____
Results (passed/failed)	_____	_____

(b) *Skin reaction test*

	Vaccine	*Reference*
Number of guinea-pigs	_____	_____
Dilutions injected	_____	_____
Observation period	_____	_____
Mean diameter of lesions (for each dilution)	_____	_____

4. Total bacterial content

Method of estimation _____

Result (per ml) _____

5. Tests for viability

(a) *Culturable particles*

Medium _____

	Before lyophilization	*After lyophilization*
No. of containers tested	_____	_____
Mean count of culturable particles per ml	_____	_____
Mean survival rate (%)	_____	

(b) *ATP content* (optional)

Mean survival rate (%) _____

6. Test of thermostability

	Vials	
	Unheated	*Heated*
No. of containers tested	_____	_____
Culturable particles in each container per ml	_____	_____
Mean survival (%)	_____	

Information on Release

Is the batch satisfactory? _____

Has the lot been released by the national control authority? _____

If yes, date _____

Has a certificate been supplied by the national control laboratory? yes/no

Name and signature of head of laboratory _____

Certification by person taking overall responsibility for production of the vaccine:

I certify that lot No. _____ of BCG vaccine statisfies Part A of the WHO Requirements for BCG Vaccine.

Date _____

Signature _____

Name typed _____

49. Requirements for dried BCG vaccine (amendment 1987)

The Committee was informed that improvements in the manufacturing process for BCG vaccines since the adoption of revised requirements in 1985 (WHO Technical Report Series, No. 745, Annex 2) have resulted in vaccines with greater stability, to which it is possible to allocate a longer expiry date. The Committee therefore agreed that it was appropriate to amend further the

Requirements for Dried BCG Vaccine (Revised 1985); it adopted the proposed text (BS/87.1544) with minor modifications and agreed that it should be annexed to this report (Annex 12).

The protocol must be accompanied by a sample of the label, a copy of the leaflet, and if the vaccine is to be exported, by a copy of the national control release certificate.

Information on the Manufacturer's product

When was the vaccine last tested in humans?
Summary of results:

No. of children examined	_____
Age group	_____
Vaccination-testing interval	_____
Mean postvaccination tuberculin reaction	_____
Mean vaccination lesion size	_____
Mean percentage of lymphadenitis	_____

C. DTP VACCINE (DIPHTHERIA, TETANUS AND PERTUSSIS VACCINES)

Documents provided in this section include:

1. Sample Procurement Requirements DTP Vaccine 274
2. DTP and DT Vaccine Model Package Inserts (UNICEF) 281
3. Diphtheria Toxoid Adsorbed, *USP 23 - NF 18*, 1994 283
   Diphtheria and Tetanus Toxoids, *USP 23 - NF 18*, 1994 284
   Diphtheria and Tetanus Toxoids Adsorbed, *USP 23 - NF 18*, 1994 284
   Diphtheria and Tetanus Toxoids and Pertussis Vaccine, *USP 23 - NF 18*, 1994 284
   Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed, *USP 23 - NF 18*, 1994 284
4. Pertussis Vaccine, US Food and Drug Administration HHS 21 CFR, Part 620, Subpart A 285
5. Requirements for Diphtheria, Tetanus, Pertussis and Combined Vaccines (Requirements for Biological Substances Nos. 8 and 10, revised 1989), WHO *Technical Report Series* No. 800, 1990 289

SAMPLE

PROCUREMENT REQUIREMENTS

DTP VACCINE

A. General Description of Intended Use

This vaccine is intended for use in a public-sector health programme for immunization of children (under one year of age) in _____ [*place*] beginning on _____ [*date*].

The total requirement for this solicitation is _____ [*quantity*] doses. _____ [*name*] expects to procure additional quantities of vaccine annually beginning in _____ [*month, year*] and subsequent years as follows: _____ [*quantity*], _____ [*quantity*] and _____ [*quantity*].

B. Specific Requirements

Item: Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed for intramuscular injection

Sterile suspension prepared by mixing suitable quantities of purified diphtheria and tetanus toxoids and inactivated pertussis (whooping cough) organisms. The toxoids are adsorbed onto _____ [*specify*]. _____ [*specify*] is used as a preservative.

The potency and proportions of the components are such as to provide an immunizing dose of each product in the total dosage prescribed in the labelling.

Each dose shall contain not less than _____ [*specify the amounts of three antigens*]. The potency of the vaccine components per single human

	dose shall be at least _____ [*specify for each of three*] infective units. The vaccine shall meet _____ [*specify*] requirements for Diphtheria, Tetanus, Pertussis, and Combined Vaccines (WHO Requirements for Biological Substances Nos. 8 and 10 or equivalent requirements of the USFDA as detailed in 21 CFR, or another fully equivalent set of biological requirements). *Editors' note: It is recognised that equivalence does not mean identical, only that both provide a similar level of potency, safety and efficacy.* The vaccine shall be currently registered in the country of manufacture and shall meet all requirements of the licensing authority of the country of manufacture. The vaccine also shall be currently registered in the country of use and shall meet all requirements of the licensing authority of the country of use.
Quantity:	_____ doses from one production lot.
Dosage Size:	0.5 ml.
Dose Package:	Vials of 10 doses. Vials of 20 doses.
Filling Volume:	Each 10-dose vial should contain 5 ml plus 15% overfill. Each 20-dose vial should contain 10 ml plus 15% overfill.
Storage Temperature:	Between +2 degrees C and +8 degrees C[22]; vaccine must NOT be frozen.

[22] This temperature range is more conservative than indicated in model package insert, page 281.

Shelf Life:	30 months from beginning of last satisfactory test for potency; at least 18 months must remain after shipment.
Stability:	Stable for a minimum of _____ years at _____ to _____ degrees C; manufacturer to specify thermal stability characteristics.
Labelling:	The label on each vial shall conform to the requirements of the country of use and shall appear in the language of _____. All labelling shall withstand immersion in water and remain intact. All labels shall state the name of the vaccine, name of the manufacturer, place of manufacture, lot number, composition, concentration, dose and mode for administration, expiry date, storage temperature and any other marking that is appropriate.
Optional Labelling:	If vaccine is destined for use in public-sector immunization programs, the statement "Not for Sale; For Use Only in Government-Approved Immunization Programmes" shall appear on each label in the language of the purchasing country.
Closures:	Vaccine vials shall be fitted with closures that conform to ISO standard 8362-2 for injection vials.
Printed Materials:	Two (2) information sheets, printed in _____ [*language*], shall be included in each box of ___ vials (see Model Package Insert, attached)[23].

[23] See example, page 281.

C. Quality Assurance

Compliance: The Supplier shall guarantee that the products as packed for shipment (a) comply with all provisions of the specification and related documents; (b) meet internationally recognized standards for safety, efficacy and quality; (c) are fit for the purposes made known to the Seller and (d) are free from defects in workmanship and in materials.

Evidence: The Supplier shall provide objective evidence, acceptable to the Purchaser, of the satisfaction of the requirements of this document for which no specific inspection has been mentioned.

The Supplier shall provide a copy of the manufacturing record and procedures to the Purchaser for each lot intended for shipment.

The Supplier shall provide a copy of the batch record and all quality assurance documentation to the Purchaser for each lot being supplied.

The Supplier shall provide a copy of the Certificate of Analysis to the Purchaser for each lot intended for shipment.

The Supplier shall provide to the Purchaser a copy of the approval of each source material, constituent material and component for each lot intended for shipment.

The Supplier shall retain a sample of twenty (20) vials from each lot shipped for two years beyond the printed expiration date.

Chemical, physical and microbiological test data for in-process and finished product testing must be on record for each lot shipped and must be

available to Purchaser's representatives when requested.

Inspection: The Purchaser may inspect and sample, or cause to be sampled, the product at the Supplier's factory and/or warehouse at a mutually agreeable time prior to shipment of the product.

Testing: The Purchaser may cause independent laboratory testing to be performed as deemed necessary to assure that the goods conform to prescribed requirements. Said testing laboratory shall be of the Purchaser's choice and suitably equipped and qualified to conduct quality assurance tests on biological products.

D. Packing

Storage: Supplier shall state storage volume occupied per dose of vaccine (storage volume includes the vaccine vial, packet containing the vaccine vial, and any intermediate packaging).

Inner Boxes: _____ [*number*] individual vials will be contained in sturdy white cardboard boxes outfitted with individual segments for protecting and separating each vial.

Cold Chain Monitor Cards: One cold chain monitor card (description attached)[24] in _____ language shall be packed with each ___ doses of vaccine. [_____ freeze watch indicators shall be included with each carton of vaccine.]

Overpacking: Boxes shall be overpacked so that the vaccine remains refrigerated at between +2 and +8 degrees C and does not freeze. The containers

[24] See example in Supplement VI.G.1.a., page 783.

must be suitable for export shipment in accordance with WHO/EPI Guidelines on International Packaging and Shipping of Vaccines. They must have adequate insulation and/or sufficient refrigerant to ensure that the warmest storage temperature of the vaccine does not rise above +8 degrees C in continuous outside ambient temperature of +43 degrees C nor fall below +2 degrees C in continuous outside ambient temperature of -20 degrees C during transit and for a period of at least 48 hours after arrival at the airport of destination.

Additional cushioning shall be provided, sufficient to protect the vials from breakage during transit and handling.

Exterior Shipping Cartons: Product and printed materials, packaged as specified above, shall be packed in weather-resistant, triple-wall corrugated fibreboard cartons with a bursting test strength of not less than 1,900 kPa.

The overall dimensions of the exterior shipping cartons should be such that the product does not become damaged during transportation and storage.

E. Markings

All containers and invoices must bear the name of the vaccine, expiry dates of the vaccine and appropriate storage temperature.

Inner Boxes: The inner boxes shall be marked with the following information in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Manufacturer's name and registered address
- Manufacturer's national registration number

- Lot or batch number
- Composition and concentration
- Number of vials contained in box
- Expiration date (month and year)
- Instructions for storage and handling†
- Place of manufacture (Made in _____)

Exterior Shipping Cartons:

The following information shall be stencilled or labelled on the exterior shipping cartons on two opposing sides in bold letters at least __ mm high with waterproof ink in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Lot or batch number
- Expiration date (month and year)
- Manufacturer's name and registered address
- Manufacturer's national registration number
- Destination country license or registration number
- Consignee's address and emergency phone number
- Destination airport
- Contract number
- Number of vials contained in the carton
- Gross weight of each carton (in kg)
- Carton #___ of ___
- Instructions for storage and handling†
- Place of manufacture (Made in _____)

F. Documentation

Supplier shall provide to Purchaser a copy of the batch record, including all quality assurance documentation for the vaccine being supplied.

† To be provided by Purchaser.

DTP VACCINE

DESCRIPTION
The vaccine contains purified diphtheria and tetanus toxoids and inactivated whooping cough organisms. The toxoids are adsorbed onto ___(specify). _____ is used as a preservative. The potency of the vaccine components per single human dose is at least _____ (specify in International Units for three components).

ADMINISTRATION
The vaccine should be shaken before use in order to get a homogeneous suspension. It should be injected intramuscularly or deep subcutaneously. The anterolateral aspect of the upper thigh is the preferred site of injection. (An injection into a child's buttocks may cause injury to the sciatic nerve and is not recommended.)

It must not be injected into the skin as this may give rise to a local reaction. 1 dose is 0.5 ml. A sterile needle and sterile syringe should be used for each injection. Once opened, multi-dose vials should be kept between 0° and +8°C. Opened vials of vaccine may be used in subsequent immunization sessions until a new shipment of vaccine arrives providing that the conditions described in WHO/EPI/LH15/95.1 are met.

IMMUNIZATION SCHEDULE
In countries where pertussis is of particular danger to young infants, DTP immunization should be started as soon as possible with the first dose given as early as 6 weeks and two subsequent doses given at 4-week intervals.

DPT vaccine can be given safely and effectively at the same time as BCG, measles, polio vaccines (OPV and IPV), Hepatitis B and Yellow Fever Vaccine.

SIDE EFFECTS
Some transitional swelling, tenderness and redness at the site of injection together with fever occur in a large proportion of cases. Occasionally severe reactions of high fever, irritability and screaming develop within 24 hours of administration. Very rare neurological complications allegedly due to the pertussis component have been observed but are rare in comparison with those observed in the course of the disease.

CONTRAINDICATIONS
There are few contraindications to the first dose of DTP. Fits or abnormal cerebral signs in the newborn period or other serious neurological abnormality are contraindications to the pertussis component. DT should be given instead. A second or subsequent dose of DTP should not be given to a child who suffered a severe reaction to the previous dose. The pertussis component should be omitted and DT given for the remainder of the course.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED INFANTS: HIV infected infants, both asymptomatic and symptomatic, should be immunized with DTP vaccine according to standard schedules.

STORAGE
DTP vaccine should be stored and transported between 0° and +8°C. IT MUST NOT BE FROZEN.

PRESENTATION
The vaccine comes in vial of ____ doses.

ADSORBED DT VACCINE FOR CHILDREN

DESCRIPTION
The vaccine contains purified diphtheria and tetanus toxoids. The toxoids are absorbed onto _____ (specify). _____ is used as a preservative. One dose of 0.5 ml contains _____ LF of diphtheria toxoid and _____ LF of tetanus toxoid. The potency of vaccine components per single human dose is: at least 30 IU (International Units) of potency for diphtheria toxoid and at least 40 IU of potency for tetanus toxoid.

ADMINISTRATION
The vaccine should be shaken before use in order to get a homogeneous suspension. It should be injected intramuscularly or deep subcutaneously.

A sterile syringe and a sterile needle should be used for each injection.

DT vaccine is recommended for children below 8 years of age. For persons 8 years and older, a special absorbed vaccine for adults, Td, is recommended.

Opened vials of vaccine may be used in subsequent immunization sessions until a new shipment of vaccine arrives providing that the conditions described in WHO/EPI/LH15/95.1 are met.

IMMUNIZATION SCHEDULE
Recommended for use in childhood immunization instead of DTP when contraindications to pertussis component exist. To complete the course, two intramuscular injections of 0.5 ml at least four weeks apart for children. It may be given at the same time as BCG, measles, polio vaccines (OPV and IPV), Hepatitis B and Yellow Fever vaccine.

SIDE EFFECTS
Some transitional tenderness and redness at the site of the injection and occasional fever.

CONTRAINDICATIONS
A second or subsequent dose of DT should not be given to a child who suffered a severe reaction to the previous dose.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED CHILDREN: HIV infected children, both asymptomatic and symptomatic, should be immunized with DT vaccine according to standard schedules.

STORAGE
DT should be stored and transported between 0° and +8°C. IT MUST NOT BE FROZEN.

PRESENTATION
The vaccine comes in vial of _____ doses.

Diphtheria Toxoid Adsorbed

» Diphtheria Toxoid Adsorbed conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile preparation of plain diphtheria toxoid that meets all of the requirements for that product with the exception of those for antigenicity, and that has been precipitated or adsorbed by alum, aluminum hydroxide, or aluminum phosphate adjuvants. It meets the requirements of the specific guinea pig antigenicity test in the production of not less than 2 units of antitoxin per mL based on the U.S. Standard Diphtheria Antitoxin and a diphtheria test toxin. It meets the requirements of the specific guinea pig detoxification test.

Packaging and storage—Preserve at a temperature between 2° and 8°.

Expiration date—The expiration date is not later than 2 years after date of issue from manufacturer's cold storage (5°, 1 year).

Labeling—Label it to state that it is to be well shaken before use and that it is not to be frozen.

Aluminum content—It contains not more than 0.85 mg per single injection, determined by analysis, or not more than 1.14 mg calculated on the basis of the amount of aluminum compound added.

Diphtheria Toxoids Adsorbed for Adult Use, Tetanus and—*see* Tetanus and Diphtheria Toxoids Adsorbed for Adult Use

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Diphtheria and Tetanus Toxoids

» Diphtheria and Tetanus Toxoids conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile solution prepared by mixing suitable quantities of fluid diphtheria toxoid and fluid tetanus toxoid. The antigenicity or potency and the proportions of the toxoids are such as to provide an immunizing dose of each toxoid in the total dosage prescribed in the labeling, and each component meets the requirements for those products. It contains not more than 0.02 percent of residual free formaldehyde.

Packaging and storage—Preserve at a temperature between 2° and 8°.

Expiration date—The expiration date is not later than 2 years after date of issue from manufacturer's cold storage (5°, 1 year).

Labeling—Label it to state that it is not to be frozen.

Diphtheria and Tetanus Toxoids Adsorbed

» Diphtheria and Tetanus Toxoids Adsorbed conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile suspension prepared by mixing suitable quantities of plain or adsorbed diphtheria toxoid and plain or adsorbed tetanus toxoid, and an aluminum adsorbing agent if plain toxoids are used. The antigenicity or potency and the proportions of the toxoids are such as to provide an immunizing dose of each toxoid in the total dosage prescribed in the labeling, and each component meets the requirements for those products. It contains not more than 0.02 percent of residual free formaldehyde.

Packaging and storage—Preserve at a temperature between 2° and 8°.

Expiration date—The expiration date is not later than 2 years after date of issue from manufacturer's cold storage (5°, 1 year).

Labeling—Label it to state that it is to be well shaken before use and that it is not to be frozen.

Diphtheria and Tetanus Toxoids and Pertussis Vaccine

» Diphtheria and Tetanus Toxoids and Pertussis Vaccine conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile suspension prepared by mixing suitable quantities of pertussis vaccine component of killed pertussis bacilli (*Bordetella pertussis*), or a fraction of this organism, fluid diphtheria toxoid, and fluid tetanus toxoid. The antigenicity or potency and the proportions of the components are such as to provide an immunizing dose of each product in the total dosage prescribed in the labeling, and each component meets the requirements for those products.

Packaging and storage—Preserve at a temperature between 2° and 8°.

Expiration date—The expiration date is not later than 18 months after date of issue from manufacturer's cold storage (5°, 1 year).

Labeling—Label it to state that it is to be well shaken before use and that it is not to be frozen.

Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed

» Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile suspension prepared by mixing suitable quantities of plain or adsorbed diphtheria toxoid, plain or adsorbed tetanus toxoid, plain or adsorbed pertussis vaccine, and an aluminum adsorbing agent if plain antigen components are used. The antigenicity or potency and the proportions of the components are such as to provide an immunizing dose of each product in the total dosage prescribed in the labeling, and each component meets the requirements for those products.

Packaging and storage—Preserve at a temperature between 2° and 8°.

Expiration date—The expiration date is not later than 18 mont after date of issue from manufacturer's cold storage (5°, 1 year).

Labeling—Label it to state that it is to be well shaken before use and that it is not to be frozen.

Subpart A—Pertussis Vaccine

§ 620.1 Pertussis Vaccine.

The proper name of this product shall be "Pertussis Vaccine", which shall be an aqueous preparation of killed whole *Bordetella pertussis* bacteria. The vaccine may be precipitated or adsorbed and may be combined with other antigens.

[56 FR 63410, Dec. 4, 1991]

§ 620.2 Production.

(a) *Propagation of bacteria.* Human blood shall not be used in culture medium for propagating bacteria either for seed or for vaccine. The culture medium for propagating bacteria for vaccine shall not contain ingredients known to be capable of producing allergenic effects in human subjects, except blood or blood products from lower animals other than the horse. When blood or a blood product is used, it shall be removed by washing the harvested bacteria. The bacterial concentrate shall be free of extraneous bacteria, fungi, and yeasts, as demonstrated by microscopic examination and cultural methods.

(b) *Bacterial content.* (1) The opacity of the bacterial concentrate shall be determined in terms of the U.S. Opacity Standard not later than 2 weeks after the harvest of the bacteria and before any treatment capable of altering the opacity of the bacterial concentrate.

(2) The total immunizing dose of a vaccine prepared with whole bacteria shall contain (i) in the case of nonadsorbed vaccine no more bacteria than the equivalent of 60 opacity units and (ii) in the case of adsorbed vaccine no more than the equivalent of 48 opacity units.

(c) *Detoxification.* After removing a sample for purity testing, the bacteria shall be killed and detoxified either (1) by heating, (2) by addition of a chemical agent and appropriate aging, or (3) by any combination of the stated procedures. The procedure used shall be one that has been shown to have no adverse effect on required safety, purity, and potency.

(d) *Preservative.* The vaccine shall contain a preservative.

§ 620.3 U.S. Standard preparations.

(a) The U.S. Standard Pertussis Vaccine shall be used for determining the potency of Pertussis Vaccine.

(b) The U.S. Opacity Standard shall be used in estimating the bacterial content of the vaccine and of the challenge culture.

§ 620.4 Potency test.

The number of protective units of the total human immunizing dose shall be estimated for each lot of vaccine from the results of simultaneous intracerebral mouse protection tests of the vaccine under test and the U.S. Standard Pertussis Vaccine. The potency test shall be performed as follows:

(a) *Mice.* Healthy mice shall be used, all from a single strain and of the same sex, or an equal number of each sex in each group, with individual weight varying no more than 4 grams in a single test. In no event shall any of the mice weigh less than 10 grams or more than 20 grams. A system of randomization shall be used to distribute the mice into the groups, with respect to shelf position and to determine the order of challenge. There shall be at least 3 groups consisting of no less than 16 mice each, for each vaccine. In addition, there shall be at least 4 groups consisting of no less than 10 mice each, for control purposes: one group for the challenge dose and 3 groups for titrating the virulence of the challenge dose.

(b) *Vaccination.* (1) Five-fold serial dilutions of the vaccine to be tested and of the standard vaccine shall be made in 0.85 percent sodium chloride solution. The dilutions of the vaccine under test shall have the same protective unitage, based on an estimate of 12 units per total human immunizing dose, as the unitage of the corresponding dilution of the standard vaccine. Each mouse in each group for vaccination shall be injected intraperitoneally with 0.5 ml. of the appropriate dilution.

(2) The interval between vaccination and challenge shall be 14 to 17 days. At least 87.5 percent of the mice in each group shall survive the period between vaccination and challenge and each mouse challenged shall appear healthy.

(c) *The challenge.* (1) The challenge culture of *Bordetella pertussis* for each test shall be taken from a batch of cultures which have been maintained by a method, such as freeze-drying, that retains constancy of virulence.

(2) The challenge and virulence titration doses shall be prepared as follows: The bacteria shall be harvested from a 20 to 24 hour culture grown on Bordet-Gengou medium seeded from a rapidly growing culture less than 48 hours old and uniformly suspended in a solution containing 1.0 percent casein peptone and about 0.6 percent sodium chloride at pH 7.1±0.1. The suspension, freed from agar particles and clumps of bacteria, and adjusted to an opacity of 10 units, shall be diluted in the solution used for suspending the bacteria, to provide in a volume of 0.03 ml. (i) a challenge dose of 0.0001 opacity units (1:3000) and (ii) virulence titration doses of $1/50$, $1/250$ and $1/1250$ respectively of the challenge dose.

(3) Each vaccinated mouse shall be injected intracerebrally with the challenge dose. The four groups of control mice shall be injected intracerebrally with the challenge dose and its three dilutions, respectively. The challenge-dose control mice shall be injected last. The interval between the removal of the bacteria from the culture medium and the injection of the last mouse shall not exceed 2½ hours.

(d) *Recording the results.* The mice shall be observed for 14 days. Mice dying within 72 hours after challenge shall be excluded from the test. Records shall be maintained of the number of mice that die after 72 hours and of the number of mice showing both paralysis and enlargement of the head at the end of 14 days. All mice that show both paralysis and enlargement of the head shall be considered as deaths for the purposes of determining the ED_{50}.

(e) *Validity of the test.* The test shall be valid provided (1) the ED_{50} of the vaccine under test and the standard vaccine is between the largest and smallest vaccinating doses; (2) the limits of one standard deviation of each ED_{50} fall within the range of 64 percent to 156 percent; (3) the protective response is graded in relation to the vaccinating doses; (4) the dose-response curves of the vaccine under test and the standard vaccine are parallel; (5) the challenge dose contains approximately 200 LD_{50}; (6) the LD_{50} contains no more than 300 colony forming units; and (7) the $1/1250$ dilution of the challenge dose contains no less than 10 and no more than 50 colony forming units.

(f) *Estimate of the potency.* The ED_{50} of each vaccine shall be calculated by a

method that provides an estimate of the standard deviation. The protective unit value per total human immunizing dose of the vaccine under test shall be calculated in terms of the unit value of the standard vaccine.

(g) *Potency requirements.* The vaccine shall have a potency of 12 units per total human immunizing dose based upon either a single test estimate of no less than 8 units or a two-, three- or four-test geometric mean estimate of no less than 9.6, 10.8, or 12 units, respectively, except that for the vaccine in a multiple antigen product containing Poliovirus Vaccine Inactivated, the estimate shall be no less than 14 units. In no event shall the estimate be more than 36 units.

(h) *Test design variation.* Variations in the design of the potency test may be permitted providing the results are demonstrated to be of equal or greater precision.

[38 FR 32064, Nov. 20, 1973, as amended at 50 FR 4137, Jan. 29, 1985]

§ 620.5 Mouse toxicity test.

The final vaccine shall be demonstrated to be free from toxicity by the following test:

A group of no less than 10 mice, each mouse weighing 14 to 16 grams, shall have free access to food and water for no less than 2 hours before injection. The group weight of the mice shall be determined immediately prior to injection. Each mouse shall be injected intraperitoneally with a test dose of one-half of the largest recommended single human dose of the final vaccine in a volume of no less than 0.5 ml. nor more than 0.75 ml. The group weight of the mice shall be determined at the end of 72 hours and at the end of 7 days after injection. At the end of 72 hours the average weight per mouse may be no less than the average weight per mouse immediately preceding the injection; at the end of 7 days the average weight gain per mouse may be no less than 3.0 grams; and at the end of 7 days there may be vaccine-related deaths of no more than 5 percent of the total number of mice in all the toxicity tests performed.

§ 620.6 General requirements.

(a) *Safety.* Each lot of product containing Pertussis Vaccine shall be tested for safety by the procedures prescribed in § 610.11 of this chapter except that the test shall consist of the intraperitoneal injection of no less than one-half of the recommended largest individual human dose into each of the mice, and either the intraperitoneal injection of no less than three times the recommended largest individual human dose, or the subcutaneous injection of 5.0 milliliters into each of the guinea pigs.

(b) *Dose.* These additional standards are based on a single injection of 0.5 ml., 1.0 ml., or 1.5 ml., and a total human immunizing dose of three single injections of a nonadsorbed vaccine, and two or three single injections of an adsorbed vaccine.

(c) *Product characteristics.* Recommendations shall be made through appropriate labeling that the product after issue should not be frozen and should be well shaken immediately prior to use.

(d) *Labeling.* In addition to the items required by other applicable labeling provisions of this part, the package label shall give the following information:

(1) For a vaccine containing a precipitant or an adsorbent, the word "Adsorbed" shall follow the proper name in the same style of type and prominence as the proper name.

(2) The total immunizing dose contains 12 units of pertussis vaccine.

(e) *Multiple antigen products.* The Pertussis Vaccine components of multiple antigen products shall be manufactured pursuant to these additional standards, except that the mouse toxicity test (§ 620.5) and the potency test (§ 620.4) shall be performed on the multiple antigen product.

(f) *Adsorbed vaccines.* Only aluminum compound reagents shall be introduced into the product to cause precipitation or adsorption of either Pertussis Vaccine or other antigens incorporated with Pertussis Vaccine.

(g) *Freezing prohibition.* Pertussis Vaccine and multiple antigen products of which Pertussis Vaccine is a component shall not be frozen at any time during storage.

(h) *Samples and protocols.* For each lot of vaccine, the following material shall be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892.

(1) A sample of no less than 20 milliliters of the final product for pertussis vaccine testing.

(2) Protocols showing summaries of the manufacturing processes and the results of all mouse toxicity (§ 620.5) and potency (§ 620.4) tests performed.

[38 FR 32064, Nov. 20, 1973, as amended at 41 FR 35480, Aug. 23, 1976; 48 FR 13025, Mar. 29, 1983; 49 FR 23834, June 8, 1984; 51 FR 15610, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

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World Health Organization, Technical Report Series, No. 800, 1990

Annex 2

REQUIREMENTS FOR DIPHTHERIA, TETANUS, PERTUSSIS AND COMBINED VACCINES

(Requirements for Biological Substances Nos. 8 and 10) (Revised 1989)

	Page
Introduction	88
Requirements for diphtheria vaccine (adsorbed)	91
General considerations	91
Part A. Manufacturing requirements	94
A.1 Definitions	94
A.2 General manufacturing requirements	95
A.3 Production control	95
A.4 Filling and containers	104
A.5 Control of final product	104
A.6 Records	105
A.7 Samples	106
A.8 Labelling	106
A.9 Distribution and transport	107
A.10 Stability, storage and expiry date	107
Part B. National control requirements	108
B.1 General	108
B.2 Official release and certification by the national control authority	108
Requirements for tetanus vaccine (adsorbed)	109
General considerations	109
Part A. Manufacturing requirements	111
A.1 Definitions	111
A.2 General manufacturing requirements	113
A.3 Production control	113
A.4 Filling and containers	121
A.5 Control of final product	121
A.6 Records	123
A.7 Samples	123
A.8 Labelling	123
A.9 Distribution and transport	124
A.10 Stability, storage and expiry date	124

Part B. National control requirements 125
B.1 General 125
B.2 Official release and certification by the national control authority 125
Requirements for pertussis vaccine 127
General considerations 127
Part A. Manufacturing requirements 129
A.1 Definitions 129
A.2 General manufacturing requirements 130
A.3 Production control 131
A.4 Filling and containers 138
A.5 Control of final product 139
A.6 Records 140
A.7 Samples 141
A.8 Labelling 141
A.9 Distribution and transport 141
A.10 Stability, storage and expiry date 142
Part B. National control requirements 143
B.1 General 143
B.2 Official release and certification by the national control authority 143
Requirements for combined vaccines (adsorbed) 144
General considerations 144
Part A. Manufacturing requirements 145
A.1 Tests for DT and DTP vaccines 145
A.2 Special tests for DTP vaccine 146
A.3 Special tests for DT vaccine 147
Part B. National control requirements 148
Authors 149
Acknowledgements 150
References 151
Appendix 1. Summary protocol for diphtheria vaccine (adsorbed) production and testing 152
Appendix 2. Model certificate for the release of vaccines 158
Appendix 3. Summary protocol for tetanus vaccine (adsorbed) production and testing 159
Appendix 4. Summary protocol for pertussis vaccine production and testing. 165
Appendix 5. Summary protocol for diphtheria, tetanus and pertussis vaccine (adsorbed) production and testing 169

INTRODUCTION

The WHO Requirements for Pertussis (P) Vaccine were formulated in 1963 (*1*) (Requirements for Biological Substances

No. 8) and those for Diphtheria (D) and Tetanus (T) Toxoid Vaccines in 1964 (*2*) (Requirements for Biological Substances No. 10); the Requirements for the three vaccines (DPT) were revised in 1978 (*3*) (Requirements for Biological Substances Nos. 8 and 10). Diphtheria and tetanus toxoids and pertussis vaccine are most commonly used in a combined form, and the 1978 revision covered all three vaccines in a single document.

At the end of May 1988, WHO organized a three-day scientific consultation in Geneva during which the changes in the production and control methods for diphtheria, pertussis and tetanus vaccines that had occurred over the previous 10 years were reviewed (*4*). Special emphasis was placed on methods of determining the potency of diphtheria and tetanus (DT) toxoid vaccines that would require a smaller number of animals. While methods for determining the antigen content of DT vaccines have been available for many years, their results do not necessarily indicate whether the vaccines are of adequate potency. This is not only because antigens that cannot be eluted from adjuvants may not be determined by these methods but also because they do not take into account certain important characteristics of vaccines, such as those associated with the conditions of inactivation, purification and adsorption, which may influence protective capacities. Immunogenicity tests in animals are therefore necessary for assessing the potency of toxoid vaccines. There are, however, different opinions on the way such tests should be conducted; in some countries, tests are carried out in such a way that potencies cannot be expressed in International Units of toxoid.

These revised Requirements were prepared by a group of experts and staff members (see p. 149) who met immediately after the 1988 consultation and reviewed the regulations for D, T, P and combined vaccines in force in a number of countries. In addition, comments have been received from a number of experts, whose assistance is gratefully acknowledged (see p. 150).

The main changes made to the 1978 revision are as follows:

(1) The term "toxoid" is replaced where appropriate by "vaccine" and the order in which pertussis and tetanus vaccines are dealt with has been reversed.
(2) Greater flexibility is allowed in the conduct of sterility tests so as to permit the use of satisfactory methods adopted by national control authorities.

(3) Attention is drawn to the need to ensure that the purification methods used exclude from final products substances derived from the medium that are likely to have sensitizing properties.
(4) The need to ensure that vaccines still possess the minimum potency at the expiry date is emphasized in a paragraph in the sections on final products; this requires the determination of the loss of potency during storage at the recommended temperature and the successful testing of three consecutive batches of vaccines.

Other changes in the requirements not common to the three vaccines are mentioned in the general considerations for each product.

In a more general way, the experts who prepared these Requirements agreed that methods of production and testing other than those described in them should be acceptable provided that they have been properly validated and that they yield products as safe and protective as those prepared using the methods described here.

Diphtheria, tetanus and pertussis vaccines are often used in combination and usually contain an adjuvant. The formulations commonly used include:

- tetanus vaccine adsorbed,
- diphtheria and tetanus vaccine adsorbed,
- tetanus and diphtheria vaccine adsorbed for adult use (Td),
- pertussis vaccine adsorbed,
- diphtheria, tetanus and pertussis vaccine adsorbed.

Plain tetanus vaccine is sometimes used for primary immunization but there is no agreed potency expressed in International Units for this type of preparation. The potency of such vaccines should therefore be approved by the national control authority.

The requirements for diphtheria vaccine, tetanus vaccine, pertussis vaccine and combined vaccines (DT and DTP) are dealt with in separate sections. As in the previous Requirements, the separate section on combined vaccines has been included since certain special tests are applicable only to such formulations.

REQUIREMENTS FOR DIPHTHERIA VACCINE (ADSORBED)

GENERAL CONSIDERATIONS

Diphtheria toxoid was one of the earliest vaccines available for protection against a bacterial disease and its use, when of proved efficacy and when immunization schedules known to give good antitoxin responses are used, has markedly reduced the incidence of the disease.

The early developments leading to the formulation of the first Requirements for Diphtheria Toxoid are described in detail in the Requirements for Biological Substances No. 10 (*2*).

The Parke Williams 8 strain of *Corynebacterium diphtheriae* has been shown to be satisfactory for producing potent diphtheria vaccines, and no purpose would seem to be served by suggesting a change of strain. The approach adopted in diphtheria vaccine production is to obtain the greatest possible quantity of toxin during the growth phase of the microorganisms and thereafter to convert the toxin into stable toxoid by the most efficient method.

One of the most important achievements in drawing up the first Requirements for Diphtheria Toxoid was the agreement reached on the formulation of requirements for the assay of potency. In 1964, almost all countries had adopted their own requirements and there were considerable differences between them. By 1978, a number of countries had adopted requirements based on a comparison of the protection afforded to laboratory animals by graded doses of the test vaccine and that provided by a reference vaccine that had been calibrated in International Units. A requirement involving the immunization of guinea-pigs followed either by a lethal challenge test or an intradermal challenge test in which graded doses of toxin are administered was therefore included in the 1978 revision of the Requirements. The former indicated that the immunized animals were protected against a single challenge dose of toxin, whereas the latter test, by virtue of the graded doses of challenge toxin used, gave a quantitative measure of the animals' level of immunity.

Following the publication of the 1978 revised Requirements, it became apparent that the large numbers of animals, particularly of guinea-pigs, required for the potency test made conformity difficult

to achieve in many countries. Means of reducing the number of animals required, without prejudice to the principle of expressing potency in terms of International Units, have therefore been sought, the emphasis being on the use of the minimum number of animals necessary to provide assurance that the potency of the vaccine is indeed greater than the minimum required. A step was taken in that direction in 1986 (5) when an addendum to Requirements Nos. 8 and 10 specified that ranges of 95% confidence intervals greater than 50–200% were acceptable provided that the lower limit of the 95% confidence interval was still above the minimum potency required in each single human dose.

A further method of reducing the number of animals used in three-dilution assay systems is to determine the individual titres of antitoxin of laboratory animals such as mice or guinea-pigs by toxin neutralization tests in cell cultures. Further development of a variety of methods specific for the assay of diphtheria antitoxin should also result in a reduction in the number of laboratory animals used.

The number of animals used in tests based on challenge can also be reduced by assaying both the test and reference vaccine at a single dilution, provided that the test is performed by laboratories with extensive experience of vaccines on which three-dilution assays have been regularly and sucessfully performed.

Although the available data are insufficient to permit a correlation between a potency level observed in a laboratory assay and protection in humans or the duration of immunity, some evidence is available from which a potency level can be specified above which a vaccine may be considered to be of acceptable potency. It is important, therefore, for the potency of diphtheria vaccines to be expressed in International Units. In some countries, potency tests based on the ability of the vaccine to induce specified levels of antitoxin are used, but such assays are often performed on pooled sera and without the use of calibrated reference materials, and do not allow a valid statistical evaluation of the results to be made. Although data are available that demonstrate that vaccines meeting such requirements can induce significant levels of antitoxin response in humans, the use of more quantitative types of assays is recommended in these revised Requirements for Diphtheria Vaccine (Adsorbed).

In addition, whenever new vaccines are produced, long-term studies should be carried out to confirm that, in 90% of the target population, diphtheria antitoxin levels are above 0.01 IU/ml five years after the completion of primary immunization in previously unvaccinated individuals.[1]

These Requirements call for the product to be purified, since diphtheria toxoid in the unpurified form is liable to give rise to severe vaccination reactions in humans; much work has therefore been done in developing purified material in order to avoid them. Even with purified products, however, untoward reactions may occur in adults. In view of the risk of reversion to toxicity, especially when a toxin is detoxified after purification, the Requirements have been formulated so as to exclude this risk and, in the irreversibility test, the incubation period at 37 °C of the purified toxoid has been increased to six weeks. There is evidence that purification may sometimes reduce the immunizing activity of diphtheria toxoid, probably as a result of the removal of substances having an adjuvant effect. Purified products, if intended for primary immunization, must therefore be combined with a mineral adjuvant, although they may be used uncombined for reinforcing immunity. In these revised Requirements, the maximum number of "limit of flocculation" or Lf units per single human dose of diphtheria vaccine (adsorbed) has been reduced to 30. These requirements do not apply to plain diphtheria vaccines, although such vaccines are sometimes used for primary immunization.

Each of the following sections constitutes a recommendation. Those parts of each section printed in large type have been written in the form of requirements, so that, if a health administration so desires, they may be adopted as they stand as definitive national requirements. Those parts of each section printed in small type are comments and/or recommendations for guidance.

Individual countries may wish to adopt these Requirements as the basis of their national regulations on diphtheria vaccines. If national requirements differ from these requirements, it is recommended that the former should be shown to ensure that the vaccine is at least as safe and as potent as that prepared in accordance with the requirements formulated below. It is desirable that the World Health Organization should be kept informed of any such differences.

[1] Primary immunization usually consists of an initial course of two or three injections at intervals of 4–6 weeks, followed by a further injection 7–12 months later. Immunity can be reinforced or "boosted" by subsequent single injections, usually given a number of years later.

PART A. MANUFACTURING REQUIREMENTS

A.1 Definitions

A.1.1 International name and proper name

The international name shall be *Vaccinum diphtheriae adsorbatum*. The proper name shall be the equivalent of the international name in the language of the country of use.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

A.1.2 Descriptive definition

Vaccinum diphtheriae adsorbatum is a preparation of diphtheria toxoid prepared by treating diphtheria toxin by chemical means so as to render it nontoxic without destroying its immunogenic potency. The toxoid is adsorbed on to a suitable adjuvant. The preparation shall satisfy the requirements formulated below.

The most common method of preparing toxoids from toxin is by means of formaldehyde.

A.1.3 International reference materials

The first International Reference Reagent of Diphtheria Toxoid for Flocculation Tests was established in 1988 (*6*).

The second International Standard of Diphtheria Toxoid, Adsorbed, was established in 1978 (*3*) for determining the potencies of vaccines containing adsorbed diphtheria toxoid.

The International Standard for Diphtheria Antitoxin was established in 1934; it is made from horse hyperimmune serum.

The above-mentioned international reference materials are in the custody of the International Laboratory for Biological Standards, State Serum Institute, Copenhagen. Samples are distributed free of charge, on request, to national control laboratories. The international reference materials are intended for the calibration of national reference materials for use in the manufacture and laboratory control of diphtheria antitoxin and vaccines.

A.1.4 Terminology

Seed lot: A quantity of bacterial suspension that is derived from one strain, has been processed as a single lot and has a uniform

composition. It is used for preparing the inoculum for the production medium.

Single harvest: The toxic filtrate or toxoid obtained from one batch of cultures inoculated, harvested and processed together.

Bulk purified toxoid: The processed purified material prepared from either a single harvest or a pool of a number of single harvests. It is the parent material from which the final bulk is prepared.

Final bulk: The homogeneous final vaccine present in a single container from which the final containers are filled either directly or through one or more intermediate containers.

Final lot: A collection of sealed final containers that are homogeneous with respect to the risk of contamination during filling. A final lot must therefore have been filled from a single container in one continuous working session.

A.2 General manufacturing requirements

The general requirements for manufacturing establishments contained in the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*7*) shall apply to establishments manufacturing diphtheria vaccine with the addition of the following:

Written descriptions of procedures for the preparation and testing of diphtheria vaccine adopted by a manufacturer together with appropriate evidence that each production step has been validated shall be submitted for approval to the national control authority. Proposals for modifications of the manufacturing and/or control methods shall also be submitted for approval to the national control authority before such modifications are implemented.

A.3 Production control

A.3.1 Control of source materials

A.3.1.1 *Strains of Corynebacterium diphtheriae*

Strains of *C. diphtheriae* used in preparing diphtheria toxoid shall be identified by a record of their history and of all tests made periodically to verify strain characters. The strain shall be maintained as a freeze-dried culture.

A highly toxinogenic strain of *C. diphtheriae* should be used. A strain that has proved satisfactory in many laboratories is the Parke Williams 8 strain.

A.3.1.2 *Seed lot system*

The production of diphtheria toxin shall be based on a seed lot system. Cultures of the working seed shall have the same characteristics as those of the strain from which the parent seed lot was derived. The preparation of seed lots shall comply with the requirements of Part A, section A.3.2.

A.3.1.3 *Culture medium for production of toxin*

It is particularly important to ensure that the final product is free from substances likely to cause toxic or allergic reactions in humans.

The method of detecting these substances should be approved by the national control authority.

If the medium is prepared from a protein digest, e.g., casein hydrolysate or digested muscle, precautions should be taken to ensure that digestion has proceeded sufficiently. Established limits, if any, for mammalian protein and human blood-group substances in the final vaccine should not be exceeded.

A.3.2 Production precautions

The general production precautions, as formulated in Part A, section 3, of Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*7*), shall apply to the manufacture of diphtheria vaccine.

Suitable methods for the production of diphtheria vaccine are given in the *Manual for the production and control of vaccines: diphtheria toxoid* (*8*).

Personnel employed in production and quality control shall be adequately trained and immunized.

A.3.3 Control of single harvests

Consistency of production shall be demonstrated.

Consistency may be demonstrated by measuring, e.g., the bacterial growth rate, pH and rate of toxin production.

Any culture showing anomalous growth characteristics shall be investigated and shown to be satisfactory before being accepted as a single harvest.

A.3.3.1 *Control of bacterial purity*

Samples of cultures used for preparing single harvests of toxoid shall be tested for bacterial purity by microscopic examination of stained smears or by inoculation into appropriate culture media. Single harvests shall not be used for preparing bulk material if contamination has occurred at any stage in their production.

A.3.3.2 *Filtration*

After having been sampled for the control of purity, cultures shall be sterilized by means of filtration. A preservative may be added, but phenol shall not be used for this purpose.

Cultures should be filtered as soon as possible after the end of their incubation period. To facilitate filtration, cultures may be centrifuged, provided that suitable precautions are taken to avoid the formation of potentially hazardous aerosols. A filter aid may be added beforehand.

In some countries, no filter capable of shedding fibres may be used.

A.3.3.3 *Determination of antigen concentration*

The supernatant of the culture prior to inactivation shall be tested by a method approved by the national control authority.

It is advisable to determine the antigen content by measuring the toxin content. This is usually done *in vivo;* however, *in vitro* methods are acceptable if validated.

Another suitable method for determining the antigen concentration is the flocculation test which is described in the *Manual for the production and control of vaccines: diphtheria toxoid* (*8*); it should be performed on both the supernatant and, for purposes of comparison, a reference material calibrated against the International Reference Reagent of Diphtheria Toxoid for Flocculation Tests, or an equivalent reference preparation approved by the national control authority.

It is preferable for culture filtrates used in preparing purified toxoid to contain at least 50 Lf/ml.

Antigen content is a good indicator of consistency of production.

A.3.3.4 *Detoxification and purification of toxin*

Purification may either precede or follow detoxification. Purification before detoxification results in a purer product, but particular care must be taken to avoid reversion to toxin, which may also occur when detoxification precedes purification. The method and agent used for detoxification and the method of purification shall be approved by the national control authority.

Amino acids such as lysine are frequently added during detoxification and help to prevent reversion.

After detoxification has been completed, the detoxifying agent shall be removed or neutralized by a method approved by the national control authority.

The method of purification shall be such that no substances are incorporated into the final product that are likely to cause untoward reactions in humans. The rate of detoxification may vary and shall be monitored. Harvests shall not be transferred from the detoxification area until the detoxification has been shown to be complete.

A.3.4 Control of bulk purified toxoid

A.3.4.1 *Preparation*

The bulk purified toxoid shall be prepared from either a single harvest or a pool of single harvests, and shall be sterile. Phenol shall not be used as a preservative.

It is advisable to sterilize the bulk purified toxoid by filtration. A preservative approved by the national control authority may be added to the bulk toxoid.

A.3.4.2 *Sterility*

Each bulk purified toxoid shall be tested for bacterial and mycotic sterility in accordance with the requirements of Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (9) or by a method approved by the national control authority. If a preservative has been added to the purified bulk, appropriate measures shall be taken to prevent any interference by it in the sterility test.

A.3.4.3 *Specific toxicity*

Each bulk purified toxoid shall be tested for the presence of diphtheria toxin. A suitable test consists of injecting the toxoid into at least five guinea-pigs, each weighing 250–350 g. Each guinea-pig shall be given a subcutaneous injection of 1 ml of a dilution of purified toxoid containing at least 500 Lf of toxoid. Animals that die shall be autopsied and examined for symptoms of diphtheria intoxication (red adrenals). The bulk purified toxoid shall pass the test if no guinea-pig shows symptoms of specific intoxication within six weeks of injection and if at least 80% of the animals survive the test period. The guinea-pigs shall not have been used previously for experimental purposes.

Some manufacturers carry out, in addition, a test for determining whether diphtheria toxin is present by injecting intradermally into rabbits or guinea-pigs at least 20 Lf of purified toxoid and observing the injection sites for specific erythema.

Alternatively, a cell-culture test system may be used; in this case, the sensitivity of the test shall have been demonstrated to be not less than that of the guinea-pig test, and the test procedures shall be approved by the national control authority.

A.3.4.4 *Reversion to toxicity*

Each bulk purified toxoid shall be tested to ensure that reversion to toxicity cannot take place on storage. The bulk purified toxoid shall be diluted in order to obtain the same concentration and chemical environment as those present in the final bulk vaccine, except for the presence of adjuvant.

To determine whether reversion has occurred, diluted toxoids that have been stored at 37 °C for six weeks shall be tested. The test employed shall be approved by the national control authority and should be sufficiently sensitive to detect very small amounts of toxin. No toxicity shall be detected.

In one country, the test is performed on toxoids that have been stored at 34 °C.

Similar dilutions of toxoid held at 2–8 °C during the same period of time as those held at 34 °C or 37 °C may be tested as controls.

Intradermal tests in guinea-pigs and cell-culture tests are both considered to be suitable.

A.3.4.5 *Antigenic purity*

Each bulk purified toxoid shall be tested for antigenic purity by determining the antigen concentration in Lf units and the concentration of protein (nondialysable) nitrogen. The antigen concentration shall be determined by comparison with a reference material calibrated against the International Reference Reagent of Diphtheria Toxoid for Flocculation Tests, or an equivalent reference preparation approved by the national control authority. The method of testing shall be approved by the national control authority. The bulk purified toxoid shall pass the test if it contains no fewer than 1500 Lf per mg of protein (nondialysable) nitrogen.

Preparation of toxoid containing more than 1500 Lf per mg is both feasible and desirable.

An indication of the antigenic quality of the toxoid may be obtained by measuring the total combining power and expressing it in relation to the number of Lf units. A suitable method for measuring the total combining power is given in the *Manual for the production and control of vaccines: diphtheria toxoid* (8).

A.3.5 Control of final bulk

A.3.5.1 *Preparation*

The final bulk shall be prepared from bulk purified toxoid. The number of Lf in a single human dose shall be approved by the national control authority but shall not exceed 30.

A.3.5.2 *Preservative*

If the vaccine is to be dispensed into multidose containers, a suitable antimicrobial preservative shall be added. The amount of preservative in the final bulk shall have been shown to have no deleterious effect on the toxoid or on other vaccine components with which the toxoid may be combined, and to cause no unexpected adverse reactions in humans. The preservative and its concentration shall be approved by the national control authority.

Phenol shall not be used as a preservative.

A.3.5.3 *Adjuvants*

The adjuvants used, their purity and their concentration shall be approved by the national control authority.

Aluminium or calcium compounds are generally used as mineral carriers.

The concentration of aluminium shall not exceed 1.25 mg and that of calcium 1.3 mg per single human dose.

In some countries, these upper limits for the concentrations of mineral carriers are considered to be too high and the limits are set at about half those given above.

In some countries, the adsorbent is precipitated in the presence of the toxoid.

The formulation shall be such that the vaccine remains suspended for a reasonable time after shaking.

A.3.5.4 *Sterility*

Each final bulk shall be tested for bacterial and mycotic sterility in accordance with the requirements of Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (9) or by a method approved by the national control authority. If a preservative has been added to the final bulk, appropriate measures shall be taken to prevent any interference by it in the sterility test.

A.3.5.5 *Specific toxicity*

In some countries, each final bulk is tested for specific toxicity in at least five guinea-pigs, each weighing 250–350 g. Each guinea-pig is given a subcutaneous injection of a quantity equivalent to at least five single human doses. Animals that die are autopsied and examined for symptoms of diphtheria intoxication (red adrenals). The final bulk passes the test if no guinea-pig shows symptoms of specific intoxication within six weeks of injection and if at least 80% of the animals survive the test period. The guinea-pigs must not have been used previously for experimental purposes.

A.3.5.6 *Potency*

The immunizing potency of each final bulk shall be determined by comparison with an appropriate reference material calibrated against the International Standard for Diphtheria Toxoid, Adsorbed. The determination shall involve the inoculation of guinea-pigs or mice with appropriate doses or dilutions of both the product and the reference material. After immunization, mice shall

be bled, and guinea-pigs bled or challenged either by the subcutaneous or by the intradermal route (*10*). When animals are bled, the antitoxin levels of the individual animals may be titrated by means of toxin neutralization tests performed using *in vivo* or *in vitro* serological methods[1] that have been validated on vaccines of the types being tested. Appropriate statistical methods shall be used to calculate the potency of the final bulk (*10*). The method adopted and the interpretation of the results shall be approved by the national control authority.

Care should be taken to ensure that diluents are inert and not pyrogenic. Phosphates might interfere with the adsorption of toxoid.

When consistency of production and testing have been established, the numbers of animals injected with each dilution of product may be reduced to levels substantially lower than those originally needed for the three-dilution assays described in the *Manual of details of tests required on final vaccines used in the WHO Expanded Programme on Immunization* (*10*), provided that the resulting assays are statistically valid. Methods based on individual quantification of antitoxin or, in the case of guinea-pigs, scores of responses to intradermal challenge, allow the use of fewer animals than are needed in lethal challenge tests.

Depending on the purpose, two types of potency assays may be considered.

Three-dilution assays may be used to test consistency of production and product stability, and to calibrate reference preparations.

One-dilution assays,[2] based on the same principles for evaluating the response as the three-dilution assays, may be used at the discretion of the national control authority for the routine testing of vaccine lots of a given formulation as soon as the production process has been established and consistency in production and control has been demonstrated. The assay involves the selection of a dose of the reference vaccine, expressed as a fraction of 30 IU (i.e., of the minimum potency of a single human dose), that elicits a minimal protective effect in guinea-pigs, and comparing its effect with the response elicited by the same fraction of a human dose of the test vaccine. If the response to the latter is significantly greater than that to the former ($P \leq 0.05$), the potency of the test vaccine is satisfactory. One-dilution tests offer advantages only when vaccine potencies are consistently and substantially in excess of 30 IU per single human dose.

[1] Information on potency determination of the diphtheria component of D, DT and DTP vaccines in mice based on antitoxin assay by toxin neutralization in Vero cell cultures is given in unpublished WHO document BS/89.1613, which can be obtained from Biologicals, World Health Organization, Geneva, Switzerland.

[2] Information on one-dilution assay methods is given in document BS/89.1618, available on request from Biologicals, World Health Organization, Geneva, Switzerland.

The potency of the final bulk shall be approved by the national control authority. The potency of diphtheria vaccine used for the immunization of children shall not be less than 30 IU per single human dose. For three-dilution assays, the limits of the 95% confidence intervals of the estimate of potency shall be within 50–200% of the estimated potency unless the lower limit of the 95% confidence interval of the estimated potency is greater than 30 IU per single human dose. When one-dilution tests are performed, the potency of the test vaccine shall be demonstrated to be significantly greater than 30 IU per human dose.

In some countries, vaccines intended for the booster immunization of adults contain less than 30 IU per dose.

In some countries, potency testing is not carried out on each final bulk but on each final lot.

A.3.5.7 *Amount of residual free detoxifying agent*

The amount of residual free detoxifying agent in each final bulk shall be determined by a method approved by the national control authority, and, if formaldehyde has been used, the residual content shall be not more than 0.2 g/l.

The colorimetric determination of the reaction product of formaldehyde and fuchsin-sulfurous acid is a suitable method.

In some countries, the amount of residual free detoxifying agent is determined in the purified bulk.

If applicable, appropriate tests for the presence of other detoxifying agents (e.g., glutaraldehyde) shall be performed. The tests used and the maximum permissible concentrations of such chemicals shall be approved by the national control authority.

A.3.5.8 *pH*

The pH of the final bulk shall be measured.

The pH should be between 6.0 and 7.0.

A.4 Filling and containers

The requirements applicable to filling and containers given in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*7*) shall apply.

Single-dose or multiple-dose containers may be used. Vaccine in multidose containers shall contain a suitable antimicrobial preservative.

A.5 Control of final product

A.5.1 Identity

An identity test shall be performed on at least one labelled container from each final lot.

Flocculation in solution, immunoprecipitation of the toxoid in gels or any other specific interaction between the vaccine and diphtheria antitoxin may serve as an identity test. Tests on toxoids adsorbed on to aluminium or calcium carrier may be performed after the carrier has been dissolved, or the adsorbed toxoid wholly or partially eluted by sodium citrate at pH 9.

If adequate quantities of toxoid cannot be recovered from the adsorbed vaccine, specific antitoxin may be sought in the sera of animals used in the innocuity test.

A.5.2 Sterility

Final containers shall be tested for bacterial and mycotic sterility by a method approved by the national control authority.

Many countries have regulations governing the sterility testing of the final product. Where these do not exist, the requirements published by WHO shall be met (*9*). If a preservative has been added to the purified bulk, appropriate measures shall be taken to prevent any interference by it in the sterility test.

A.5.3 Potency

A potency test shall be carried out, as provided in Part A, section A.3.5.6, on each final lot, if such a test has not been performed on the final bulk.

A.5.4 Innocuity

Each final lot shall be tested for abnormal toxicity by the injection by the intraperitoneal route of one human dose, but not more than 1 ml, into each of five mice (weighing 17–22 g) and at least one human dose, but not more than 1 ml, into each of two guinea-pigs (weighing 250–350 g). The tests shall be approved by the national control authority. The final product shall be considered innocuous if the animals survive for at least seven days without showing significant signs of toxicity.

A.5.5 Adjuvant content

The adjuvant content of each final lot shall be determined by a method approved by the national control authority (see Part A, section A.3.5.3).

In some countries, this test is used to verify the homogeneity of filling.

A.5.6 Preservative content

The preservative content of each final lot shall be determined (see Part A, section A.3.5.2). The method used shall be approved by the national control authority.

In some countries, this test is applied to the final bulk only.

A.5.7 pH

The pH of each final lot shall be measured.

The pH should be between 6.0 and 7.0.

A.5.8 Inspection of final containers

Each container in each final lot shall be inspected visually, and those showing abnormalities—such as improper sealing, lack of integrity, clumping or the presence of particles shall be discarded.

A.6 Records

The requirements given in Part A, section 6, of the revised Requirements for Biological Substances No. 1 (General Require-

ments for Manufacturing Establishments and Control Laboratories) (7) shall apply.

Written records shall be kept of all tests, irrespective of their results. The records shall be of a type approved by the national control authority.

A model of a suitable summary protocol to be used for diphtheria vaccines is given in Appendix 1.

A.7 Samples

The requirements given in Part A, section 7, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply.

A.8 Labelling

The label printed on or affixed to each container and the label on the carton enclosing one or more containers shall show as a minimum:

- the words *Vaccinum diphtheriae adsorbatum* and/or the proper name of the product,
- the name and address of the manufacturer,
- the number of the final lot,
- the recommended storage temperature and the expiry date if kept at that temperature, and
- the recommended single human dose and route of administration.

In addition, the label printed on or affixed to the container, or the label on the cartons, or the leaflet accompanying the container shall contain the following:

- a statement that the vaccine satisfies the requirements of this document,
- the nature and amount of any preservative present in the vaccine,
- the nature and amount of the adsorbing agent,
- the recommended temperature for storage and transport,
- a warning that the adsorbed vaccine should not be frozen,
- a warning that the adsorbed vaccine should be shaken before use, and

- instructions for the use of the vaccine and information on contraindications and the reactions that may follow vaccination.

A.9 Distribution and transport

The requirements given in Part A, section 9, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply.

A.10 Stability, storage and expiry date

A.10.1 Stability

Tests shall be conducted to determine the loss of potency to be expected during storage. The stability of the vaccine shall be demonstrated to the satisfaction of the national control authority; final containers from at least three lots derived from different lots of purified bulk toxoid shall be tested on the expiry date to demonstrate stability during storage. The vaccine shall meet the requirements for final product (see Part A, sections A.5.3, A.5.4, A.5.7 and A.5.8) up to the expiry date, provided that it has been stored at the recommended temperature. When any changes are made in the production procedure that may affect the stability of the product, the vaccine produced by the new method shall be shown to be stable.

The statements concerning storage temperature and expiry date appearing on the label, as required in Part A, section 8, shall be based on experimental evidence and shall be submitted for approval to the national control authority.

A.10.2 Storage conditions

Storage at a temperature of 5 °C ± 3 °C has been found to be satisfactory.

Adsorbed vaccines shall not be frozen.

A.10.3 Expiry date

The expiry date shall be approved by the national control authority based on the stability studies referred to in section A.10.1

and shall relate to the date of the last satisfactory potency determination, performed in accordance with Part A, section A.5.3, i.e., the date on which the test animals were immunized with the vaccine.

PART B. NATIONAL CONTROL REQUIREMENTS

B.1 General

The general requirements for control laboratories contained in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*7*) shall apply.

The detailed production and control procedures and any significant changes in them shall be discussed with, and approved by, the national control authority, which shall obtain the International Standard for Diphtheria Toxoid, Adsorbed and establish a national working reference preparation by comparison with it.

B.2 Official release and certification by the national control authority

A vaccine shall be released only if it satisfies Part A of the present Requirements.

A statement signed by the appropriate official of the national control authority shall be provided at the request of the manufacturing establishment and shall certify that the lot of vaccine in question satisfies all national requirements as well as Part A of the present Requirements. The certificate shall state the number under which the lot was released by the national control authority, and the number appearing on the labels of the containers. The official national release document shall be provided to importers of diphtheria vaccines.

The purpose of the certificate is to facilitate the exchange of diphtheria vaccines between countries. A model of a suitable certificate is given in Appendix 2.

REQUIREMENTS FOR TETANUS VACCINE (ADSORBED)

GENERAL CONSIDERATIONS

Tetanus toxoid is one of the most immunogenic antigens available for protection against an infectious disease. In the developed countries, its use has markedly decreased the incidence of tetanus and the demand for tetanus antitoxin, but in the developing world much needs to be done to increase its use. This is particularly important where neonatal tetanus can be eliminated by the immunization of pregnant women.

The developments leading to the formulation of the first Requirements for Tetanus Toxoid are described in detail in the Requirements for Biological Substances No. 10 (*2*). The purpose of the present General Considerations section is to draw attention to the significant developments that have taken place since those Requirements were revised in 1978 (*3*).

As with diphtheria vaccine, the most important development is the agreement reached on the formulation of requirements for the assay of potency. It has now been accepted that the potency of tetanus vaccine can be measured by an active challenge test and that either guinea-pigs or mice may be used. In studies of the use of a lethal challenge dose as compared with a paralytic challenge dose, it was found that they give similar results when the potency of a test vaccine is compared with that of a reference preparation. It is important to note, however, that when pertussis vaccine is mixed with tetanus toxoid and when the potency assay is carried out in mice, there is a significant adjuvant effect due to the whole-cell pertussis component. Allowance must be made for this effect in the assay of a combined vaccine to determine the potency of the tetanus component. The minimum acceptable level of potency expressed in International Units must be specified by the national control authority.

The statements made in the section on diphtheria vaccines dealing with the reduction in the number of laboratory animals used in potency tests based on challenge (see pp. 91–92) are equally applicable to tetanus vaccines. Further development of a variety of specific methods for the assay of tetanus antitoxin should also result in a reduction in the utilization of laboratory animals.

Although there are few data on which a correlation between the potency level determined by a biological assay and protection in humans could be based, and even fewer on which to base a correlation between the potency level and the duration of immunity, some evidence is available that makes it possible to specify a level above which a vaccine may be considered to be of acceptable potency. Such a level is included in the present Requirements. It is important, therefore, for countries to adopt the principle of expressing the potency of tetanus vaccines in International Units. In some countries, potency tests based on the ability of vaccines to induce specified levels of antitoxin are used, but such assays are often performed on pooled sera and without the use of calibrated reference materials, so that a valid statistical evaluation of the results cannot be made. Although data are available that demonstrate that vaccines meeting such requirements can induce significant levels of antitoxin response in humans, the use of more quantitative types of assays is recommended in these revised Requirements for Tetanus Vaccine (Adsorbed).

In addition, it is desirable that, whenever new vaccines are produced, long-term studies are carried out to confirm that, in 90% of the target population, tetanus antitoxin levels are above 0.01 IU/ml five years after the completion of primary immunization in previously unimmunized individuals.[1]

These Requirements call for the product to be purified, since tetanus toxoid in the unpurified form is liable to give rise to vaccination reactions in humans; much work has therefore been done in developing purified material in order to avoid them. Even with purified products, however, untoward reactions may occur in adults. There is evidence that purification, although enabling more highly concentrated preparations to be used, may sometimes reduce the immunizing activity of tetanus vaccine, probably as a result of the removal of substances having an adjuvant effect. Such purified products may be used for primary immunization after combination with an adjuvant. In these revised Requirements, the maximum number of Lf per single human dose of tetanus vaccine (adsorbed) has been reduced to 25 if more than one dose is recommended for primary immunization.

[1] Primary immunization usually consists of an initial course of two or three injections at an interval of 4–6 weeks, followed by a further injection 7–12 months later. Immunity can be reinforced or "boosted" by subsequent single injections, usually given a number of years later.

Each of the following sections constitutes a recommendation. Those parts of each section printed in large type have been written in the form of requirements, so that, if a health administration so desires, they may be adopted as they stand as definitive national requirements. Those parts of each section printed in small type are comments and/or recommendations for guidance.

Individual countries may wish to adopt these Requirements as the basis of their national regulations on tetanus vaccine. If national requirements differ from these requirements, it is recommended that the former should be shown to ensure that the vaccine is at least as safe and as potent as that prepared in accordance with the requirements formulated below. It is desirable that the World Health Organization should be kept informed of any such differences.

PART A. MANUFACTURING REQUIREMENTS

A.1 Definitions

A.1.1 International name and proper name

The international name shall be *Vaccinum tetani adsorbatum*. The proper name shall be the equivalent of the international name in the language of the country of use.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

A.1.2 Descriptive definition

Vaccinum tetani adsorbatum is a preparation of tetanus toxoid prepared by treating tetanus toxin by chemical means to render it nontoxic without losing its immunogenic potency. The toxoid is adsorbed on to a suitable adjuvant. The preparation shall satisfy the requirements formulated below.

The most common method of preparing toxoids from toxins is by means of formaldehyde.

A.1.3 International reference materials

The first International Reference Reagent of Tetanus Toxoid for Flocculation Tests was established in 1988 (*6*).

The second International Standard for Tetanus Toxoid, Adsorbed, was established in 1981 (*11*) for determining the potencies of vaccines containing tetanus toxoid. In view of the fact that different results may be obtained when potency tests are carried out in mice instead of guinea-pigs, tests in mice of vaccines containing tetanus toxoid should be performed using reference vaccines calibrated against the International Standard by means of potency tests on guinea-pigs; however, this is not an entirely satisfactory procedure.

The second International Standard for Tetanus Antitoxin was established in 1969 (*12*); it has an *in vivo/in vitro* ratio of antitoxin activity of 1.4 and is made of purified hyperimmune horse serum.

The above-mentioned international reference materials are in the custody of the International Laboratory for Biological Standards, State Serum Institute, Copenhagen. Samples are distributed free of charge on request to national control laboratories. The international reference materials are intended for the calibration of national reference materials for use in the manufacture and control of tetanus antitoxin and vaccine.

A.1.4 Terminology

Seed lot: A quantity of bacterial suspension that is derived from one strain, has been processed as a single lot and has a uniform composition. It is used for preparing the inoculum for the production medium.

Single harvest: The toxic filtrate or toxoid obtained from one batch of cultures inoculated, harvested and processed together.

Bulk purified toxoid: The processed purified material, prepared from either a single harvest or a pool of a number of single harvests. It is the parent material from which the final bulk is prepared.

Final bulk: The final homogeneous vaccine present in a single container from which the final containers are filled either directly or through one or more intermediate containers.

Final lot: A collection of sealed final containers that are homogeneous with respect to the risk of contamination during filling. A final lot must therefore have been filled from a single container in one continuous working session.

A.2 General manufacturing requirements

The general requirements for manufacturing establishments contained in the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*7*) shall apply to establishments manufacturing tetanus vaccine with the addition of the following:

All manufacturing processes up to and including the completion of detoxification shall be carried out in completely separate areas and by means of separate equipment.

Written descriptions of procedures for the preparation and testing of tetanus vaccine adopted by a manufacturer together with appropriate evidence that each production step has been validated shall be submitted for approval to the national control authority. Proposals for modifications of the manufacturing and/or control methods shall also be submitted for approval to the national control authority before such modifications are implemented.

A.3 Production control

A.3.1 Control of source materials

A.3.1.1 *Strains of Clostridium tetani*

Strains of *C. tetani* used in preparing tetanus toxoid shall be identified by a record of their history and of all tests made periodically to verify strain characters. The strain shall be maintained as a freeze-dried culture.

A highly toxinogenic strain of *C. tetani* should be used. A strain that has proved satisfactory in many laboratories is the Harvard strain.

A.3.1.2 *Seed lot system*

The production of tetanus toxin shall be based on a seed lot system. Cultures of the working seed shall have the same characteristics as those of the strain from which the parent seed lot was derived. The preparation of the seed lot shall comply with the requirements of Part A, section A.3.2.

A.3.1.3 *Culture medium for production of toxin*

It is particularly important to ensure that the final product is free from substances likely to cause toxic or allergic reactions in humans.

The method of detecting these substances should be approved by the national control authority.

If the medium is prepared from a protein digest, e.g., casein hydrolysate or digested muscle, precautions should be taken to ensure that digestion has proceeded sufficiently. Established limits, if any, for mammalian protein and human blood-group substances in the final vaccine should not be exceeded.

A.3.2 Production precautions

The general production precautions, as formulated in the requirements of Part A, section 3, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*7*), shall apply to the manufacture of tetanus vaccine.

Suitable methods for the production of tetanus vaccine are given in the *Manual for the production and control of vaccines: tetanus toxoid* (*13*).

Personnel employed in production and quality control must be adequately trained and immunized.

A.3.3 Control of single harvests

Consistency of production shall be demonstrated.

Consistency may be demonstrated by measuring, e.g., the bacterial growth rate, pH and rate of toxin production.

Any culture showing anomalous growth characteristics shall be investigated and shown to be satisfactory before being accepted as a single harvest.

A.3.3.1 *Control of bacterial purity*

Samples of cultures used for preparing single harvests of toxoid shall be tested for bacterial purity by microscopic examination of stained smears or by inoculation into appropriate culture media. Single harvests shall not be used for preparing bulk materials if contamination has occurred at any stage in their production.

A.3.3.2 *Filtration*

After having been sampled for control of purity, cultures shall be sterilized by means of filtration. A preservative may be added, but phenol shall not be used for this purpose.

Cultures should be filtered as soon as possible after the end of their incubation period. To facilitate filtration, cultures may be centrifuged, provided that suitable precautions are taken to avoid the formation of potentially hazardous aerosols. A filter aid may be added beforehand.

In some countries, no filter capable of shedding fibres may be used.

A.3.3.3 *Determination of antigen concentration*

The supernatant of the culture prior to inactivation shall be tested by a method approved by the national control authority.

It is advisable to determine the antigen content by measuring the toxin content. This is usually done *in vivo*.

Another suitable method for determining the antigen concentration is the flocculation test described in the *Manual for the production and control of vaccines: tetanus toxoid* (*13*); it should be performed both on the supernatant and, for purposes of comparison, a reference material calibrated against the International Reference Reagent of Tetanus Toxoid for Flocculation Tests, or an equivalent reference material approved by the national control authority.

It is preferable for culture filtrates used in preparing purified toxoid to contain not less than 40 Lf/ml.

Antigen content is a good indicator of consistency of production.

A.3.3.4 *Detoxification and purification of toxin*

Purification may either precede or follow detoxification. Purification before detoxification results in a purer product, but particular care must be taken to avoid reversion to toxin, which may also occur when detoxification precedes purification. The method and agent used for detoxification and the method of purification shall be approved by the national control authority.

After detoxification has been completed, the detoxifying agent shall be removed or neutralized by a method approved by the national control authority.

The method of purification shall be such that no substances are incorporated into the final product that are likely to cause untoward

reactions in humans. The rate of detoxification may vary and shall be monitored. Harvests shall not be transferred from the detoxification area until the detoxification has been shown to be complete.

A.3.4 Control of bulk purified toxoid

A.3.4.1 *Preparation*

The bulk purified toxoid shall be prepared from either a single harvest or a pool of single harvests, and shall be sterile. Phenol shall not be used as a preservative.

It is advisable to sterilize the bulk purified toxoid by filtration. A preservative approved by the national control authority may be added to the bulk toxoid.

A.3.4.2 *Sterility*

Each bulk toxoid shall be tested for bacterial and mycotic sterility in accordance with the requirements of Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances (*9*)) or by a method approved by the national control authority. If a preservative has been added to the purified bulk, appropriate measures shall be taken to prevent any interference by it in the sterility test.

A.3.4.3 *Specific toxicity*

Each bulk purified toxoid shall be tested for the presence of tetanus toxin by injection into at least five guinea-pigs, each weighing 250–350 g. Each guinea-pig shall be given a subcutaneous injection of 1 ml of a dilution of purified toxoid containing at least 500 Lf of toxoid. Each guinea-pig shall be observed daily and closely examined weekly for signs of tetanic paralysis. Animals that die shall be examined by autopsy. The bulk purified toxoid shall pass the test if no guinea-pig shows symptoms of specific paralysis or any other signs of tetanus within 21 days of injection and if at least 80% of the animals survive the test period. The guinea-pigs shall not have been used previously for experimental purposes.

A.3.4.4 *Reversion to toxicity*

Each bulk purified toxoid shall be tested to ensure that reversion to toxin cannot take place on storage. The bulk toxoid shall be diluted in order to obtain the same concentration and chemical environment as those present in the final bulk vaccine, except for the presence of adjuvant.

To determine whether reversion has occurred, diluted toxoids which have been stored at 37 °C for six weeks shall be tested. The test employed shall be approved by the national control authority and shall be sufficiently sensitive to detect very small amounts of toxin. No toxicity shall be detected.

In one country, the test is performed on toxoids that have been stored at 34 °C.

Similar dilutions of toxoid held at 2–8 °C during the same period of time as those held at 34 °C or 37 °C may be tested as controls.

Mice are not as sensitive to tetanus toxin as guinea-pigs but may be used for the test, subject to the approval of the national control authority.

A.3.4.5 *Antigenic purity*

Each bulk purified toxoid shall be tested for antigenic purity by determining the Lf value and the concentration of protein (nondialysable) nitrogen. The Lf value shall be determined by comparison with a reference material calibrated against the International Reference Reagent of Tetanus Toxoid for Flocculation Tests or an equivalent reference preparation approved by the national control authority. The method of testing shall be approved by the national control authority. The bulk purified toxoid shall pass the test if it contains no fewer than 1000 Lf per mg of protein (nondialysable) nitrogen.

Preparation of toxoid containing more than 1500 Lf per mg is both feasible and desirable.

An indication of the antigenic quality of the toxoid may be obtained by measuring the total combining power and expressing it in relation to the number of Lf units. A suitable method for measuring the total combining power is given in the *Manual for the production and control of vaccines: tetanus toxoid* (*13*).

A.3.5 Control of final bulk

A.3.5.1 *Preparation*

The final bulk shall be prepared from bulk purified toxoid. The number of Lf in a single human dose shall be approved by the national control authority but shall not exceed 25 if more than one dose is recommended for primary immunization.

A.3.5.2 *Preservative*

If the vaccine is to be filled in multidose containers, a suitable antimicrobial preservative shall be added. The amount of preservative in the final bulk shall have been shown to have no deleterious effect on the toxoid or on other vaccine components with which the toxoid may be combined, and to cause no unexpected adverse reactions in humans. The preservative and its concentration shall be approved by the national control authority.

Phenol shall not be used as a preservative.

A.3.5.3 *Adjuvants*

The adjuvants used, their purity and their concentration shall be approved by the national control authority.

Aluminium or calcium compounds are generally used as mineral carriers.

The concentration of aluminium shall not exceed 1.25 mg and that of calcium 1.3 mg per single human dose.

In some countries, these upper limits for the concentration of mineral carriers are considered to be too high and the limits are set at about half those given above.

In some countries, the adsorbent is precipitated in the presence of the toxoid.

The formulation shall be such that the vaccine remains suspended for a reasonable time after shaking.

A.3.5.4 *Sterility*

Each final bulk shall be tested for bacterial and mycotic sterility in accordance with the requirements of Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General

Requirements for the Sterility of Biological Substances, revised 1973) (*9*) or by a method approved by the national control authorities. If a preservative has been added to the final bulk, adequate measures shall be taken to prevent any interference by it in the sterility test.

A.3.5.5 *Specific toxicity*

In some countries, each final bulk is tested for specific toxicity in at least five guinea-pigs, each weighing 250-350 g. Each guinea-pig is given a subcutaneous injection of a quantity equivalent to at least five single human doses, and is then observed daily and examined closely every week for signs of tetanic paralysis. Animals that die are examined by autopsy. The final bulk passes the test if no guinea-pig shows paralysis or any other signs of tetanus within 21 days of injection and if at least 80% of the animals survive the test period. The guinea-pigs must not have been used previously for experimental purposes.

A.3.5.6 *Potency*

The immunizing potency of each final bulk shall be determined by comparison with an appropriate reference material properly calibrated against the International Standard for Tetanus Toxoid, Adsorbed. The test shall involve the inoculation of guinea-pigs or mice with appropriate doses or dilutions of both the final product and the reference material. After immunization, the animals shall be bled or challenged by the subcutaneous route (*10*). If animals are bled, the antitoxin levels of the individual animals may be titrated by means of toxin neutralization tests performed using *in vivo* or *in vitro* serological methods that have been validated on vaccines of the type being tested. Appropriate statistical methods shall be used to calculate the potency of the final bulk. The method adopted and the interpretation of the results shall be approved by the national control authority.

Care should be taken to ensure that diluents are inert and not pyrogenic. Phosphates might interfere with the adsorption of toxoid.

When consistency of production and testing have been established, the numbers of animals injected with each dilution of product may be reduced to levels substantially lower than those originally needed for the three-dilution assays described in the *Manual of details of tests required on final vaccines used in the WHO Expanded Programme on Immunization* (*10*), provided

that the resulting assays are statistically valid. Test methods based on individual quantification of antitoxin allow the use of fewer animals than are needed in challenge tests.

Depending on the purpose, two types of potency assays may be consideree.

Three-dilution assayc m!y be used to test consistency of production and product stacility, and to calibrate reference preparations.

One-dilution assays[1] for evaluating the response based on the same principle as the three-dilution assays may be used at the discretion of the national control authority for the routine testing of vaccine lots of a given formulation as soon as the production process has been established and consistency in production and control has been demonstrated. The test involves the selection of a dose of reference vaccine, expressed as a fraction of 40 IU (i.e., of the minimum potency of a single human dose) that elicits a minimal protective effect in mice or guinea-pigs, and comparing its effect with the response elicited by the same fraction of a human dose of the test vaccine. If the response to the latter is significantly greater than that to the former ($P \leqslant 0.05$), the potency of the test vaccine is satisfactory. One-dilution tests offer advantages only when vaccine potencies are consistently and substantially in excess of 40 IU per single human dose.

The potency of the final bulk shall be approved by the national control authority. The potency of tetanus vaccine used for the immunization of children shall not be less than 40 IU per single human dose. For three-dilution assays, the limits of the 95% confidence intervals of the estimate of potency shall be within 50–200% of the estimated potency unless the lower limit of the 95% confidence interval of the estimated potency is greater than 40 IU per single human dose. When one-dilution tests are performed, the potency of the test vaccine shall be demonstrated to be significantly greater than 40 IU per human dose.

In some countries, potency testing is not carried out on each final bulk but on each final lot.

A.3.5.7 *Amount of residual free detoxifying agent*

The amount of residual free detoxifying agent in each final bulk shall be determined by a method approved by the national control

[1] Information on one-dilution assay methods is given in document BS/89.1618, available on request from Biologicals, World Health Organization, Geneva, Switzerland.

authority and, if formaldehyde has been used, the residual content shall be not more than 0.2 g/l.

The colorimetric determination of the reaction product of formaldehyde and fuchsin-sulfurous acid is a suitable method.

In some countries, the amount of residual free detoxifying agent is determined in the purified bulk.

If applicable, appropriate tests for the presence of other detoxifying agents (e.g., glutaraldehyde) shall be performed. The tests used and the maximum permissible concentrations of such chemicals shall be approved by the national control authority.

A.3.5.8 *pH*

The pH of the final bulk shall be measured.

The pH should be between 6.0 and 7.0.

A.4 Filling and containers

The requirements applicable to filling and containers given in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply.

Single-dose or multiple-dose containers may be used. Vaccines in multidose containers shall contain a suitable antimicrobial preservative.

A.5 Control of final product

A.5.1 Identity

An identity test shall be performed on at least one labelled container from each final lot.

Flocculation in solution, immunoprecipitation of the toxoid in gels or any other specific interaction between the vaccine and tetanus antitoxin may serve as an identity test. Tests on toxoids adsorbed on to aluminium or calcium carriers may be performed after the carrier has been dissolved, or the adsorbed toxoid wholly or partially eluted by sodium citrate at pH 9.

If adequate quantities of toxoid cannot be recovered from the adsorbed vaccine, specific antitoxin may be sought in the sera of animals used in the innocuity test.

A.5.2 Sterility

Final containers shall be tested for bacterial and mycotic sterility by a method approved by the national control authority.

Many countries have regulations governing the sterility testing of the final product. Where these do not exist, the requirements published by WHO shall be met (*9*). If a preservative has been added to the vaccine, appropriate measures shall be taken to prevent any interference by it in the sterility test.

A.5.3 Potency

A potency test shall be carried out as provided in Part A, section A.3.5.6, on each final lot, if such a test has not been performed on the final bulk.

A.5.4 Innocuity

Each final lot shall be tested for abnormal toxicity by the injection by the intraperitoneal route of one human dose, but not more than 1 ml, into each of five mice (weighing 17–22 g) and at least one human dose, but not more than 1 ml, into each of two guinea-pigs (weighing 250–350 g). The tests shall be approved by the national control authority. The final product shall be considered innocuous if the animals survive for at least seven days without showing significant signs of toxicity.

A.5.5 Adjuvant content

The adjuvant content of each final lot shall be determined by a method approved by the national control authority (see Part A, section A.3.5.3).

In some countries, this test is used to verify the homogeneity of filling.

A.5.6 Preservative content

The preservative content of each final lot shall be determined (see Part A, section A.3.5.2). The method used shall be approved by the national control authority.

In some countries, this test is applied to the final bulk only.

A.5.7 pH

The pH of each final lot shall be measured.

The pH should be between 6.0 and 7.0.

A.5.8 Inspection of final containers

Each container in each final lot shall be inspected visually, and those showing abnormalities—such as improper sealing, lack of integrity, clumping or the presence of particles—shall be discarded.

A.6 Records

The requirements given in Part A, section 6, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*7*) shall apply.

Written records shall be kept of all tests, irrespective of their results. The records shall be of a type approved by the national control authority.

A model of a suitable summary protocol to be used for tetanus vaccines is given in Appendix 3.

A.7 Samples

The requirements given in Part A, section 7, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*7*) shall apply.

A.8 Labelling

The label printed on or affixed to each container and the label on the carton enclosing one or more containers shall show as a minimum:

- the words *Vaccinum tetani adsorbatum* and/or the proper name of the product,
- the name and address of the manufacturer,

- the number of the final lot,
- the recommended storage temperature and the expiry date if kept at that temperature, and
- the recommended single human dose and route of administration.

In addition, the label printed on or affixed to the container, or the label on the cartons, or the leaflet accompanying the container shall contain the following:

- a statement that the vaccine satisfies the requirements of this document,
- the nature and amount of any preservative present in the vaccine,
- the nature and amount of the adsorbing agent,
- the recommended temperature for storage and transport,
- a warning that the adsorbed vaccine should not be frozen,
- a warning that the adsorbed vaccine should be shaken before use, and
- instructions for the use of the vaccine and information on contraindications and the reactions that may follow vaccination.

A.9 Distribution and transport

The requirements given in Part A, section 9, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply.

A.10 Stability, storage and expiry date

A.10.1 Stability

Tests shall be conducted to determine the loss of potency to be expected during storage. The stability of the vaccine shall be demonstrated to the satisfaction of the national control authority; final containers from at least three lots derived from different lots of purified bulk toxoid shall be tested on the expiry date to demonstrate stability during storage. The vaccine shall meet the requirements for the final product (see Part A, sections A.5.3, A.5.4, A.5.7 and A.5.8) up to the expiry date, provided that it has been stored at the recommended temperature. When any changes are made in the

production procedure that may affect the stability of the product, the vaccine produced by the new method shall be shown to be stable.

The statements concerning storage temperature and expiry date appearing on the label, as required in Part A, section A.8, shall be based on experimental evidence and shall be submitted for approval to the national control authority.

A.10.2 Storage conditions

Storage at a temperature of 5 ± 3 C has been found to be satisfactory.

Adsorbed vaccines shall not be frozen.

A.10.3 Expiry date

The expiry date shall be approved by the national control authority based on the stability studies referred to in section A.10.1 and shall relate to the date of the last satisfactory potency determination, performed in accordance with Part A, section A.5.3, i.e., the date on which the test animals were immunized with the vaccine.

PART B. NATIONAL CONTROL REQUIREMENTS

B.1 General

The general requirements for control laboratories contained in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply.

The detailed production and control procedures and any significant changes in them shall be discussed with and approved by the national control authority, which shall obtain the International Standard for Tetanus Toxoid, Adsorbed and establish a national working reference preparation by comparison with it.

B.2 Official release and certification by the national control authority

A vaccine shall be released only if it satisfies Part A of the present Requirements.

A statement signed by the appropriate official of the national control authority shall be provided at the request of the manufacturing establishment and shall certify that the lot of vaccine in question satisfies all national requirements as well as Part A of the present Requirements. The certificate shall state the number under which the lot was released by the national control authority, and the number appearing on the labels of the containers. The official national release document shall be provided to importers of tetanus vaccines.

The purpose of the certificate is to facilitate the exchange of tetanus vaccines between countries. A model of a suitable certificate is given in Appendix 2.

REQUIREMENTS FOR PERTUSSIS VACCINE

GENERAL CONSIDERATIONS

The formulation of the Requirements for [whole-cell] Pertussis Vaccine and the events leading up to their formulation have already been described (*1*). These vaccines have been in use on a wide scale for almost 30 years and, where vaccines of adequate potency have been administered correctly in accordance with correct schedules, the incidence of whooping cough has decreased markedly.

However, two factors have given rise to concern among public health administrators, namely the toxicity of the vaccines and the lack of efficacy of some of them. Whole-cell pertussis vaccines consist of a suspension of killed organisms, and their inoculation has been temporally associated with adverse events. There appears to be no likelihood that vaccines, either whole-cell or acellular, that are effective but cause few adverse reactions can be produced in the immediate future. The aim of these Requirements is both to encourage the production of such vaccines in the longer term and to indicate where further research may be helpful.

Before release, whole-cell pertussis vaccines must be shown to be potent by the mouse protection test. The best evidence that this test is a good indicator of clinical efficacy was provided by the Medical Research Council trial carried out in the United Kingdom from 1951 to 1959, from which it was concluded that vaccines shown to protect mice against intracerebral challenge also protected immunized children against whooping cough when such children were exposed to the disease in the home by infection from a sibling. The establishment of this correlation between test results and efficacy was a marked advance in the development of whole-cell pertussis vaccines against whooping cough, but a number of anomalies still demand an explanation.

A great deal of effort has been put into attempts to obtain greater reproducibility in mouse protection tests. The use of healthy mice selected at random for their place in the test has improved the reproducibility of the results, which are also affected by the particular strain of mice selected, so that great attention should be paid to these parameters of the test.

Although the mouse protection test is still the only recognized test, further research aimed at the establishment of alternative or

supplementary tests should be actively encouraged. Until an alternative test has been shown to be good indicator of efficacy in humans, however, the mouse protection test will remain the only recognized test for the measurement of the potency of whole-cell pertussis vaccines. A description of the test is included in these Requirements.

The presence of agglutinogens 1, 2 and 3 in whole-cell pertussis vaccines is believed to contribute to their protective efficacy, and a test has been introduced in these revised Requirements for the purpose of determining whether such agglutinogens are present before adjuvant is added.

An immunizing dose of pertussis vaccine is the minimum number of killed organisms that have been shown to give an adequate antigenic stimulus and thus provide protection. The number of killed organisms required for this purpose is indicated by the opacity of the bacterial suspension, determined before the bacteria are killed.

Tests for toxicity continue to pose a problem, since a large number of toxins may be produced during the growth of pertussis organisms. Some of these, such as the heat-labile (i.e., dermonecrotic) toxin can be measured quite accurately, whereas others are difficult to quantify. The present Requirements can do no more than specify the mouse weight-gain test adopted by many manufacturers and required by many control authorities as an "in process" control measure. This should not be an impediment to further developments in the measurement of the toxicity of pertussis vaccines.

These revised Requirements propose tests for the heat-labile toxin, the lymphocytosis promoting factor and the endotoxin for use in monitoring detoxification processes, as well as for validating methods used for detoxification and establishing consistency of production.

Since the requirement previously included for the abnormal toxicity test (intraperitoneal injection of mice with a single human dose) gives rise to excessive vaccine-related symptoms of toxicity, these revised Requirements specify the intraperitoneal injection of only half a single human dose.

Each of the following sections constitutes a recommendation. Those parts of each section printed in large type have been written in the form of requirements so that, if a health administration so desires, they may be adopted as they stand as definitive national

requirements. Those parts of each section printed in small type are comments and/or recommendations for guidance.

Individual countries may wish to adopt these Requirements as the basis of their national regulations on pertussis vaccines. If national requirements differ from these requirements, it is recommended that the former should be shown to ensure that the vaccine is at least as safe and as potent as that prepared in accordance with the requirements formulated below. It is desirable that the World Health Organization should be kept informed of any such differences.

PART A. MANUFACTURING REQUIREMENTS

A.1 Definitions

A.1.1 International name and proper name

The international name shall be *Vaccinum pertussis*. The proper name shall be the equivalent of the international name in the language of the country of use.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

A.1.2 Descriptive definition

Vaccinum pertussis is a saline suspension of the whole cells of one or more strains of killed *Bordetella pertussis* which have been appropriately treated to minimize toxicity and retain potency. The preparation shall satisfy all the requirements formulated below.

A.1.3 International reference materials

The second International Standard for Pertussis Vaccine was established in 1980 (*14*). It is intended for the calibration of national standards used for determining the potencies of pertussis vaccines. It is in the custody of the International Laboratory for Biological Standards, State Serum Institute, Copenhagen.

The fifth International Reference Preparation of Opacity (*15*) consists of plastic rods simulating the optical properties of a bacterial suspension and defined as having an opacity of 10 International Units of opacity. Countries are invited to use the

International Reference Preparation of Opacity so that the opacities of suspensions of pertussis bacteria can be expressed in International Units of opacity. A bacterial suspension having the same opacity as the International Reference Preparation of Opacity has a bacterial concentration providing 10 IU of opacity. The relationship between such units and actual numbers of bacterial cells may vary from vaccine to vaccine.

The International Reference Preparation of Opacity is in the custody of the International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Potters Bar, Herts., England.

International reference materials are distributed free of charge, on request, to national control laboratories.

A.1.4 Terminology

Seed lot: A quantity of bacterial suspension that is derived from one strain, has been processed as a single lot and has a uniform composition. It is used for preparing the inoculum for the production medium.

Single harvest: A suspension of bacteria prepared from cultures of one strain of *B. pertussis* inoculated, harvested and processed together.

Final bulk: The homogeneous finished vaccine present in a single container from which the final containers are filled either directly or through one or more intermediate containers.

Final lot: A collection of sealed final containers that are homogeneous with respect to the risk of contamination during filling. A final lot must therefore have been filled from a single container in one continuous working session.

A.2 General manufacturing requirements

The general requirements for manufacturing establishments contained in the Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply to establishments manufacturing pertussis vaccine, with the addition of the following:

Written descriptions of procedures for the preparation and testing of pertussis vaccine adopted by a manufacturer together with appropriate evidence that each production step has been validated shall be submitted for approval to the national control authority. Proposals for modifications of the manufacturing and/or control method shall also be submitted for approval to the national control authority before such modifications are implemented.

A.3 Production control

A.3.1 Control of source materials

A.3.1.1 *Strains of Bordetella pertussis*

Strains of *B. pertussis* used in preparing vaccines shall be identified by a full record of their history, including their origin, characters on isolation, and particulars of all tests made periodically to verify strain characters. The strains shall be chosen in such a way that the final vaccine includes agglutinogens 1, 2 and 3.

The strains shall be maintained by a method that will preserve their ability to yield potent vaccine.

Freeze-drying or storage in liquid nitrogen is a satisfactory method of maintaining strains.

A.3.1.2 *Seed lot system*

The production of pertussis vaccine shall be based on a seed lot system. Cultures of the working seed shall have the same characteristics as those of the strain from which the parent seed lot was derived.

A.3.1.3 *Culture medium for production of bacteria*

The medium shall enable *B. pertussis* to grow and to retain agglutinogens and potency. Human blood or blood products shall not be used in culture media for propagating bacteria, either for seed or for vaccine. When animal blood or blood products are used, they shall be removed by washing the harvested bacteria, and the final product shall be demonstrated to be free of contaminating antigens and allergenic substances.

The absence of sensitizing animal proteins in final vaccines may be demonstrated by passive cutaneous anaphylaxis or other suitably sensitive and specific procedures. In some countries, the use in the medium of blood from any source is not permitted.

A.3.2 Production precautions

The general production precautions, as formulated in the requirements of Part A, section 3, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories (7) shall apply to the manufacture of pertussis vaccine.

A.3.3 Control of single harvests

Consistency of production shall be demonstrated.

Consistency may be evaluated by measuring the bacterial growth rate and testing for the presence of agglutinogens in the cultured organisms.

Any culture showing anomalous growth characteristics shall be investigated and shown to be satisfactory before being accepted as a single harvest.

A.3.3.1 *Control of bacterial purity*

Samples of single harvests taken before killing shall be tested for purity by microscopic examination of stained smears or by inoculation into appropriate culture media. Single harvests shall not be used for the final bulk if contamination has occurred at any stage in their production.

A.3.3.2 *Control of opacity*

The opacity of each single harvest shall be measured not later than two weeks after harvesting and before the bacterial suspension has been subjected to any process capable of altering its opacity. It shall be measured by comparison with the International Reference Preparation of Opacity or an equivalent reference preparation approved by the national control authority.

A.3.3.3 *Killing and detoxification*

After samples of single harvests have been taken for purposes of purity control and opacity measurement, the bacteria shall be killed and detoxified by a method approved by the national control authority. If chemicals are used for this purpose, they shall be approved by the national control authority. In order to ensure that

the organisms have been killed, a sample shall be tested in an appropriate culture medium.

B. pertussis can be killed by a number of methods whose effectiveness depends on the concentration of the chemicals used and the temperature, time and pH at which killing is carried out. The aim in killing and detoxification is to achieve complete killing and an appropriate level of detoxification without adversely affecting the potency or the physical characteristics of the vaccine. The methods used should be validated to the satisfaction of, and approved by, the national control authority.

After killing and detoxification, the opacity of the suspension will be different from what it was originally. Each single harvest should, however, still be regarded as containing the same number of bacteria.

No biologically active heat-labile toxin (dermonecrotic toxin) should be detectable in a vaccine. The method of manufacture should have been shown to ensure that active dermonecrotic toxin is not present in the final product. The method of detoxification used should minimize the bioactivity of lymphocytosis promoting factor while retaining immunogenicity. Since endotoxin (lipopolysaccharide, LPS) is an intrinsic part of the cell envelope of *B. pertussis*, it is best controlled by reducing the number of bacteria needed to achieve an acceptable level of potency (see also section A.3.4.7). In the present state of knowledge, it is not possible to recommend limits for levels of lymphocytosis promoting factor, endotoxin, tracheal cytotoxin and adenylate cyclase in whole-cell pertussis vaccines.

A.3.4 Control of final bulk

A.3.4.1 *Preparation*

The final bulk shall be prepared by pooling a number of single harvests. Where vaccine is prepared from two or more strains, consecutive batches of the final bulk shall be consistent with respect to the proportions of each strain present, as measured in opacity units. The concentration of bacteria in the final bulk, when prepared as a formulation for a single human dose of 1 ml, shall correspond to an opacity (before killing) of not more than 20 IU. The concentration of bacteria in the final bulk, when prepared as a formulation for a single human dose of 0.5 ml, shall correspond to an opacity (before killing) of not more than 40 IU. The number of IU of opacity in the final bulk shall be calculated from that found in the tests (see Part A, section A.3.3.2) performed on the single harvests.

It is advisable to use as few IU of opacity as possible while still satisfying the potency requirements prescribed in section A.3.4.7. Potent vaccines which contain in a single human dose the equivalent of a 1-ml suspension having 5–10 IU of opacity can be produced consistently and manufacturers should be encouraged to produce potent vaccines of even lower opacity. In some countries, vaccines containing an aluminium carrier must not contain in a single human dose more than the equivalent of 1 ml of a suspension having 16 IU of opacity.

A.3.4.2 *Agglutinogens*

Each bulk shall be examined for the presence of agglutinogens 1, 2 and 3 before adjuvant is added.

A.3.4.3 *Preservative*

If the vaccine is to be dispensed into multidose containers, a suitable antimicrobial preservative shall be added. The amount of preservative in the final bulk shall have been shown not to have any deleterious effect on the pertussis immunogen or on other vaccine components with which pertussis vaccine may be combined, and not to cause any unexpected adverse reactions in humans. The preservative and its concentration shall be approved by the national control authority.

Phenol shall not be used as a preservative.

A.3.4.4 *Adjuvants*

Adjuvants may be added to the vaccine; their nature, purity and concentration shall be approved by the national control authority.

Aluminium or calcium compounds may be used as mineral carriers.

The concentration of aluminium shall not exceed 1.25 mg and that of calcium 1.3 mg per single human dose.

In some countries, an upper limit of 1.25 mg of aluminium is considered to be excessive for products containing a pertussis component and such vaccines contain only 0.1–0.3 mg of aluminium per single human dose.

The formulation shall be such that the vaccine remains suspended for a reasonable time after shaking.

A.3.4.5 *Sterility*

Each final bulk shall be tested for bacterial and mycotic sterility in accordance with the requirements given in Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*9*) or by a method approved by the national control authority. If a preservative has been added to the vaccine, appropriate measures shall be taken to prevent any interference by it in the sterility test.

A.3.4.6 *Specific toxicity*

Each final bulk shall be tested for toxicity by a validated method approved by the national control authority.

Toxicity tests in animals are required by many national control authorities, but in most tests the results obtained depend to some extent on factors independent of the vaccine, such as the strain of animal used and the conditions under which the animals are kept.

Little information is available on the relationship between the toxicity of vaccines in animals and the occurrence of untoward reactions following vaccination in humans.

Mouse weight-gain test. One of the tests most widely used is the mouse weight-gain test; it has been of some value in ensuring the production of vaccines which, in general, are satisfactory in that they cause minimal untoward reactions in humans. The test may be performed as follows. No fewer than 10 healthy mice each weighing 14–16 g are used for each sample and for the saline control. They should have access to food and water for at least 2 h before injection and continuously after injection for the duration of the test. The total weight of the group of mice is determined immediately before injection. The mice used for testing the vaccine(s) and the control group of mice should be of the same sex. If both sexes are used, they should be equally distributed in all groups. Each mouse is given an intraperitoneal injection of 0.5 ml of a suspension containing a volume of the final bulk under test equivalent to not less than half the volume recommended as a single human dose. A similar control group of mice is inoculated with 0.5 ml of physiological saline, preferably containing the same amount of preservative as the inoculum injected into the test mice. The total weight of each group of mice is determined 72 h and seven days after injection. The final bulk is considered to be satisfactory if: (*a*) at the end of 72 h the total weight of the group is not less than it was before the injection; (*b*) at the end of seven days, the average weight gain per mouse is not less than 60% of that of the control group

of mice; and (*c*) not more than 5% of the total number of injected mice die.

Other tests. Cell harvests of *B. pertussis* to be used in the manufacture of pertussis vaccine contain a number of biologically active molecules which may contribute to the toxicity of final product. Assays for some of these substances can be used to monitor and validate the methods used for detoxification and may also be useful in assessing final products. In the process of validating the manufacturing procedures, manufacturers are encouraged to measure at least one of the following:

(1) *Heat-labile toxin* (dermonecrotic toxin). Subcutaneous injection of test materials into the nuchal area of suckling mice is the most sensitive and useful method of detecting heat-labile toxin. The suckling mouse test should be used to validate the manufacturing process but need not be performed on the final bulk or final container materials. Pertussis vaccine should not contain biologically active heat-labile toxin.

(2) *Lymphocytosis promoting factor.* Many different assays can be used to measure the diverse activities of lymphocytosis promoting factor but many suffer from technical problems which make their use as routine control procedures difficult. The induction of lymphocytosis in mice is not a sensitive method but is an adequate means of monitoring the level of active factor in bulk vaccine. Tests for histamine sensitizing activity in mice may also be used.

(3) *Endotoxin.* The endotoxin content of vaccines can be determined by the limulus amoebocyte lysate assay, the rabbit pyrogen test and the silver staining of electrophoretic gels. However, there is no agreement as to what constitutes an acceptable level of endotoxin in whole-cell pertussis vaccines.

A.3.4.7 *Potency*

The potency of each final bulk (or of each final lot) shall be determined by comparison with that of a reference vaccine calibrated against the International Standard for Pertussis Vaccine or an equivalent standard vaccine approved by the national control authority. The assay shall be performed by the intracerebral mouse protection test. The assay method and the method of calculating the results shall be approved by the national control authority. The potency of the final bulk shall be not less than 4.0 IU in the volume recommended as a single human dose.

A satisfactory method of carrying out the assay is as follows:

(*a*) *Mice.* Healthy mice, preferably from a strain and colony capable of giving an adequate immune response, are used. They should preferably be of the same sex but, if this is not possible, both sexes should be distributed equally throughout the test and the sexes segregated. Each mouse should weigh at least 10 g but not more than 18 g and, in a single test, the mice should not differ in weight by more than 4 g.

The mice are randomly allocated to the different groups, and the shelf position of the cages, the order of immunization, and the order of challenge are also randomized. Groups of at least 16 mice should be used for each dilution of the standard vaccine and of the vaccines under test, and at least 16 mice should be used for each dilution of the culture in the determination of the challenge dose.

(*b*) *Immunization of mice.* At least three dilutions of the reference vaccine and of each lot of vaccine should be tested. Serial dilutions, not greater than five-fold, of the vaccine to be tested and of the standard vaccine should be made in a suitable diluent. The median effective dose (ED_{50}) for each preparation should be bracketed by the dilutions used. Each mouse in each immunization group should be injected intraperitoneally with 0.5 ml of the appropriate dilution.

The interval between immunization and challenge should be 14–17 days. At least 94% of the mice immunized by each dilution of both the reference vaccine and the vaccines under test should survive until challenged, and each mouse challenged should appear healthy prior to challenge.

(*c*) *The challenge.* The strain used for challenge (generally *B. pertussis* 18323) should be approved by the national control authority. To ensure constancy of virulence from test to test, a large working challenge lot prepared from the master culture is dispensed into ampoules and freeze-dried or stored in liquid nitrogen.

The bacterial suspension used for challenge is prepared from a 20–24-h culture grown on Bordet-Gengou medium, or other suitable medium that has been seeded from a rapidly growing culture not more that 30 h old. Alternatively, aliquots of the challenge suspension may be frozen and kept in liquid nitrogen; after thawing and dilution, they can be used directly as the challenge culture. The suspension is diluted with a diluent in which the organisms will remain viable, e.g., an aqueous solution containing 10 g/l casein peptone and 6 g/l sodium chloride adjusted to a pH of 7.1 ± 0.1. The suspension, free from particles of agar or clumps of bacteria, is adjusted in such a way that each challenge dose of not more than 0.03 ml contains 100–1000 times the median lethal dose (LD_{50}).

Mice immunized with the reference vaccine and the vaccines under test are challenged at random under mild narcosis by intracerebral injection of the challenge dose. To obtain an estimate of the LD_{50}, dilutions of the challenge dose are then injected into control mice by the intracerebral route and an appropriate dilution of the challenge dose is cultured on Bordet-

Gengou medium to determine the number of colony-forming units contained therein.

(*d*) *Recording of results*. The mice are observed for 14 days. Mice dying within 72 hours should be excluded from the test. To determine the ED_{50} of the vaccines, records should be kept of the number of mice that die after 72 hours.

(*e*) *Calculation of results*. The ED_{50} values for each preparation are determined by a statistical method that includes the transformation of the mouse survival data into a form capable of consistently producing a linear regression. Probits, logits and angle transformation have been shown to be suitable. Similar methods should be used to determine the LD_{50} of the challenge suspension.

(*f*) *Validity of the test*. The test is valid if the ED_{50} of each vaccine is intermediate between the largest and the smallest immunizing doses, and the regressions do not show significant deviations from linearity and parallelism ($P \leqslant 0.05$). The challenge dose should contain 100–1000 LD_{50} and the LD_{50} should contain no more than 300 colony-forming units.

(*g*) *Estimate of potency*. The ED_{50} of the vaccine under test and the standard vaccine are calculated by a method that provides an estimate of the limits of the 95% confidence intervals (*10*). The potency is estimated in terms of International Units in the volume recommended for a single human dose, unless the national control authority decides otherwise. The vaccine passes the requirements for potency if the result of a statistically valid test shows that the estimated potency of the vaccine is not less than 4.0 IU in the volume recommended for a single human dose and if the lower fiducial limit ($P = 0.95$) of the estimated potency is not less than 2.0 IU. Additional tests may be carried out, but in this case the results of all valid tests must be combined in the geometric mean estimate and its lower fiducial limit. In some countries, an upper limit of potency is also specified.

A.3.4.8 *pH*

The pH of the final bulk shall be measured.

A.4 Filling and containers

The requirements applicable to filling and containers given in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*7*) shall apply.

Single-dose and multiple-dose containers may be used. Vaccine in multidose containers shall contain a suitable antimicrobial preservative.

A.5 Control of final product

A.5.1 Identity

An identity test shall be performed on at least one container from each final lot.

Agglutination of the organisms with specific antipertussis serum may serve as an identity test. Vaccines may also be inoculated into animals in order to show that pertussis agglutinins are produced in their serum.

A.5.2 Sterility

Final containers shall be tested for sterility by a method approved by the national control authority.

Many countries have regulations governing the sterility testing of the final product. Where these do not exist, the requirements published by WHO shall be met (*9*). If a preservative has been added to the vaccine, appropriate measures shall be taken to prevent any interference by it in the sterility test.

A.5.3 Potency

A potency test shall be carried out as provided in Part A, section A.3.4.7, on each final lot, if such a test has not been made on the final bulk.

A.5.4 Innocuity

Each final lot shall be tested for abnormal toxicity by the injection by the intraperitoneal route of half a human dose, but not more than 1 ml, into each of five mice (weighing 17–22 g) and at least one human dose, but not more than 1 ml, into each of two guinea-pigs (weighing 250–350 g). The tests shall be approved by the national control authority. The final product shall be considered innocuous if the animals survive for at least seven days without showing significant signs of toxicity.

A.5.5 Adjuvant content

If an adjuvant has been added to the final bulk, the adjuvant content of the final lot shall be determined by a method approved by the national control authority (see Part A, section A.3.4.4).

In some countries, this test is applied to verify the homogeneity of filling.

A.5.6 Preservative content

If preservative has been added to the final bulk, the preservative content of the final lot shall be determined (see Part A, section A.3.4.3). The test method shall be approved by the national control authority.

In some countries, this test is applied to the final bulk only.

A.5.7 pH

The pH of each final lot shall be measured.

The permitted range of pH values shall be approved by the national control authority.

In some countries, this test is applied to the final bulk only.

A.5.8 Inspection of final containers

Each container in each final lot shall be inspected visually, and those showing abnormalities—such as improper sealing, lack of integrity, clumping or the presence of particles—shall be discarded.

A.6 Records

The requirements given in Part A, section 6, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply, with the addition of the following:

Written records shall be kept of all tests, irrespective of their results. The records shall be of a type approved by the national control authority.

A model of a suitable summary protocol to be used for pertussis vaccines is given in Appendix 4.

A.7 Samples

The requirements given in Part A, section 7, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply.

A.8 Labelling

The label printed on or affixed to each container, and the label on the carton enclosing one or more containers, shall show as a minimum:

- the words *Vaccinum pertussis* and/or the proper name of the product,
- the word "adsorbed", if applicable,
- the name and address of the manufacturer,
- the recommended storage temperature and the expiry date if kept at that temperature, and
- the recommended single human dose and route of administration.

In addition, the label printed on or affixed to the container, or the label on the carton, or the leaflet accompanying the container shall contain the following:

- a statement that the vaccine satisfies the requirements of this document,
- the nature and amount of any preservative present in the vaccine (if there is no preservative in single-dose containers, this should be stated),
- the nature and amount of the adsorbing agent, if applicable,
- the nature and amount of any substances added to the vaccine,
- the recommended conditions for storage and transport,
- a warning that the vaccine should not be frozen,
- a warning that the vaccine should be shaken before use, and
- instructions for the use of the vaccine and information on contraindications and the reactions that may follow vaccination.

A.9 Distribution and transport

The requirements given in Part A, section 9, of the revised Requirements for Biological Substances No. 1 (General Require-

ments for Manufacturing Establishments and Control Laboratories) (7) shall apply.

A.10 Stability, storage and expiry date

A.10.1 Stability

Tests shall be conducted to determine the loss of potency to be expected during storage. The stability of the vaccine shall be demonstrated to the satisfaction of the national control authority; final containers from at least three batches of vaccine derived from different bulks shall be tested on the expiry date to demonstrate stability during storage. The vaccine shall meet the requirements for final vaccine (see Part A, sections A.3.4.7, A.5.4, A.5.7 and A.5.8) up to the expiry date, provided that it has been stored at the recommended temperature. When any changes are made in the production procedure which may affect the stability of the product, the vaccine produced by the new method shall be shown to be stable.

The statement concerning storage temperature and expiry date appearing on the label, as required in Part A, section 8, shall be based on experimental evidence and shall be submitted for approval to the national control authority.

A.10.2 Storage conditions

The manufacturer shall recommend conditions of storage and transport that shall ensure that the vaccine satisfies the potency requirements until the expiry date stated on the label.

> Storage at a temperature of 5 °C ± 3 °C has been found to be satisfactory.

The vaccine shall not be frozen.

A.10.3 Expiry date

The expiry date shall be fixed with the approval of the national control authority based on the stability studies referred to in section A.10.1 and shall not be more than 2½ years after the date of the last satisfactory potency test, i.e., the date on which the animals were immunized with the vaccine.

PART B. NATIONAL CONTROL REQUIREMENTS

B.1 General

The general requirements for control laboratories contained in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply.

> In view of the lack of information on the relationship between the toxicity of vaccines in animals and the production of untoward reactions in humans after vaccination, and the sometimes wide limits of the 95% confidence interval of the potency data (Part A, section A.3.4.7), the degree of consistency in producing satisfactory final bulk vaccines is an important factor in ensuring the safety and efficacy of a particular manufacturer's product. Definite requirements in this respect cannot be formulated, but the national control authorities should satisfy themselves, on the basis of the method of manufacture and the results of tests on a series of consecutive vaccines, that the manufacturer is able to produce, with satisfactory consistency, a product of the required quality.

The detailed production and control procedures and any significant changes in them shall be discussed with and approved by the national control authority, which shall obtain the International Standard for Pertussis Vaccine and establish a national working reference preparation by comparison with it.

B.2 Official release and certification by the national control authority

A vaccine shall be released only if it satisfies Part A of the present Requirements.

A statement signed by the appropriate official of the national control authority shall be provided at the request of the manufacturing establishment and shall certify that the lot of vaccine in question satisfies all national requirements as well as Part A of the present Requirements. The certificate shall state the number under which the lot was released by the national control authority, and the number appearing on the labels of the containers. The official national release document shall be provided to importers of pertussis vaccines.

> The purpose of the certificate is to facilitate exchange of pertussis vaccines between countries. A model of a suitable certificate is given in Appendix 2.

REQUIREMENTS FOR COMBINED VACCINES (ADSORBED)

GENERAL CONSIDERATIONS

In order to satisfy the need for combined vaccines, national control authorities have combined the appropriate tests for the individual components of diphtheria and tetanus (DT) and diphtheria, tetanus and pertussis (DTP) vaccines, but have not adopted a uniform approach in so doing.

The requirements for a combined vaccine must include the tests applicable to the various components incorporated into the final product, but further tests are also required after blending. The need for tests at each stage in the production is important because of possible interaction between antigens, as well as the effects that both adjuvants and preservatives may have on the potency and stability of the final product.

The tests carried out on the combined vaccines are on the whole the same as those on the individual components, so that, in requirements, a cross-reference to the tests specified for these components, including the "one-dilution" tests, is all that is necessary in many cases. However, certain tests—such as the tetanus potency assay of DTP vaccine—need special consideration when mice are used in the assay.

No international reference materials specific for the combined vaccines exist, and the potency of each component is expressed in International Units by comparison with reference materials calibrated against the reference materials for the individual components. This is not an ideal situation, because dose–response relationships in animals may differ when the components are combined. Nevertheless, meaningful potency data can be obtained.

Filling, sampling, labelling, transportation, distribution and storage have not been dealt with here since they have been adequately covered for the individual (diphtheria, tetanus, pertussis) vaccines. However, it should be noted that, as with single adsorbed vaccines, combined DT and DTP vaccines must not be frozen and that the expiry date of combined vaccines is determined by the component with the shortest shelf-life.

A number of manufacturers and control authorities have experienced difficulties in reporting the results of tests of combined

vaccines, and a composite protocol for this purpose has therefore been included for DTP (Appendix 5).

These Requirements cover only the two combined vaccines DT and DTP, since these combinations are the most widely used. Reference is also made to tetanus and diphtheria vaccine for adults (Td) for which the potency requirements for the diphtheria component are reduced. No attempt has been made to include other combinations, including those with *Haemophilus influenzae* and *Neisseria meningitidis* polysaccharides and poliomyelitis vaccines. Requirements for these vaccines should therefore include the relevant tests for the individual components, and special attention should be given to possible interactions between the components.

Each of the following sections constitutes a recommendation. Those parts of each section printed in large type have been written in the form of requirements so that, if a health administration so desires, they may be adopted as they stand as definitive national requirements. Those parts of each section printed in small type are comments and/or recommendations for guidance.

Individual countries may wish to adopt these Requirements as the basis of their national regulations on combined vaccines. If national requirements differ from these requirements, it is recommended that the former should be shown to ensure that the vaccine is at least as safe and as potent as that prepared in accordance with the requirements formulated below. It is desirable that the World Health Organization should be kept informed of any such differences.

PART A. MANUFACTURING REQUIREMENTS

A.1 Tests for DT and DTP vaccines

A.1.1 Final bulk

The following tests on the final bulk are common to diphtheria, tetanus and pertussis vaccines (DTP) and to diphtheria and tetanus vaccines (DT): the test for adjuvant content, the sterility test and the test for free detoxifying agent.

A.1.2 Final lot

The following tests on the final lot are common to both DTP and DT: the sterility test, the test for adjuvant content, the test for preservative content and the inspection of the final containers. The pH of DT and DTP vaccines shall be 6.0–7.0.

A.2 Special tests for DTP vaccine

A.2.1 Final bulk

The following tests shall be carried out on the final bulk.

A.2.1.1 *Potency test*

For the diphtheria component, the requirements for diphtheria vaccine (section A.3.5.6, p. 101) shall apply.

When the test for the potency of the tetanus component is performed in guinea-pigs, the requirements for tetanus vaccine (section A.3.5.6, p. 119) shall apply. When mice are used, the potency of the tetanus component shall be 60 IU per single human dose. The 95% confidence interval of the tests shall be within 50–200%. If the interval is greater than 50–200%, the lower 95% fiducial limit of the estimate of potency must be greater than 60 IU per dose. In one-dilution tests in mice, the dose of reference vaccine injected shall be expressed as a fraction of 60 IU.

For the pertussis component, the requirements for pertussis vaccine (section A.3.4.7, p. 136) shall apply.

A.2.1.2 *Specific toxicity test*

For the specific toxicity test for the diphtheria component, the requirements for diphtheria vaccine (section A.3.5.5, p. 101) apply.

For the specific toxicity test for the tetanus component, the requirements for tetanus vaccine (section A.3.5.5, p. 119) apply.

The same animals are used for these two tests and are observed for six weeks in order to cover the observation period specified for diphtheria vaccine (section A.3.5.5).

For the mouse weight-gain test for pertussis toxicity, the requirements for pertussis vaccine (section A.3.4.6, p. 135) apply.

A.2.2 Final lot

The following tests shall be carried out on the final lot.

A.2.2.1 *Identity test*

Identity tests on the components of a triple vaccine shall be carried out after they have been eluted from the mineral carrier with sodium citrate and the pertussis organisms and residual carrier sedimented by centrifugation. The tests specified in the requirements for diphtheria vaccine (section A.5.1, p. 104), tetanus vaccine (section A.5.1, p. 121) and pertussis vaccine (section A.5.1, p. 139) shall apply.

A.2.2.2 *Potency test*

If a potency test has not been performed on the triple vaccine in the final bulk, the tests specified in section A.2.1.1 (p. 146) shall apply.

A.2.2.3 *Innocuity test*

The innocuity test shall be performed in accordance with the requirements for pertussis vaccine (section A.5.4, p. 139).

A.3 Special tests for DT vaccine

A.3.1 Final bulk

The following tests shall be carried out on the final bulk.

A.3.1.1 *Potency test*

The test for the potency of the diphtheria component shall be that specified for diphtheria vaccine (section A.3.5.6, p. 101).

In some countries, tetanus and diphtheria vaccine for use in adults (Td) is released with a diphtheria potency of less than 30 IU per dose.

The test for the potency of the tetanus component of DT and Td vaccines shall be that specified for tetanus vaccine (section A.3.5.6, p. 119).

A.3.1.2 *Specific toxicity test*

The tests for the specific toxicity of the diphtheria and tetanus components are as specified in section A.3.5.5, pp. 101 and 119, with an observation period of six weeks in order to cover the period specified for diphtheria vaccine.

A.3.2 Final lot

The following tests shall be carried out on the final lot.

A.3.2.1 *Identity test*

Identity tests on the components of DT vaccine shall be carried out after they have been eluted from the mineral carrier with sodium citrate. The tests specified for diphtheria vaccine (section A.5.1, p. 104) and tetanus vaccine (section A.5.1, p. 121) shall apply.

A.3.2.2 *Potency test*

If a potency test has not been performed on the final bulk, the tests specified for the individual vaccines (section A.3.5.6, pp. 101 and 119) shall apply.

A.3.2.3 *Innocuity test*

The innocuity test shall be performed in accordance with the requirements for diphtheria and tetanus vaccines (section A.5.4, pp. 105 and 122).

PART B. NATIONAL CONTROL REQUIREMENTS

In addition to its responsibilities in respect of each individual vaccine, including release and certification, the national control authority shall approve:

- the formulation of the combined vaccine in order to ensure that the components are present at concentrations appropriate to its use,
- the formulation including the preservative and adjuvant in order to ensure that the stability of the vaccine is such that it remains effective up to the expiry date, provided that it has been stored at the recommended temperature, and

- the protocols for reporting the results of tests on the combined vaccine (a suggested protocol for this purpose is given in Appendix 5).

AUTHORS

The first drafts of the revised Requirements for Diphtheria, Tetanus, Pertussis and Combined Vaccines were prepared in 1988 by Dr P. Knight (Wellcome Biotech, Beckenham, England) and Dr C. Manclark (Laboratory of Pertussis, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, USA).

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ACKNOWLEDGEMENTS

Acknowledgements are due to the following experts for their comments and advice on the second draft of these Requirements: Dr R.H. Ali, President, Egyptian Organization for Biological Products and Vaccines, Agouza, Cairo, Egypt; Dr J. Cameron, North York, Ontario, Canada; Dr R. Donikian, Mérieux Institute, Marcy l'Etoile, France; Dr V. Garic, Head, Control Department, Institute of Immunology and Virology, Torlak, Belgrade, Yugoslavia; Dr E.H. Ghaly, Director General of Production, Egyptian Organization for Biological Products and Vaccines, Agouza, Cairo, Egypt; Dr D.K. Gill, Director, Regulatory Affairs, International, Connaught Laboratories Limited, Willowdale, Ontario, Canada; Dr E. Griffiths, Department of Bacteriology, National Institute for Biological Standards and Control, Potters Bar, Herts., England; Dr C. Guthrie, Operations Director, Commonwealth Serum Laboratories, Parkville, Victoria, Australia; Professor D.K. Hazra, Head, Nuclear Medicine and Radio-immunoassay Unit, Post Graduate Department of Medicine, S.N. Medical College, Agra, India; Dr P. Knight, Wellcome Biotech, Beckenham, Kent, England; Dr Ko Ko Gyi, Director, Development Centre for Pharmaceutical Technology, Rangoon, Myanmar; Dr M. Krasselt, National Institute for Public Health and Environmental Protection, Bilthoven, Netherlands; Dr J. G. Kreeftenberg, Head, Laboratory for Control of Bacterial Vaccines, National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands; Dr D. Labert, Pasteur Vaccines, Val de Reuil, France; Dr S. Langlois, Connaught Laboratories Limited, Willowdale, Ontario, Canada; Mr W. Latham, Massachusetts Public Health Biological Laboratories, Boston, MA, USA; Dr Lehmann, Behringwerke, Marburg, Federal Republic of Germany; Mr J. Lyng, Department of Biological Standardization, State Serum Institute, Copenhagen, Denmark; Mr E. McCarthy, Managing Director, Wyeth-Ayerst Laboratories, Marietta, PA, USA; Dr K. McClintock, Director, Regulatory and Technical Affairs, Lederle Laboratories, Pearl River, NY, USA; Dr D. McKay, National Biological Standards Laboratory, Canberra, Australia; Dr T. Matuhasi, Consulting Research Scientist, Okinaka Memorial Institute for Medical Research, Tokyo, Japan; Dr L. Milcke, Behringwerke, Marburg, Federal Republic of Germany; Dr J. Milstien, Scientist, Biologicals, World Health Organization, Geneva, Switzerland; Dr G. Nyerges, Department of Control of Vaccines and Sera, National Institute of Hygiene, Budapest, Hungary; Dr M. Quevillon, Armand Frappier Institute, Quebec, Canada; Dr K. Readhead, National Institute for Biological Standards and Control, Potters Bar, Herts., England; Dr P. Rousseau, Armand Frappier Institute, Quebec, Canada; Dr S.N. Saxena, Director, Central Research Institute, Kasauli, India; Dr E.B. Seligmann Jr, Consultant, Biologics Manufacturing, Frederick, MD, USA; Dr D.W. Stainer, Conpharma Vaccines Ltd., North York, Ontario, Canada; Dr G.R.E. Swaniker, Faculty of Medicine, The University of Papua New Guinea, Boroko, Papua New Guinea; Dr V. Takla-Rizk, Egyptian Organization for Biological Products and Vaccines, Agouza, Cairo, Egypt; Dr S.J. Varallyay, Swiss Vaccine and Serum Institute, Bern, Switzerland; Dr J. Vose, Assistant Vice-President, Regulatory and Technical Affairs, Connaught Research Institute, Willowdale, Ontario, Canada; Dr J.J. Walsh, Quality Assurance and Animal Services Director, Commonwealth Serum Laboratories, Parkville, Victoria, Australia; Dr J.C.W. Weber, Director, Regulatory Affairs, Connaught Laboratories Limited, Willowdale, Ontario, Canada; Dr Yu Yong Pin, National Institute for Control of Pharmaceutical and Biological Products, Temple of Heaven, Beijing, China.

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[1] Available to readers on request from Biologicals, World Health Organization, 1211 Geneva 27, Switzerland.

Appendix 1

SUMMARY PROTOCOL FOR DIPHTHERIA VACCINE (ADSORBED) PRODUCTION AND TESTING

Summary information on final lot

Name and address of manufacturer .....
.....
.....

Lot No. .....

Date of filling .....

Volume of each recommended single human dose .....

No. of doses per final container .....

No. of final containers .....

Expiry date .....

Detailed information on manufacture and control

Strain

Identity of *C. diphtheriae* strain used for vaccine production .....

Reference No. of seed lot .....

Date(s) of reconstitution of ampoule(s) for manufacture .....

Single harvests used for preparing the bulk purified toxoid

List the single harvests and indicate the medium, dates of inoculation, temperature of incubation, dates of harvests, volumes, results of tests for bacterial purity, method of inactivation and yields.

Bulk purified toxoid

Reference No. .....

Volume and Lf/ml .....

Date and result of test for antigenic purity (Lf/mg of protein nitrogen) .....

Test of irreversibility

Lf/ml of test toxoid solution .....

Temperature of incubation of toxoid .....

Dates of beginning and end of incubation .....

If a test on guinea-pigs was used, indicate:

No. of guinea-pigs injected, route and date of injection .....

Date of end of observation .....

Result of test .....

If cell culture was used, provide suitable information on the test system and give results

Specific toxicity test

If a test on guinea-pigs was used, indicate:

No. of guinea-pigs injected and date of injection .....

No. of Lf per guinea-pig and route of injection .....

Date of end of observation .....

Result of test .....

If cell culture was used, provide suitable information on the test system and give results

Final bulk

Identification .....

Volume .....

Lf/ml .....

Sterility test

Date and result of test .....

Specific toxicity test (optional)

No. of guinea-pigs injected and date of injection .....

Volume and route of injection .....

Date of end of observation .....

Result of test .....

Potency test[1]

(1) Based on lethal or paralytic challenge

(i) Three-dilution assays

(*a*) Lethal challenge

Weight of guinea-pigs .....

Date of immunization and volume of dilutions administered .....

Date of challenge .....

Challenge dose .....

Date of end of observation .....

Results .....

	Dilution	*No. of survivors/No. of animals injected*	*Median effective dose (ED_{50})*
Reference vaccine (... IU/ml)	.....	.....	..... ml
	.....	.....	
	.....	.....	
Test vaccine	.....	.....	..... ml
	.....	.....	
	.....	.....	

Potency of test toxoid is ... IU per single human dose. Limits of 95% confidence interval (in %) are ...

(*b*) Multiple intradermal challenge

Report all relevant information on animals and date of immunization, challenge and end of observation.

Results

	Dilution	*Mean score*
Reference vaccine (... IU/ml)	.....	.....
	.....	.....
	.....	.....
Test vaccine	.....	.....
	.....	.....
	.....	.....

Potency of test toxoid is ... IU per single human dose. Limits of 95% confidence interval (in %) are ...

(ii) One-dilution challenge test

Date of performance of last satisfactory three-dilution test .....

[1] Only one of the potency tests listed need be performed.

Nature and reference No. of product tested (specify also whether it was a final bulk or a final product) .....

Provide relevant information validating the one-dilution assay system.

Identity and titre (IU/ml) of reference vaccine .....

Weight of guinea-pigs .....

Date of immunization .....

Date of challenge .....

Challenge dose .....

Date of end of observation .....

Results

	Reference vaccine	*Test vaccine*
Dilution used for immunization	.....	.....
No. of survivors/No. injected	.....	.....

P value indicating the probability that the test vaccine contains more than 30 IU per single human dose .....

(2) Test based on measuring antitoxin induction as an alternative to lethal or intradermal challenge

(i) Toxin neutralization on cell cultures

Provide separately all relevant information such as identity and titre (in IU/ml) of the reference vaccine, species of animals immunized, dilutions of vaccines, date of immunization, date of bleeding, dilution at which immune sera were assayed, amount of toxin added to dilutions of immune sera and, for each dilution: No. of wells (or tubes) where cells survived/No. of wells inoculated with toxin–antitoxin mixtures or (control) toxin, median effective doses (ED_{50}s), titre per single human dose, limits of 95% confidence intervals, and any other relevant information.

(ii) Other test, e.g., competitive enzyme-linked immunosorbent assay (ELISA)

Provide separately all relevant information, including the data by which the method was validated.

Potency of test vaccine ... IU per single human dose. Limits of 95% confidence interval (in %) are ...

Test for residual free detoxifying agent

Detoxifying agent (formaldehyde or glutaraldehyde) .....

Date of test .....

Result (in g/l) .....

pH

Date of measurement .....

Result .....

Final product

Identity test

Date of test .....

Type of test and result .....

Sterility test

No. of times the test had to be performed .....

No. of containers tested .....

Media and temperatures of incubation .....

Date of inoculation .....

Date of end of observation .....

Result of last test .....

Potency test

If this test was not performed on the final bulk, indicate this and report the data obtained on the final product in the space provided for potency tests in the "final bulk" section.

Innocuity test	*Mice*	*Guinea-pigs*
No. of animals	.....	.....
Route of injection	.....	.....
Volume of injection	.....	.....
Date of start of test	.....	.....
Date of end of test	.....	.....
Results	.....	.....

Test for adjuvant

Date of test .....

Nature and concentration of adjuvant per single human dose .....

Test for preservative

Date of test .....

Nature and concentration of preservative .....

pH

Date of measurement .....

Result .....

Inspection of final containers

Date of inspection .....

Result .....

Stability test[1]

Indicate separately all relevant details and (as a percentage) the calculated losses of potency per year and half-lives at different temperatures as determined by accelerated degradation tests, and actual titres[2] (with 95% limits of confidence intervals) after storage for the maximum period claimed for the product at the recommended temperature.

Certification by the manufacturer

Name of head of production (typed) .....

Certification by person from the control laboratory of the manufacturing company taking overall responsibility for the production and control of the vaccine

I certify that lot No. ... of diphtheria vaccine (adsorbed), whose number appears on the label of the final containers, meets all national requirements[3] and satisfies Part A of the diphtheria vaccine section of Requirements for Biological Substances Nos. 8 and 10, revised 1989 and (if applicable) addenda 19...

Signature .....

Name (typed) .....

Date .....

Certification by the national control authority

If the vaccine is to be exported, attach a certificate from the national control authority, a model of which is shown in Appendix 2, a label from a final container, and an instruction leaflet for users.

[1] Not required in summary protocols of every batch.

[2] Needed only for three batches to validate the production method.

[3] If any national requirement(s) is (are) not met, specify which one(s) and indicate why release of the lot has nevertheless been authorized.

Appendix 2

MODEL CERTIFICATE FOR THE RELEASE OF VACCINES

This certificate is to be provided by the national control authority of the country where the vaccines have been manufactured, upon request by the manufacturer

The following lots of .....[1] vaccine produced by .....[2] in .....,[3] whose numbers appear on the labels of the final containers, meet all national requirements,[4] Part A of the .....[1] section of the Requirements for Biological Substances Nos. 8 and 10 (Requirements for Diphtheria, Tetanus, Pertussis and Combined Vaccines, revised 1989 [if applicable, addendum 19...])[5] and the Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (revised 1965).[6]

Lot No.	Date of the last potency test by the manufacturer	Expiry date	Lot No.	Date of the last potency test by the manufacturer	Expiry date
.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....

As a minimum, this certificate is based on an examination of the manufacturing protocol.

The number of this certificate is .....

The Director of the National Control Laboratory (or Authority as appropriate)[7]

Name (typed) .....

Signature .....

Date .....

[1] Indicate type of vaccine (e.g., tetanus, diphtheria-tetanus, diphtheria-tetanus-pertussis).

[2] Name of manufacturer.

[3] Country.

[4] If any national requirement(s) is (are) not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the national control authority.

[5] With the exception of the provisions on shipping, which the national control authority may not be in a position to control.

[6] Published in WHO Technical Report Series, No. 323, 1966.

[7] Or his or her representative.

Appendix 3

SUMMARY PROTOCOL FOR TETANUS VACCINE (ADSORBED) PRODUCTION AND TESTING

Summary information on final lot

Name and address of manufacturer .....
.....
.....

Lot No. .....

Date of filling .....

Volume of each recommended single human dose .....

No. of doses per final container .....

No. of final containers .....

Expiry date .....

Detailed information on manufacture and control

Strain

Identity of *C. tetani* strain used for vaccine production .....

Reference No. of seed lot .....

Date(s) of reconstitution of ampoule(s) for manufacture .....

Single harvests used for preparing the bulk purified toxoid

List the single harvests and indicate the medium, dates of inoculation, temperature of incubation, dates of harvests, volumes, results of tests for bacterial purity, method of inactivation and yields.

Bulk purified toxoid

Reference No. .....

Volume and Lf/ml .....

Date and result of test for antigenic purity (Lf/mg of protein nitrogen) .....

Test of irreversibility

Lf/ml of test toxoid solution .....

Temperature of incubation of test toxoid .....

Dates of beginning and end of incubation .....

No. of guinea-pigs injected, route and date of injection .....

Date of end of observation .....

Result of test .....

Specific toxicity test

No. of guinea-pigs injected and date of injection .....

No. of Lf per guinea-pig and route of injection .....

Date of end of observation .....

Result of test .....

Final bulk

Identification .....

Volume .....

Lf/ml .....

Sterility test

Date and result of test .....

Specific toxicity test (optional)

No. of guinea-pigs injected and date of injection .....

Volume and route of injection .....

Date of end of observation .....

Result of test .....

Potency test[1]

(1) Based on lethal or paralytic challenge

(i) Three-dilution assays

Species and weight of animals .....

Date of immunization and volume of dilutions administered .....

Date of challenge .....

Challenge dose (indicate whether lethal or paralytic) .....

[1] Only one of the potency tests listed need be performed.

Date of end of observation .....

Results

	Dilution	*No. of survivors (or of animals not paralysed)/No. of animals injected*	*Median effective dose (ED_{50})*
Reference vaccine (... IU/ml)	.....	.....	..... ml
	.....	.....	
	.....	.....	
Test vaccine	.....	.....	..... ml
	.....	.....	
	.....	.....	

Potency of test vaccine is ... IU per single human dose. Limits of 95% confidence interval (in %) are ...

(ii) One-dilution challenge test

Date of performance of last satisfactory three-dilution test .....

Nature and reference No. of product tested (specify also whether it was a final bulk or a final product) .....

Provide relevant information validating the one-dilution assay system.

Identity and titre (IU/ml) of reference vaccine .....

Animal species and weight of animals .....

Date of immunization .....

Date of challenge .....

Challenge dose (specify whether lethal or paralytic) .....

Date of end of observation .....

Results

	Reference vaccine	*Test vaccine*
Dilution used for immunization	.....	.....
No. of survivors (or of animals not paralysed)/No. injected	.....	.....

P value indicating the probability that the test vaccine contains more than 40 IU/ single human dose .....

(2) Test based on measuring antitoxin induction as an alternative to lethal or intradermal challenge

Provide separately all relevant information on other tests, e.g., competitive enzyme-linked immunosorbent assay (ELISA) or passive haemagglutination, including the data by which the method was validated.

Potency of test vaccine ... IU per single human dose. Limits of 95% confidence interval (in %) are ...

Test for residual free detoxifying agent

Detoxifying agent (formaldehyde or glutaraldehyde) .....

Date of test .....

Result (in g/l) .....

pH

Date of measurement .....

Result .....

Final product

Identity test

Date of test .....

Type of test and result .....

Sterility test

No. of times the test had to be performed .....

No. of containers tested .....

Media and temperatures of incubation .....

Date of inoculation .....

Date of end of observation .....

Result of last test .....

Potency test

If the test was not performed on the final bulk, indicate this and report the data in the space provided for potency tests in the "final bulk" section.

Innocuity test	*Mice*	*Guinea-pigs*
No. of animals	.....	.....
Route of injection	.....	.....

	Mice	*Guinea-pigs*
Volume of injection	.....	.....
Date of start of test	.....	.....
Date of end of test	.....	.....
Results	.....	.....

Test for adjuvant

Date of test .....

Nature and concentration of adjuvant per single human dose .....

Test for preservative

Date of test .....

Nature and concentration of preservative .....

pH

Date of measurement .....

Result .....

Inspection of final containers

Date of inspection .....

Result .....

Stability test[1]

Indicate separately all relevant details and (as a percentage) the calculated losses of potency per year at different temperatures as determined by accelerated degradation tests, and actual titres[2] (with limits of 95% confidence intervals) after storage for the maximum period claimed for the product at the recommended temperature.

[1] Not required in summary protocols of every batch.
[2] Needed only for three batches to validate the production method.

Certification by the manufacturer

Name of head of production (typed) .....

Certification by person from the control laboratory of the manufacturing company taking overall responsibility for the production and control of the vaccine

I certify that lot No. ... of tetanus vaccine (adsorbed), whose number appears on the label of the final containers, meets all national requirements[1] and satisfies Part A of the tetanus vaccine section of Requirements for Biological Substances Nos. 8 and 10, revised 1989 and (if applicable) addenda 19...

Signature .....

Name (typed) .....

Date .....

Certification by the national control authority

If the vaccine is to be exported, attach a certificate from the national control authority as shown in Appendix 2, a label from a final container and an instruction leaflet for users.

[1] If any national requirement(s) is (are) not met, specify which one(s) and indicate why release of the lot has nevertheless been authorized.

Appendix 4

SUMMARY PROTOCOL FOR PERTUSSIS VACCINE PRODUCTION AND TESTING

Summary information on final lot

Name and address of manufacturer .....

.....

.....

Lot No. .....

Date of filling .....

Nature of final product (plain or absorbed) .....

Volume of each recommended single human dose .....

No. of doses per final container .....

No. of final containers .....

Expiry date .....

Detailed information on manufacture and control

Strain

Identity of *B. pertussis* strains used in vaccine .....

Serological types of strains .....

Reference No. of seed lot .....

Date(s) of reconstitution of ampoule(s) for manufacture .....

Single harvests used for preparing final bulk

List the single harvests and indicate the medium, dates of inoculation, temperature of incubation, dates of harvests, volumes, results of tests for bacterial purity, methods and dates of inactivation, opacity, and agglutinogens present.

Final bulk

Identification .....

Volume .....

No. of opacity units (calculated from opacities of single harvests) .....

Test for agglutinogens 1, 2 and 3

Date and results (before addition of adjuvant) .....

Sterility test

Medium, date and result of test .....

Specific toxicity test (mouse weight-gain test)

Strain of mice .....

No. of animals in test group and control group .....

Date of injection .....

Volume and route of injection .....

Date of end of observation .....

Result of test: on a separate sheet of paper, give all relevant details for mice in the control and test groups (survival, mean weight on day of injection and three and seven days after it) and indicate percentage weight of test group as compared with control group.

Other specific toxicity tests

Mention here date and results of any other specific toxicity test which may have been performed (e.g., tests for heat-labile toxin, lymphocytosis promoting factor and endotoxin) .....

Potency test

Strain, weight and sex of mice .....

Date of immunization .....

LD_{50} in challenge dose .....

No. of colony-forming units in challenge dose .....

Date of challenge .....

Date of end of observation .....

Results .....

	Dilution	*No. of survivors/ No. inoculated*	*Median effective dose (ED_{50})*
Reference vaccine (... IU/ml)	.....	.....	
	.....	.....	..... ml
	.....	.....	

	Dilution	*No. of survivors No. inoculated*	*Median effective dose (ED_{50})*
Test vaccine	.....	.....	
	.....	.....	..... ml
	.....	.....	

Potency of test vaccine is ... IU per single human dose. Limits of 95% confidence interval (in %) are ...

pH

Date of measurement .....

Result .....

Final product

Identity test

Date of test .....

Type of test and result .....

Sterility test

No. of times the test had to be performed .....

No. of containers tested in each test .....

Media and temperatures of incubation .....

Date(s) of inoculation .....

Date(s) of end of observation .....

Result of each test .....

Potency test

If the test was not performed on the final bulk, indicate this and report the data obtained on the final product in the space provided for potency tests in the "final bulk" section.

Innocuity test	Mice	Guinea-pigs
No. of animals	.....	.....
Route of injection	.....	.....
Volume of injection	.....	.....
Date of start of test	.....	.....
Date of end of test	.....	.....
Results	.....	.....

Test for adjuvant

Date of test .....

Nature and concentration of adjuvant per single human dose .....

Test for preservative

Date of test .....

Nature and concentration of preservative .....

pH

Date of measurement .....

Result .....

Inspection of final containers

Date of inspection .....

Result .....

Stability test[1]

Indicate separately all relevant details and (as a percentage) the calculated losses of potency per year at different temperatures, as determined by accelerated degradation tests, and actual titres[2] (with limits of 95% confidence intervals) after storage for the maximum period claimed for the product at the recommended temperature.

Certification by the manufacturer

Name of head of production (typed) .....

Certification by person from the control laboratory of the manufacturing company taking overall responsibility for the production and control of the vaccine

I certify that lot No. ... of pertussis vaccine, whose number appears on the label of the final containers, meets all national requirements[3] and satisfies Part A of the pertussis vaccine section of Requirements for Biological Substances Nos. 8 and 10, revised 1989 and (if applicable) addenda 19...

Signature .....

Name (typed) .....

Date .....

Certification by the national control authority

If the vaccine is to be exported, attach a certificate from the national control authority as shown in Appendix 2, a label from a final container, and an instruction leaflet for users.

[1] Not required in summary protocols of every batch.

[2] Needed only for three batches to validate the production method.

[3] If any national requirement(s) is (are) not met, specify which one(s) and indicate why release of the lot has nevertheless been authorized.

Appendix 5

SUMMARY PROTOCOL FOR DIPHTHERIA, TETANUS AND PERTUSSIS VACCINE (ADSORBED) PRODUCTION AND TESTING[1]

Summary information on final lot

Name and address of manufacturer .....

.....

.....

Lot No. .....

Date of filling .....

Volume of each recommended single human dose .....

No. of doses per final container .....

No. of final containers .....

Expiry date .....

Detailed information on manufacture and control

Strains, single harvests, bulks

Diphtheria vaccine

Strain

Identity of *C. diphtheriae* strain used for vaccine production .....

Reference No. of seed lot .....

Date(s) of reconstitution of ampoule(s) for manufacture .....

Single harvests used for preparing bulk purified toxoid

List the single harvests and indicate the medium, dates of inoculation, temperature of incubation, dates of harvests, volumes, results of tests for bacterial purity, method of inactivation and yields.

[1] For diphtheria–tetanus vaccines, delete "Pertussis" in the title of the protocol and do not fill in sections relating solely to pertussis vaccine.

Bulk purified toxoid

Reference No. .....

Volume and Lf/ml .....

Date and result of test for antigenic purity (Lf/mg of protein nitrogen) .....

Test of irreversibility

Lf/ml of test toxoid solution .....

Temperature of incubation of test toxoid .....

Dates of beginning and end of incubation .....

No. of guinea-pigs injected, route and date of injection[1] .....

Date of end of observation .....

Result of test .....

Specific toxicity test[1]

No. of guinea-pigs injected and date of injection .....

No. of Lf per guinea-pig and route of injection .....

Date of end of observation .....

Result of test .....

Tetanus vaccine

Strain

Identity of *C. tetani* strain used for vaccine production .....

Reference No. of seed lot .....

Date of reconstitution of ampoule(s) for manufacture .....

Single harvests used for preparing bulk purified toxoid

List the single harvests and indicate the medium, dates of inoculation, temperature of incubation, dates of harvests, volumes, results of tests for bacterial purity, yields and method of inactivation.

[1] If a cell-culture system was used, provide all appropriate information.

Bulk purified toxoid

Reference No. .....

Volume and Lf/ml .....

Date and result of test for antigenic purity (Lf/mg of protein nitrogen) .....

Test of irreversibility

Lf/ml of test toxoid solution .....

Temperature of incubation of test toxoid .....

Dates of beginning and end of incubation .....

No. of guinea-pigs injected, route and date of injection .....

Date of end of observation .....

Result of test .....

Specific toxicity test

No. of guinea-pigs injected and date of injection .....

No. of Lf per guinea-pig and route of injection .....

Date of end of observation .....

Result of test .....

Pertussis vaccine

Strain

Identity of *B. pertussis* strain used in vaccine .....

Serological type of strain .....

Reference No. of seed lot .....

Date(s) of reconstitution of ampoule(s) for manufacture .....

Single harvests used for preparing the bulk material

List the single harvests and indicate the medium, dates of inoculation, temperature of incubation, dates of harvests, volumes, results of tests for bacterial purity and presence of agglutinogen, methods and dates of inactivation, and opacity.

Bulk material

Identification .....

Volume and opacity/ml .....

Date, results of, and medium used in test for living organisms .....

Date of test for presence of agglutinogens 1, 2 and 3 and results .....

Information on blending

Diphtheria toxoid component

Reference No. .....

Lf/ml .....

Volume .....

Tetanus toxoid component

Reference No. .....

Lf/ml .....

Volume .....

Pertussis vaccine component

Reference No. .....

Opacity units (calculated from opacities of single harvests) .....

Volume .....

Adjuvant

Nature and concentration (Al or Ca in mg/ml) .....

Volume .....

Preservatives

Nature and concentration .....

Volume .....

Buffer

Nature and concentration .....

Volume .....

Tests on final bulk

Reference No. .....

Date of completion .....

Volume .....

Sterility test

Date and result of test .....

Specific toxicity test

Tetanus and diphtheria (optional)

No. of guinea-pigs injected and date of injection .....

Number of single human doses injected per guinea-pig, volume and route of injection .....

Date of end of observation .....

Result of test .....

Pertussis

(i) Mouse weight-gain test
Strain of mice .....

No. of animals in test group and control group .....

Date of injection .....

Volume and route of injection .....

Date of end of observation .....

Results of test: on a separate sheet of paper, give all relevant details for mice in the control and test groups (survival, mean weight before injection and three and seven days after it) and indicate percentage weight gain of test group as compared with control group.

(ii) Other tests
Mention here date and results of any other specific toxicity test which may have been performed (e.g., tests for heat-labile toxin, lymphocytosis promoting factor, pertussis toxin on cell cultures and endotoxin).

Potency test

Diphtheria[1]

(i) Tests based on challenge
(*a*) Three-dilution assays

Lethal challenge

Weight of guinea-pigs .....

Date of immunization and volume of dilutions administered .....

Date of challenge .....

[1] Only one of the potency tests described here need be performed.

Challenge dose .....

Date of end of observation .....

Results

	Dilution	*No. of survivors/No. of animals injected*	*Median effective dose (ED_{50})*
Reference vaccine (... IU/ml)	.....	.....	
	.....	.....	..... ml
	.....	.....	
Test vaccine	.....	.....	
	.....	.....	..... ml
	.....	.....	

Potency of test vaccine is ... IU per single human dose. Limits of 95% confidence interval (in %) are ...

Multiple intradermal challenge

Report all relevant information on animals, and dates of immunization, challenge, and end of observation

Results

	Dilution	*Mean score*
Reference vaccine (... IU/ml)	.....	.....
	.....	.....
	.....	.....
Test vaccine	.....	.....
	.....	.....
	.....	.....

Potency of test vaccine is ... IU per single human dose. Limits of 95% confidence interval (in %) are ...

(*b*) One-dilution challenge test

Date of performance of last satisfactory three-dilution test .....

Nature and reference No. of product tested (specify also whether it was a final bulk or a final product) .....

Provide relevant information validating the one-dilution assay system.

Identity and titre (IU/ml) of reference toxoid .....

Weight of guinea-pigs .....

Date of immunization .....

Date of challenge .....

Challenge dose .....

Date of end of observation .....

Results

	Reference vaccine	*Test vaccine*
Dilution used for immunization	.....	.....
No. of survivors/No. injected	.....	.....

P value indicating the probability that the test vaccine contains more than 30 IU per single human dose .....

(ii) Tests not based on challenge

(*a*) Toxin neutralization on cell cultures

Provide separately all relevant information such as identity and titre (in IU/ml) of the reference vaccine, species of animals immunized, dilutions of vaccines, date of immunization, date of bleeding, dilution at which immune sera were assayed, amount of toxin added to dilutions of immune sera and, for each dilution: No. of wells (or tubes) where cells survived/No. of wells inoculated with toxin antitoxin mixtures or (control) toxin, median effective doses (ED_{50}s), titre per single human dose, confidence interval, etc.

(*b*) Other tests, e.g., competitive enzyme-linked immunosorbent assay (ELISA)

Provide separately all relevant information, including data by which the method was validated.

Potency of test vaccine ... IU per single human dose. Limits of 95% confidence interval (in %) are ...

Tetanus[1]

(i) Tests based on lethal or paralytic challenge

(*a*) Three-dilution assays

Species and weight of animals .....

Date of immunization and volume of dilutions administered .....

Date of challenge .....

Challenge dose (indicate whether lethal or paralytic) .....

Date of end of observation .....

[1] Only one of the potency tests listed here need be performed.

Results

	Dilution	*No. of survivors (or of animals not paralysed)/No. of animals injected*	*Median effective dose (ED_{50})*
Reference vaccine (... IU/ml)	.....	.....	
	.....	.....	..... ml
	.....	.....	
Test vaccine	.....	.....	
	.....	.....	..... ml
	.....	.....	

Potency of test vaccine is ... IU per single human dose. Limits of 95% confidence interval (in %) are ...

(*b*) One-dilution challenge test

Date of performance of last satisfactory three-dilution test .....

Nature and reference No. of product tested (specify also whether it was a final bulk or a final product) .....

Provide relevant information validating the one-dilution assay system.

Identity and titre (IU/ml) of reference toxoid .....

Animal species and weight of animals .....

Date of immunization .....

Date of challenge .....

Challenge dose (specify whether lethal or paralytic) .....

Date of end of observation .....

Results

	Reference vaccine	*Test vaccine*
Dilution used for immunization	.....	.....
No. of survivors (or of animals not paralysed)/No. injected	.....	.....

P value indicating the probability that the test vaccine contains more than 40 IU/single dose (60 IU if DTP vaccine is assayed in mice) .....

(ii) Tests not based on challenge

Provide separately all relevant information on other tests, e.g., competitive enzyme-linked immunosorbent assay (ELISA) or passive haemagglutination, including the data by which the method was validated.

Potency of test toxoid is ... IU per single human dose. Limits of 95% confidence interval (in %) are ...

Pertussis

Strain, weight and sex of mice .....

Date of immunization .....

LD_{50} in challenge dose .....

No. of colony-forming units in challenge dose .....

Date of challenge .....

Date of end of observation .....

Results

	Dilution	*No. of survivors/ No. inoculated*	*Median effective dose (ED_{50})*
Reference vaccine (... IU/ml)	.....	.....	
	.....	.....	..... ml
	.....	.....	
Test vaccine	.....	.....	
	.....	.....	..... ml
	.....	.....	

Potency of test vaccine is ... IU per single human dose. Limits of 95% confidence interval (in %) are ...

Test for residual free detoxifying agent

Detoxifying agent (formaldehyde or glutaraldehyde) .....

Date of test .....

Result (in g/l) .....

pH

Date of measurement .....

Result .....

Tests on final product

Identity test

Test for diphtheria toxoid: method, date and results .....

Test for tetanus toxoid: method, date and results .....

Test for pertussis vaccine: method, date and results .....

Sterility test

No. of times the test had to be performed .....

No. of containers tested .....

Media and temperatures of incubation .....

Date of inoculation .....

Date of end of observation .....

Result of the (last) test .....

Potency test

If this test was not performed on the final bulk, indicate this and report the data obtained on the final product in the space provided for potency tests in the "final bulk" section.

Innocuity test	*Mice*	*Guinea-pigs*
No. of animals	.....	.....
Route of injection	.....	.....
Volume of injection	.....	.....
Date of injection	.....	.....
Date of end of observation	.....	.....
Results	.....	.....

Test for adjuvant

Date of test .....

Nature and concentration of adjuvant per single human dose .....

Test for preservative

Date of test .....

Nature and concentration of preservative .....

pH

Date of measurement .....

Result .....

Inspection of final containers

Date of inspection .....

Result .....

Stability test[1]

For each component of the vaccine, indicate separately all relevant details and (as a percentage) the calculated losses of potency per year at different temperatures as determined by accelerated degradation tests, and actual titres[2] (with the limits of 95% confidence intervals) after storage for the maximum period claimed for the product at the recommended temperature.

Certification by the manufacturer

Name of head of production (typed) .....

Certification by person from the control laboratory of the manufacturing company taking overall responsibility for the production and control of the vaccine

I certify that lot No. ... of diphtheria, tetanus and pertussis vaccine, whose number appears on the label of the final containers, meets all national requirements[3] and satisfies Part A of the combined vaccines section of Requirements for Biological Substances Nos. 8 and 10, revised 1989 and (if applicable) addenda 19...

Signature .....

Name (typed) .....

Date .....

Certification by the national control authority

If the vaccine is to be exported, attach a certificate from the national control authority as shown in Appendix 2, a label from a final container, and an instruction leaflet for users.

[1] Not required in summary protocols of every batch.

[2] Needed only for three batches to validate the production method.

[3] If any national requirement(s) is (are) not met, specify which one(s) and indicate why release of the lot has nevertheless been authorized.

D. MEASLES VACCINE

Documents provided in this section include:

1. Sample Procurement Requirements Measles Vaccine 337
2. Measles Vaccine Model Package Insert (UNICEF) 345
3. Measles Virus Vaccines Live, *USP 23 - NF 18*, 1994 346
4. Measles Virus Vaccines Live, US Food and Drug Administration HHS 21 CFR, Part 630, Subpart D 348
5. Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live) (Requirements for Biological Substances No. 47), WHO *Technical Report Series* No. 840, Annex 3, 1994 355

SAMPLE

PROCUREMENT REQUIREMENTS

MEASLES VACCINE

A. General Description of Intended Use

This vaccine is intended for use in a public-sector health programme for immunization of children (under one year of age) in _____ [*place*] beginning on _____ [*date*].

The total requirement for this solicitation is _____ [*quantity*] doses. _____ [*name*] expects to procure additional quantities of vaccine annually beginning in _____ [*month, year*] and subsequent years as follows: _____ [*quantity*], _____ [*quantity*] and _____ [*quantity*].

B. Specific Requirements

Item: Measles Vaccine for subcutaneous injection, live, attenuated, freeze-dried with sterile diluent packed separately.

Bacterially sterile preparation of live attenuated measles virus grown in a suitable cell culture; tested for neurovirulence in monkeys, safety and immunogenicity; free from all demonstrable viable microbial agents except unavoidable bacteriophage; and found suitable for human immunization.

The vaccine may contain a suitable stabilising agent with antimicrobial properties.

Each dose shall contain not less than _____ [*specify*] infective units of viral vaccine strain _____ [*specify*], and not more than

_____ gm _____ residual antibiotic [*specify the antibiotic*].

The vaccine shall meet _____ [*specify*] requirements for Measles Vaccines (Live) (WHO Requirements for Biological Substances No. 12, revised 1987 or equivalent requirements of the USFDA as detailed in 21 CFR, or another fully equivalent set of biological requirements). *Editors' note: It is recognized that equivalence does not mean identical, only that both provide the same level of potency, safety and efficacy.*

The vaccine shall be currently registered in the country of manufacture and shall meet all requirements of the licensing authority of the country of manufacture. The vaccine also shall be currently registered in the country of use and shall meet all requirements of the licensing authority of the country of use.

Quantity:	_____ doses from one production lot.
Dosage Size:	0.5 ml.
Dose Package:	Vials of 10 doses with diluent in corresponding quantity; container must protect vaccine from exposure to light.
Filling Volume:	Final reconstituted product should contain one dose in 0.5 ml + 15% overfill.
Tolerance:	Volume shall not vary by more than 5%. Weight of the antigen shall not vary by more than 5%.
Storage Temperature:	Below +8 degrees C; may be frozen. Diluent need not be refrigerated.

Shelf Life:	The shelf life of the product provided under this solicitation will be at least 24 months from date of manufacture when stored below +8 degrees C. The supplier will provide manufacturer's stability test data substantiating this 24-month shelf life in the proposed vial. At the time of inspection or acceptance for delivery to the country of destination, no more than six (6) months shall have expired since the date of manufacture (or date of the beginning of the last satisfactory test for potency) shown on the Certificate of Analysis.
Stability:	Stable for a minimum of _____ years at _____ to _____ degrees C; manufacturer to specify thermal stability characteristics.
Labelling:	The label on each vial shall conform to the requirements of the country of use and shall appear in the language of _____. All labelling shall withstand immersion in water and remain intact. All labels shall state the name of the vaccine, name of the manufacturer, place of manufacture, lot number, composition, concentration, dose and mode of administration, expiry date, storage temperature and any other marking that is appropriate.
Optional Labelling:	If vaccine is destined for use in public-sector immunization programs, the statement "Not for Sale; For Use Only in Government-Approved Immunization Programmes" shall appear on each label in the language of the purchasing country.

Closures:	Vaccine vials shall be fitted with closures that conform to ISO standard 8362-2.
Printed Materials:	Two (2) information sheets, printed in _____ [language], shall be included in each box of ___ vials (see Model Package Insert, attached).[25]

C. Quality Assurance

Compliance:	The Supplier shall guarantee that the products as packed for shipment (a) comply with all provisions of the specification and related documents; (b) meet internationally recognized standards for safety, efficacy and quality; (c) are fit for the purposes made known to the Seller and (d) are free from defects in workmanship and in materials.
Evidence:	The Supplier shall provide objective evidence, acceptable to the Purchaser, of the satisfaction of the requirements of this document for which no specific inspection has been mentioned. The Supplier shall provide a copy of the manufacturing record and procedures to the Purchaser for each lot intended for shipment. The Supplier shall provide a copy of the batch record and all quality assurance documentation to the Purchaser for each lot being supplied. The Supplier shall provide a copy of the Certificate of Analysis to the Purchaser for each lot intended for shipment. The Supplier shall provide to the Purchaser a copy of the approval of each source material, constituent material and component for each lot intended for shipment.

[25] See example, page 345.

The Supplier shall retain a sample of twenty (20) vials from each lot shipped for two years beyond the printed expiration date.

Chemical, physical and microbiological test data for in-process and finished product testing must be on record for each lot shipped and must be available to Purchaser's representatives when requested.

Inspection: The Purchaser may inspect and sample, or cause to be sampled, the product at the Supplier's factory and/or warehouse at a mutually agreeable time prior to shipment of the product.

Testing: The Purchaser may cause independent laboratory testing to be performed as deemed necessary to assure that the goods conform to prescribed requirements. Said testing laboratory shall be of the Purchaser's choice and suitably equipped and qualified to conduct quality assurance tests on biological products.

D. Packing

Storage: Supplier shall state storage volume occupied per dose of vaccine (storage volume includes the vaccine vial, packet containing the vaccine vial and any intermediate packaging).

Inner Boxes: _____ [*number*] individual glass vials will be contained in sturdy white cardboard boxes outfitted with individual segments for protecting and separating each vial. Vaccine and diluent will be packaged to correspond; i.e., one box of diluent will constitute one box of vaccine with no deficit or excess.

Cold Chain Monitor Cards:	One cold chain monitor card (description attached)[26] in _____ language shall be packed with each ___ doses of vaccine.
Overpacking:	Boxes shall be overpacked so that the vaccine remains refrigerated below +8 degrees C. The containers must be suitable for export handling in accordance with WHO/EPI Guidelines on International Packaging and Shipping of Vaccines. They must have adequate insulation and/or sufficient refrigerant to ensure that the warmest storage temperature of the vaccine does not rise above +8 degrees C in continuous outside ambient temperature of +43 degrees C during transit and for a period of at least 48 hours after arrival at the airport of destination. Additional cushioning shall be provided, sufficient to protect the vials from breakage during transit and handling.
Exterior Shipping Cartons:	Product and printed materials, packaged as specified above, shall be packed in weather-resistant, triple-wall corrugated fibreboard cartons with a bursting test strength of not less than 1,900 kPa. The overall dimensions of the exterior shipping cartons should be such that the product does not become damaged during transportation and storage.

E. Markings

All containers and invoices must bear the name of the vaccine, expiry dates of the vaccine and appropriate storage temperature.

[26] See example in Supplement VI.G.1.a., page 783.

Inner Boxes: The inner boxes containing vaccine vials shall be marked with the following information in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Manufacturer's name and registered address
- Manufacturer's national registration number
- Lot or batch number
- Composition and concentration
- Number of vials contained in box
- Expiration date (month and year)
- Instructions for storage and handling†
- Place of manufacture (Made in _____)

Exterior Shipping Cartons: The following information shall be stencilled or labelled on the exterior shipping cartons on two opposing sides in bold letters at least __ mm high with waterproof ink in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Lot or batch number
- Expiration date (month and year)
- Manufacturer's name and registered address
- Manufacturer's national registration number
- Destination country license or registration number
- Consignee's address and emergency phone number
- Destination airport
- Contract number
- Number of vials contained in the carton
- Gross weight of each carton (in kg)
- Carton #___ of ___

† To be provided by Purchaser.

- Instructions for storage and handling†
- Place of manufacture (Made in _____)

F. Documentation

Supplier shall provide to Purchaser a copy of the batch record, including all quality assurance documentation for the vaccine being supplied.

† To be provided by Purchaser.

MEASLES VACCINE

DESCRIPTION
An attenuated live virus vaccine. Each dose contains not less than _____ (specify) infective units of viral vaccine strain _____(specify), and not more than _____ µgm _____ residual antibiotic (specify the antibiotic). This vaccine is a freeze-dried product which must be reconstituted only with 0.5 ml of the sterile diluent provided separately by the manufacturer for that purpose.

ADMINISTRATION
The immunization consists of a single dose of 0.5 ml injected SUBCUTANEOUSLY, preferably in the upper arm. A sterile needle and sterile syringe must only be used for each injection. Once the vaccine has been reconstituted, it should be used the same day, and only then if the vial has been maintained between 0° and +8° and protected from light in the meantime. The diluent MUST BE kept cool prior to use.

IMMUNIZATION SCHEDULE
A single dose of measles vaccine is sufficient to provide prolonged immunity to infection. In countries where the incidence and mortality from measles is high in the first year of life, the recommended age for immunization against measles is a soon as possible after 9 months of age (270 days). Countries where measles is less of a problem may decide on a later date for immunization. Measles vaccine can be safely and effectively given simultaneously with DT, Td, TT, BCG, polio vaccines (OPV and IPV), Hepatitis B and Yellow Fever vaccine.

SIDE EFFECTS
A mild fever and rash lasting one to three days may occur in up to 15% of vaccinees 8 to 12 days after vaccination. Encephalitis has been reported following measles vaccination, approximately one case per million doses administered.

CONTRAINDICATIONS
There are few contraindications to the administration of measles vaccine. On the contrary, it is particularly important to immunize children with malnutrition. Low grade fever, mild respiratory infections or diarrhoea and other minor illnesses should not be considered as contraindications.

It is contraindicated in patients with a known severe allergy to _____ (specify the antibiotic used as a preservative). Since the effect of the live measles virus vaccine on the fetus is not known, it is also contraindicated in pregnancy.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED PERSONS: The vaccine is contraindicated in those with immune deficiency disease or supposed immune response from leukaemia, lymphoma or generalized malignancy. However, HIV infected persons, both asymptomatic and symptomatic, should be immunized with measles vaccine according to the standard schedule.

STORAGE
Freeze-dried measles vaccine should be kept below +8°C (and if possible below 0°C) until used. Stability is even better if the vaccine (not the diluent) is kept at -20°C. The diluent should not be frozen but kept cool immediately prior to use. The vaccine should be protected from light.

PRESENTATION
The vaccine comes in vials of _____ doses.

tween 2° and 8°. Multiple-dose containers for 50 doses are adapted for use only in jet injectors, and those for 10 doses for use by jet or syringe injection.

Expiration date—The expiration date is 1 to 2 years, depending on the manufacturer's data, after date of issue from manufacturer's cold storage (−20°, 1 year).

Labeling—Label the Vaccine in multiple-dose containers to indicate that the contents are intended solely for use by jet injector or for use by either jet or syringe injection, whichever is applicable. Label the Vaccine in single-dose containers, if such containers are not light-resistant, to state that it should be protected from sunlight. Label it also to state that constituted Vaccine should be discarded if not used within 8 hours.

Measles Virus Vaccine Live

» Measles Virus Vaccine Live conforms to the regulations of the FDA concerning biologics (630.30 to 630.37) (see *Biologics* ⟨1041⟩). It is a bacterially sterile preparation of live virus derived from a strain of measles virus tested for neurovirulence in monkeys, for safety, and for immunogenicity, free from all demonstrable viable microbial agents except unavoidable bacteriophage, and found suitable for human immunization. The strain is grown for purposes of vaccine production on chicken embryo primary cell tissue cultures derived from pathogen-free flocks, meets the requirements of the specific safety tests in adult and suckling mice; the requirements of the tests in monkey kidney, chicken embryo and human tissue cell cultures and embryonated eggs; and the requirements of the tests for absence of *Mycobacterium tuberculosis* and of avian leucosis, unless the production cultures were derived from certified avian leucosis-free sources and the control fluids were tested for avian leucosis. The strain cultures are treated to remove all intact tissue cells. The Vaccine meets the requirements of the specific tissue culture test for live virus titer, in a single immunizing dose, of not less than the equivalent of 1000 $TCID_{50}$ (quantity of virus estimated to infect 50% of inoculated cultures × 1000) when tested in parallel with the U. S. Reference Measles Virus, Live Attenuated. It may contain suitable antimicrobial agents.

Packaging and storage—Preserve in single-dose containers, or in light-resistant, multiple-dose containers, at a temperature be-

Packaging and storage—Preserve in single-dose containers, or in light-resistant, multiple-dose containers, at a temperature between 2° and 8°. Multiple-dose containers for 50 doses are adapted for use only in jet injectors, and those for 10 doses for use by jet or syringe injection.

Expiration date—The expiration date is 1 to 2 years, depending on the manufacturer's data, after date of issue from manufacturer's cold storage (−20°, 1 year).

Labeling—Label the Vaccine in multiple-dose containers to indicate that the contents are intended solely for use by jet injector or for use by either jet or syringe injection, whichever is applicable. Label the Vaccine in single-dose containers, if such containers are not light-resistant, to state that it should be protected from sunlight. Label it also to state that constituted Vaccine should be discarded if not used within 8 hours.

Subpart C—[Reserved]

Subpart D—Measles Virus Vaccine Live

§ 630.30 Measles Virus Vaccine Live.

(a) *Proper name and definition.* The proper name of this product shall beMeasles Virus Vaccine Live, which shall consist of a preparation of live, attenuated, measles virus.

(b) *Criteria for acceptable strains of attenuated measles virus.* Strains of attenuated measles virus used in the manufacture of vaccine shall be identified by (1) historical records, including origin and manipulation during attenuation and (2) antigenic specificity as measles virus as demonstrated by tissue culture neutralization tests. Strains used for the manufacture ofMeasles Virus Vaccine Live, shall have been shown to be safe and potent in man by field studies with experimental vaccines. The vaccine shall have been demonstrated as safe and potent in at least 10,000 susceptible persons. Susceptibility shall be shown by the absence of neutralizing or other antibodies against measles virus, or by other appropriate methods. Seed virus used for vaccine manufacture shall be free of all demonstrable extraneous

viable microbial agents except for unavoidable bacteriophage.

(c) *Neurovirulence safety test of the virus seed strain in monkeys*—(1) *The test.* A demonstration shall be made in monkeys of the lack of neurotropic properties of the seed strain of attenuated measles virus used in the manufacture of measles virus vaccine. For this purpose and to establish consistency of manufacture of the vaccine, vaccine from each of five consecutive lots shall be tested separately in the following manner:

(i) Samples of each of the five lots of vaccine shall be tested in measles susceptible monkeys. Immediately prior to initiation of a test each monkey shall have been shown to be serologically negative for neutralizing antibodies by means of a tissue culture neutralization test with undiluted serum from each monkey tested at approximately 100 $TCID_{50}$ of Edmonston strain measles virus, or negative for measles virus antibodies as demonstrated by tests of equal sensitivity.

(ii) A test sample of vaccine removed after clarification but before final dilution for standardization of virus content shall be used for the test.

(iii) Vaccine shall be injected by combined intracerebral, intraspinal, and intramuscular routes into not less than 20 Macaca or Cercopithecus monkeys or a species found by the Director, Center for Biologics Evaluation and Research, to be equally suitable for the purpose. The animals shall be in overt good health and injected under deep barbiturate anesthesia. The intramuscular injection shall consist of 1.0 milliliter of test sample into the right leg muscles. At the same time, 200 milligrams of cortisone acetate shall be injected into the left leg muscles, and 1.0 milliliter of procaine penicillin (300,000 units) into the right arm muscles. The intracerebral injection shall consist of 0.5 milliliter of test sample into each thalamic region of each hemisphere. The intraspinal injection shall consist of 0.5 milliliter of test sample into the lumber spinal cord enlargement.

(iv) The monkeys shall be observed for 17–21 days and symptoms of paralysis as well as other neurologic disorders shall be recorded.

(v) At least 90 percent of the test animals must survive the test period without losing more than 25 percent of their weight except that, if at least 70 percent of the test animals survive the first 48 hours after injection, those animals which do not survive this 48-hour test period may be replaced by an equal number of qualified test animals which are tested pursuant to paragraphs (c)(1)(i) through (iv) of this section. At least 80 percent of the injected animals surviving beyond the first 48 hours must show gross or microscopic evidence of inoculation trauma in the thalamic area and microscopic evidence of inoculation trauma in the lumbar region of the spinal cord. If less than 70 percent of the test animals survive the first 48 hours, or if less than 80 percent of the animals meet the inoculation criteria prescribed in this paragraph, the test must be repeated.

(vi) At the end of the observation period, each surviving monkey shall (*a*) be bled and the serum tested for evidence of serum antibody conversion to measles virus and (*b*) be autopsied and samples of cerebral cortex and of cervical and lumbar spinal cord enlargements shall be taken for virus recovery and identification if needed pursuant to paragraph (c)(1)(vii) of this section. Histological sections shall be prepared from both spinal cord enlargements and appropriate sections of the brain and examined.

(vii) Doubtful histopathological findings necessitate (*a*) examination of a sample of sections from several regions of the brain in question, and (*b*) attempts at virus recovery from the nervous systems tissues previously removed from the animal.

(viii) The lot is satisfactory if the histological and other studies demonstrate no evidence of changes in the central nervous system attributable to unusual neurotropism of the seed virus or of the presence of extraneous neurotropic agents.

(2) *Wild virus controls.* As a check against the inadvertent introduction of wild measles virus, at least four uninoculated measles susceptible control monkeys shall be maintained as either cage mates to, or within the same immediate area of, the 20 inoculated test animals for each lot of vaccine for

the entire period of observation (17-21 days) and an additional 10 days. Serum samples from these control contact monkeys drawn at the time of seed virus inoculation of the test animals, and again after completion of the test, shall be shown to be free of measles neutralizing antibodies.

(3) *Test results.* (i) For each lot of vaccine under test, at least 80 percent of the monkeys must show measles antibody serological conversion (1:4 or greater) when the serum as obtained from the monkey is tested and the control contact monkeys must demonstrate no immunological response indicative of measles virus infection.

(ii) The measles virus seed has acceptable neurovirulence properties for use in vaccine manufacture only if for each of the five lots (*a*) 90 percent of the monkeys survive the observation period, (*b*) the histological and other studies produce no evidence of changes in the central nervous system attributable to unusual neurotropism of the seed virus, and (*c*) there is no evidence of the presence of extraneous neurotropic agents.

(4) *Need for additional neurovirulence safety testing.* A neurovirulence safety test as prescribed in this paragraph shall be performed on vaccine from five consecutive lots whenever a new production seed lot is introduced or whenever the source of cell culture substrate must be reestablished and recertified as prescribed in § 630.32(a) and (b) of this part.

[38 FR 32068, Nov. 20, 1973, as amended at 40 FR 11719, Mar. 13, 1975; 49 FR 23834, June 8, 1984; 50 FR 4138, Jan. 29, 1985; 55 FR 11013, Mar. 26, 1990; 55 FR 47875, Nov. 16, 1990]

§ 630.31 Clinical trials to qualify for license.

To qualify for license, the antigenicity of the vaccine shall have been determined by clinical trials of adequate statistical design, by a suitable route of administration of the product. Such clinical trials shall be conducted with five lots of measles virus vaccine which have been manufactured by the same methods. There shall be a demonstration under circumstances in which adequate clinical and epidemiological surveillance of illness has been maintained to show that the measles virus vaccine, when administered as recommended by the manufacturer, is free of harmful effect upon administration to approximately 1,000 susceptible individuals, in that there were no detectable neutralizing antibodies before vaccination and there was serological conversion after vaccination. The five lots of vaccine shall be distributed as evenly as possible among the 1,000 individuals tested. Demonstration shall be made of immunogenic effect by the production of specific measles neutralizing antibodies (i.e., sero-conversion from less than 1:4 to 1:8 or greater) in at least 90 percent of each of five groups of measles susceptible individuals, each having received a virus vaccine dose which is not greater than that which was demonstrated to be safe in field studies (§ 630.30(b)) when used under comparable conditions. Such clinical trials shall be conducted in compliance with part 56 of this chapter unless exempted under § 56.104 or granted a waiver under § 56.105, and with the requirements for informed consent set forth in part 50 of this chapter.

[55 FR 47875, Nov. 16, 1990]

§ 630.32 Manufacture of live, attenuated Measles Virus Vaccine.

(a) *Virus cultures.* Virus shall be propagated in chick embryo tissue cultures.

(b) *Virus propagated in chick embryo tissue cultures.* Embryonated chicken eggs used as the source of chick embryo tissue for the propagation of measles virus shall be derived from flocks certified to be free of Salmonella pullorum, avian tuberculosis, fowl pox, Rous sarcoma, avian leucosis, reticuloendotheliosis virus, and other adventitious agents pathogenic for chickens. If eggs are procured from flocks that are not so certified, tests shall be performed to demonstrate freedom of the vaccine from such agents. (See § 630.35(a)(8) for test for avian leucosis.)

(c) [Reserved]

(d) *Passage of virus strain in vaccine manufacture.* Virus in the final vaccine shall represent no more than ten tissue culture passages beyond the passage used to perform the clinical trials (§ 630.30(b)) which qualified the manufacturer's vaccine strain for license.

(e) *Tissue culture preparation.* Only primary cell tissue cultures shall be used in the manufacture of Measles Virus Vaccine. Continuous cell lines shall not be introduced or propagated in Measles Virus Vaccine manufacturing areas.

(f) *Control vessels.* (1) From the tissue used for the preparation of tissue cultures for growing attenuated measles virus, an amount of processed cell suspension equivalent to that used to prepare 500 ml. of tissue culture shall be used to prepare uninfected tissue control materials. This material shall be distributed in control vessels and observed microscopically for a period of no less than 14 days beyond the time of inoculation of the production vessels with measles virus; but if the production vessels are held for use in vaccine manufacture for more than 14 days, the control vessels shall be held and observed for the additional period. At the end of the observation period or at the time of virus harvest, whichever is later, fluids from the control cultures shall be tested for the presence of adventitious agents as follows:

Samples of fluid from each control vessel shall be collected at the same time as fluid is harvested from the corresponding production vessels. If multiple virus harvests are made from the same cell suspension, the control samples for each harvest shall be frozen and stored at −60° C. until the last viral harvest for that cell suspension is completed. The fluid from all the control samples from that suspension shall be pooled in proportionate amounts and at least five ml. inoculated into human and simian cell tissue culture systems and in the tissue culture system used for virus production. The cultures shall be observed for the presence of changes attributable to growth of adventitious viral agents including hemadsorption viral agents.

(2) The cell sheets of one quarter to one third of the control vessels shall be examined at the end of the observation period (14 days or longer) for the presence of hemadsorption viruses by the addition of guinea pig red blood cells. If the chick embryo cultures were not derived from a certified source (paragraph (b) of this section), the remaining tissue culture controls may be used to test for avian leucosis virus using either Rubin's procedure for detecting Resistance Inducing Factor (RIF) or a method of equivalent effectiveness.

(3) The test is satisfactory only if there is no evidence of adventitious viral agents and if at least 80 percent of the control vessels are available for observation at the end of the observation period (14 days or longer).

(g) *Test samples.* Samples of virus harvests or pools for testing by inoculation into animals, into tissue culture systems, into embryonated hens' eggs, and into bacteriological media, shall be withdrawn immediately after harvesting or pooling but prior to freezing except that samples of test materials frozen immediately after harvesting or pooling and maintained at −60° C. or below, may be tested upon thawing, provided no more than two freeze-thaw cycles are employed. The required tests shall be initiated without delay after thawing.

[38 FR 32068, Nov. 20, 1973, as amended at 40 FR 11719, Mar. 13, 1975; 47 FR 24699, June 8, 1982]

§ 630.33 Reference virus.

A U.S. Reference Measles Virus, Live, Attenuated, shall be obtained from the Center for Biologics Evaluation and Research as a control for correlation of virus titers.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§ 630.34 Potency test.

The concentration of live measles virus shall constitute the measure of potency. The titration shall be performed in a suitable cell culture system, free of wild viruses, using either the U.S. Reference Measles Virus, Live, Attenuated or a calibrated equivalent strain as a titration control. The concentration of live measles virus contained in the vaccine of each lot under test shall be no less than the equivalent of 1,000 $TCID_{50}$ of the U.S. reference per human dose.

§ 630.35 Test for safety.

(a) *Tests prior to clarification of vaccine manufactured in chick embryo tissue cultures.* Prior to clarification, the following tests shall be performed on each virus pool of chick embryo tissue culture:

(1) *Inoculation of adult mice.* Each of at least 20 adult mice each weighing 15-20 grams shall be inoculated intraperitoneally with 0.5 ml. and intracerebrally with 0.03 ml. amounts of each virus pool to be tested. The mice shall be observed for 21 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection by direct observation and subinoculation of appropriate tissue into at least five additional mice which shall be observed for 21 days. The virus pool may be used only if at least 80 percent of the original group of mice remain healthy and survive the observation period and if none of the mice show evidence of a transmissible agent or other viral infection, other than measles virus, attributable to the vaccine.

(2) *Inoculation of suckling mice.* Each of at least 20 suckling mice less than 24 hours old shall be inoculated intracerebrally with 0.01 ml. and intraperitoneally with 0.1 ml. of the virus pool to be tested. The mice shall be observed daily for at least 14 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection. Such examination shall include subinoculation of appropriate tissue suspensions into an additional group of at least five suckling mice by intracerebral and intraperitoneal routes and observed daily for 14 days. In addition, a blind passage shall be made of a single pool of the emulsified tissue (minus skin and viscera) of all mice surviving the original 14-day test. The virus pool is satisfactory for Measles Virus Vaccine only if at least 80 percent of the original inoculated mice remain healthy and survive the entire observation period, and if none of the mice used in the test show evidence of a transmissible agent or viral infection, other than measles virus, attributable to the vaccine.

(3) *Inoculation of monkey tissue cell cultures.* A volume of virus suspension of each undiluted virus pool, equivalent to at least 500 human doses or 50 milliliters, whichever represents a greater volume, shall be tested for adventitious agents in *Cercopithecus* monkey kidney tissue culture preparations or *Erythrocebus patas* monkey kidney tissue culture preparations, after neutralization of the measles virus by a high titer antiserum of nonhuman, nonsimian and nonchicken origin. The immunizing antigen used for the preparation of the measles antiserum shall be grown in tissue culture cells that shall be free of extraneous viruses which might elicit antibodies that could inhibit growth of extraneous viruses present in the measles virus pool. The tissue culture of the virus pool shall be observed for no less than 14 days. The virus pool is satisfactory for measles virus vaccine only if all the tissue culture tests fail to show evidence of any extraneous transmissible agent other than measles virus attributable to the vaccine.

(4) *Inoculation of other cell cultures.* The measles virus pool shall be tested in the same manner as prescribed in paragraph (a)(3) of this section in rhesus or cynomolgus monkey kidney, chick embryo, and human tissue cell cultures.

(5) *Inoculation of embryonated chicken eggs.* A volume of virus suspension of each undiluted virus pool, equivalent to at least 100 doses or 10 milliliters, whichever represents a greater volume, after neutralization of the measles virus by a high titer antiserum of nonhuman, nonsimian, nonavian origin shall be tested as follows:

(i) Embryonated eggs, 10 to 11 days old, shall be inoculated by the allantoic route using 0.5 milliliter per egg. Follow incubation at 35° C for 72 hours, the allantoic fluids shall be harvested, pooled, and subpassed by the same route into fresh, embryonated eggs, 10 to 11 days old, using 0.5 milliliter per egg and incubated at 35° C for 72 hours. Both the initial pool and the subpassage harvest shall be tested for the presence of hemagglutinin. The virus pool is satisfactory if the embryos appear normal and there is no evidence of hemagglutinating agents.

(ii) Embryonated eggs, 6 to 7 days old, shall be inoculated by the yolk sac route using 0.5 milliliter per egg. Following incubation at 35° C for at least 9 days, the yolk sacs shall be harvested and pooled. A 10-percent suspension of

yolk sacs shall be subpassed by the same route into fresh embryonated eggs, 6 to 7 days old, using 0.5 milliliter of inoculum per egg and incubated at 35° C for at least 9 days. The virus pool is satisfactory if the embryos in both the initial test and the subpassage appear normal.

(6) [Reserved]

(7) *Bacteriological tests.* Each virus pool shall be tested for sterility in accordance with § 610.12 of this chapter. In addition each virus pool shall be tested for the presence of M. tuberculosis, both avian and human, by appropriate culture methods.

(8) *Test for avian leucosis.* If the cultures were not derived from a certified source (§ 630.32(b)), and the control fluids were not tested for avian leucosis (§ 630.32(f)), at least 500 doses or 50 ml., whichever represents a greater volume of each undiluted vaccine pool, shall be tested and found negative for avian leucosis, using either Rubin's procedure for detecting Resistance Inducing Factor (RIF) or another method of equivalent effectiveness.

(b) [Reserved]

(c) *Clarification.* After harvesting and removal of samples for testing as prescribed above in this section, the virus fluids shall be clarified by centrifugation, by passage through filters of sufficiently small porosity, or by any other method that will assure removal of all intact tissue cells which may have been collected in the harvesting process.

[38 FR 32068, Nov. 20, 1973, as amended at 40 FR 11719, Mar. 13, 1975; 41 FR 43400, Oct. 1, 1976; 47 FR 24699, June 8, 1982]

§ 630.36 General requirements.

(a) *Final container tests.* In addition to the tests required pursuant to § 610.14 of this chapter, an immunological and virological identity test shall be performed on the final container if it was not performed on each pool or the bulk vaccine prior to filling.

(b)—(c) [Reserved]

(d) *Dose.* These standards are based on an individual human immunizing dose of no less than 1,000 $TCID_{50}$ of Measles Virus Vaccine Live, expressed in terms of the assigned titer of the U.S. reference measles virus.

(e) *Labeling.* In addition to the items required by other applicable labeling provisions of this subchapter, single-dose container labeling for vaccine which is not protected against photochemical deterioration shall include a statement cautioning against exposure to sunlight.

(f) [Reserved]

(g) *Photochemical deterioration; protection.* Vaccine in multiple dose final containers shall be protected against photochemical deterioration. Such containers may be colored, or outside coloring or protective covering may be used for this purpose, provided (1) the method used is shown to provide the required protection, and (2) visible examination of the contents is not precluded. Vaccine in single dose containers may be protected in the same manner provided the same conditions are met.

(h) *Sample and protocols.* The following materials shall be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892:

(1) For each lot of vaccine:

(i) A protocol which consists of a summary of the history of the manufacture of the lot, including all results of each test for which test results are requested by the Director, Center for Biologics Evaluation and Research.

(ii) A total of no less than two 25-milliliter volumes in a frozen state (−60° C) of preclarification bulk vaccine containing no preservative or adjuvant.

(iii) A total of no less than 30 containers of the vaccine from each filling of each bulk lot of single-dose containers. A total of no less than six 50-dose containers or ten 10-dose containers of the vaccine from each filling of each bulk lot of multiple-dose containers.

(2) In addition to the requirements of paragraph (h)(1) of this section, whenever a new production seed lot is introduced, or whenever the source of cell culture substrate must be reestablished and recertified, samples consisting of no less than 100 milliliters in 10 milliliter volumes, in a frozen state (−60° C), of postclarification bulk vaccine

containing stabilizer but no preservative or adjuvant, taken from each of 5 consecutive lots of the bulk vaccine.

[38 FR 32068, Nov. 20, 1973, as amended at 41 FR 10429, Mar. 11, 1976; 49 FR 23834, June 8, 1984; 50 FR 4138, Jan. 29, 1985; 51 FR 15610, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

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WHO Technical Report Series, No. 840, 1994

WHO/V&B/99.12

Annex 3
Requirements for measles, mumps and rubella vaccines and combined vaccine (live)
(Requirements for Biological Substances No. 47)

Introduction 102
Measles 102
Mumps 103
Rubella 103
Vaccine requirements 104

I. Requirements for measles vaccine 105

General considerations 105

Part A. Manufacturing requirements 106
A.1 Definitions 106
A.2 Certification of the strain of virus for use in vaccine production 108
A.3 General manufacturing requirements 108
A.4 Production control 109
A.5 Filling and containers 117
A.6 Control tests on final product 117
A.7 Records 118
A.8 Samples 119
A.9 Labelling 119
A.10 Distribution and shipping 119
A.11 Storage and expiry date 119

Part B. National control requirements 120
B.1 General 120
B.2 Release and certification 120

II. Requirements for mumps vaccine 121

General considerations 121

Part A. Manufacturing requirements 122
A.1 Definitions 122
A.2 Certification of the strain of virus for use in vaccine production 124
A.3 General manufacturing requirements 124
A.4 Production control 125
A.5 Filling and containers 133
A.6 Control tests on final product 133
A.7 Records 134
A.8 Samples 135
A.9 Labelling 135
A.10 Distribution and shipping 135
A.11 Storage and expiry date 135

Part B. National control requirements 136
B.1 General 136
B.2 Release and certification 136

III. Requirements for rubella vaccine 137

General considerations 137

Part A. Manufacturing requirements 138
A.1 Definitions 138
A.2 Certification of the strain of virus for use in vaccine production 140
A.3 General manufacturing requirements 140
A.4 Production control 141
A.5 Filling and containers 149
A.6 Control tests on final product 149
A.7 Records 150
A.8 Samples 150
A.9 Labelling 150
A.10 Distribution and shipping 151
A.11 Storage and expiry date 151

Part B. National control requirements 151
B.1 General 151
B.2 Release and certification 152

IV. Requirements for measles–mumps–rubella (MMR) combined vaccine 152

General considerations 152

Part A. Manufacturing requirements 153
A.1 Descriptive definition 153
A.2 Manufacturing requirements for MMR vaccine 153
A.3 Filling and containers 154
A.4 Control tests on final product 154
A.5 Records 156
A.6 Samples 156
A.7 Labelling 156
A.8 Distribution and shipping 156
A.9 Storage and expiry date 157

Part B. National control requirements 157
B.1 General 157
B.2 Release and certification 157

Authors 158

Acknowledgements 158

References 158

Appendix 1
Summary protocol for production and testing of measles vaccine (live) 160

Appendix 2
Requirements for human diploid cells used for the production of measles, mumps and rubella vaccines (live) 169

Appendix 3
Proposed assay method for determining the virus content of live measles vaccine 173

Appendix
Model certificate for release of measles, mumps, rubella or combined vaccines 175

Appendix 5
Summary protocol for production and testing of mumps vaccine (live) 176

Appendix 6
Proposed assay method for determining the virus content of live mumps vaccine 185

Appendix 7
Summary protocol for production and testing of rubella vaccine (live) 187

Appendix 8
Proposed assay method for determining the virus content of live rubella vaccine 196

Appendix 9
Summary protocol for production and testing of measles–mumps–rubella combined vaccine (live) 197

Appendix 10
Proposed assay method for determining the virus content of measles–mumps–rubella combined live vaccine 201

Introduction

Combined live vaccine for measles, mumps and rubella (MMR) is used widely for the immunization of children in certain regions of the world because of its advantages over the individual vaccines. Combined vaccine provokes an adequate immune response in children simultaneously for the three infections and facilitates the implementation of current immunization strategies.

Measles

Measles is an almost invariable clinical experience of childhood, and is often a severe disease, frequently complicated by middle-ear infection or bronchopneumonia. In some countries it is a major cause of illness and death. Encephalitis occurs in approximately one of every 2000 reported cases; survivors often have permanent brain damage and mental retardation. Death, predominantly from respiratory and neurological causes, occurs in one of every 3000 reported measles cases. The risk of death is greater for infants and adults than for children and adolescents.

Contracting measles during pregnancy increases fetal risk. Most commonly, this risk involves premature labour and moderately increased rates of spontaneous abortion and of low birth weight.

Subacute sclerosing panencephalitis, a slow virus infection of the central nervous system, is associated with measles virus. It is noteworthy that, in the United States of America, widespread use of measles vaccine has led to the virtual disappearance of this infection.

Immunization against measles has been of interest to WHO for many years, and especially since the Expanded Programme on Immunization

was launched in 1974 with measles as one of the principal diseases against which it was directed. In support of the immunization programme, reference materials for anti-measles serum and measles vaccine virus have been established. The Requirements for Measles Vaccine (Live) were adopted by the WHO Expert Committee on Biological Standardization in 1966 (*1*), with an Addendum in 1981 to include an accelerated test for stability (*2*). Since the original production of measles vaccine, the search for improved immunizing agents has continued.

The last revision of the Requirements for Measles Vaccine (Live) was adopted by the WHO Expert Committee on Biological Standardization in 1987 (*3*). In that revision, account was taken of the opinions of consultants, of the regulations and requirements for the manufacture and control of measles vaccine that had been formulated in a number of countries, and of information from published and unpublished sources. For the present revised Requirements, published together with the new Requirements for Combined Vaccine, some changes have been made to the previous revision.

Mumps

Mumps is an acute disease of children and young adults, caused by a paramyxovirus of which there is only a single serotype. Mumps virus produces no symptoms in about one-third of infected people. In those with a clinical response, glandular and nerve tissue are most often affected. The most common signs are fever and swelling of the parotid glands. Other complications, which may appear simultaneously with these signs or in any sequence, are epididymo-orchitis, meningo-encephalitis, cranial nerve involvement (especially eighth cranial nerve damage leading to hearing impairment), pancreatitis, oophoritis, mastitis and myocarditis. Frequent viruria and abnormal renal function suggest that mumps virus may infect the kidneys. In some instances, one or more of the other complications may be present in the absence of parotitis.

The most common complication of mumps in children is meningitis, sometimes associated with encephalitis, and in young adults orchitis. Most complications due to mumps infection resolve without permanent damage. Death following mumps is rare and is mostly due to mumps encephalitis. Many people reach adulthood without developing immunity and potential target populations for immunization therefore include both children and susceptible adults.

The Requirements for Mumps Vaccine (Live) were adopted by the WHO Expert Committee on Biological Standardization in 1987 (*4*), and have been included in this combined document with some changes.

Rubella

Rubella (German measles) gives rise to a mild exanthematous illness, accompanied by few constitutional symptoms, and occurs most commonly

in childhood. If the infection occurs in a woman in early pregnancy however, the virus may cross the placenta to reach the fetus, in which the infection can induce birth defects. These defects may be serious and permanent and include congenital heart disease, cataract formation, deafness and mental retardation. The prevention of fetal infection, therefore, is the primary purpose of rubella immunization.

To meet the need for an international standard for anti-rubella serum for use in the assay of rubella antibodies and in the control of specific (anti-rubella) immunoglobulins, the WHO Expert Committee on Biological Standardization established the second International Reference Preparation of Anti-Rubella Serum in 1970(*5*). The Requirements for Rubella Vaccine (Live) were adopted by the Committee in 1977(*6*), with an Addendum in 1980 (*7*), and have been included in this combined document with some changes.

Vaccine requirements

The combination of different strains of attenuated measles, mumps and rubella viruses in a single vaccine might interfere with the immune response to one or more of the components or provoke serious adverse reactions. Therefore, before use for routine immunization of children, every proposed combination of the different strains of measles, mumps and rubella viruses should be studied in clinical trials to determine the minimum effective virus dose, optimal balance of components and general safety profile.

In the present Requirements, no reference is made to the maximum permitted number of passages between the master seed and working seed; instead, it is recommended that manufacturers follow the routine production procedure employed to produce the clinical trial material that established the safety and efficacy of the vaccine. Reference to passage levels of virus may become irrelevant with the use of fermenters and of different multiplicities of infection and numbers of harvests. For each strain of virus, it is necessary to establish the relationship between the laboratory estimation of virus titre on the one hand and safety and efficacy for humans on the other. Where individual vaccines are to be used in combination with other vaccines, the dose–response curve for each component should be determined by administering it in the proposed combined form.

It is essential that every precaution be taken to exclude adventitious agents from vaccines for use in humans. In the Requirements formulated below, tests have been described for detecting adventitious agents that might be present in cell cultures used for vaccine production.

The systematic use of cells from birds maintained in closed colonies that have been subject to continuous and systematic veterinary and laboratory monitoring for the presence of infectious agents, or of cells derived from well characterized human diploid cell lines, has improved the quality of cell

substrates. However, although a group convened by WHO in 1980(*8*) concluded that, for cell cultures, it was sufficient to conduct tests for extraneous agents on control cells, tests for the absence of such agents are still mandatory for individual harvests and/or virus pools in the present Requirements. On the other hand, tests for extraneous agents on small laboratory animals have been abandoned.

Experience has shown that the maintenance of a standard operating procedure, including a standard temperature of incubation and the use of a seed lot system, results in a consistent product that shows a regular pattern of attenuation and immunogenicity.

I. Requirements for measles vaccine

General considerations

Hundreds of millions of people have been vaccinated with live attenuated measles virus vaccines produced from a variety of strains, and there is ample evidence that these vaccines are safe and effective. Production of such vaccines requires the observance of certain rules, and it is thus important that international requirements for live attenuated measles vaccines should be available to manufacturers and national control authorities.

The antibody response in people inoculated with live measles vaccine can be accurately measured serologically, and a number of studies have established that the presence of detectable levels of antibody is correlated with protection against the disease. Immunity following the use of live measles vaccine appears to be of long duration, as indicated by the persistence of neutralizing antibodies in children several years after receiving vaccines prepared from further-attenuated strains derived from the Edmonston strain of measles virus. Although persistence of antibodies to the Edmonston strain has been demonstrated, it is important that studies should be undertaken to determine the duration of immunity induced by vaccines derived from approved strains.

The optimum age for the immunization of babies may differ from one country to another. If immunization is carried out too early in life, there may be no or poor protection, because of pre-existing maternal antibodies, especially if vaccine strains have been over-attenuated. On the other hand, the pattern of incidence of measles in some developing countries is such that it may be necessary to immunize babies as early as six months of age. The minimum and maximum virus titre and the general safety of the virus strain should be defined. The poorer seroconversion rate and antibody titre when measles vaccine is administered early in life can be partially corrected by a second injection later in life.

It is obviously important that the strains of virus used to prepare live measles vaccine should show no tendency to produce neurological

complica. . of the type encountered in some cases of natural measles. Present experience indicates that the live vaccines so far used are, indeed, safe in this respect. In the absence of a more satisfactory test, the intracerebral inoculation of monkeys has been used as a laboratory test to evaluate this property; the development of more reliable methods is desirable. Post-measles encephalopathy may be the result of an immunopathogenic reaction, but the underlying mechanism is not known.

Each of the following sections constitutes a recommendation. The parts of each section printed in normal type have been written in the form of requirements so that, if a health administration so desires, they may be adopted as they stand as definitive national requirements. The parts of each section printed in small type are comments or recommendations for guidance. To facilitate the international distribution of vaccine made in accordance with these Requirements, a summary protocol for recording the results of tests is included as Appendix 1.

Should individual countries wish to adopt these Requirements as the basis of their national regulations for measles vaccines, it is recommended that modifications be made only on condition that the modified requirements ensure at least an equal degree of safety and potency of the vaccine. The World Health Organization should then be informed of the action taken.

Part A. Manufacturing requirements

A.1 Definitions

A.1.1 *International name and proper name*

The international name shall be "Vaccinum morbillorum vivum". The proper name shall be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

A.1.2 *Descriptive definition*

"Vaccinum morbillorum vivum" is a preparation of live attenuated measles virus grown in a suitable cell culture. The preparation shall satisfy all the requirements formulated below.

At present, live measles vaccines are blended with an appropriate stabilizer and lyophilized. They are available for distribution only in that form, either as monovalent vaccines or in combination with live mumps and/or live rubella vaccines.

A.1.3 *International reference materials*

The International Reference Reagent for the Assay of Measles Vaccine (Live) and the International Standard for Anti-Measles Serum are available on request from the National Institute for Biological Standards and Control, Potters Bar, England.

A.1.4 *Terminology*

The following definitions are given for the purpose of these Requirements only.

Original vaccine: A vaccine prepared according to the manufacturer's specifications and shown on administration to humans to be safe and immunogenic.

Master seed lot: A quantity of virus derived from, or used to prepare, an original vaccine; the virus suspension has been processed as a single lot to ensure a uniform composition and is fully characterized. The master seed lot is used for the preparation of working seed lots.

Working seed lot: A quantity of virus of uniform composition, fully characterized, derived from a master seed lot. The working seed lot is used for the production of vaccines.

Cell seed: A quantity of fully characterized cells of human, animal or other origin stored frozen at -70 °C or below in aliquots of uniform composition, one or more of which are used for the production of a manufacturer's working cell bank.

Manufacturer's working cell bank (MWCB): A quantity of cells of uniform composition derived from one or more ampoules of the cell seed and stored frozen at -70 °C or below in aliquots, one or more of which are used for production purposes.

In normal practice a cell seed is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer and approved by the national control authority. The cells are combined in a single pool, distributed into ampoules and preserved cryogenically to form the MWCB.

Production cell culture: A number of cell cultures derived from the same pool of cells and processed together.

Single harvest: A quantity of virus suspension derived from a batch of production cell cultures that were inoculated with the same working seed lot and processed together in a single production run.

Virus pool: A homogeneous pool of single harvests collected into a single vessel before clarification.

Final bulk: The homogeneous finished virus suspension prepared from one or more clarified virus pools in the vessel from which the final containers are filled.

Filling lot (final lot): A collection of sealed final containers of finished vaccine that are homogeneous with respect to the risk of contamination during filling and freeze-drying. All the final containers must, therefore, have been filled from a single vessel of final bulk in one working session and lyophilized under standardized conditions in a common chamber.

Cell-culture infective dose 50% ($CCID_{50}$): The quantity of a virus suspension that is estimated to infect 50% of cell cultures.

Plaque-forming unit (PFU): The smallest quantity of a virus suspension that will produce a plaque in monolayer cell cultures.

A.2 Certification of the strain of virus for use in vaccine production

The strain of measles virus used in the production of measles vaccine shall be identified by historical records that include information on the origin of the strain, its method of attenuation and the passage level at which attenuation was demonstrated by clinical evaluation.

The strain of measles virus used in the production of vaccine shall have been shown to be safe by appropriate laboratory tests (see section A.4 of these Requirements) and safe and immunogenic by tests in susceptible humans. Only strains that are certified by the national control authority shall be used.

The lowest immunizing dose of virus in the vaccine that induces seroconversion in susceptible individuals shall be established in a dose-response study. This dose shall serve as a basis for establishing parameters for stability and expiry of the vaccine.

A.3 General manufacturing requirements

The requirements of Good Manufacturing Practices for Pharmaceutical (*9*) and Biological (*10*) Products and the Requirements for Human Diploid Cells Used for the Production of Measles, Mumps and Rubella Vaccines (Live) (Appendix 2) shall apply to establishments manufacturing measles vaccine, with the addition of the following requirements.

Production areas shall be decontaminated before they are used for the manufacture of measles vaccine.

Measles vaccine shall be produced by staff who have not handled other infectious microorganisms or animals on the same working day. The staff shall consist of persons who are periodically examined medically and found to be healthy. Steps shall be taken to ensure that all personnel involved in the production areas are immune to measles. Production and control shall be organized as two separate units of the manufacturing establishment with independent responsibilities.

Only the virus seed lot and cell cultures approved by the national control authority for the production of measles vaccine shall be introduced or handled in the production area.

Persons not directly concerned with the production processes, other than official inspectors, shall not be permitted to enter the production area without valid reason and specific authorization.

Particular attention shall be given to the recommendations in Good Manufacturing Practices for Biological Products (*10*) regarding the training and experience of the persons in charge of production and testing, and of those assigned to various areas of responsibility in the manufacturing establishment, as well as to the registration of such personnel with the national control authority.

A.4 Production control

The general production precautions formulated in Good Manufacturing Practices for Biological Products (*10*) shall apply to the manufacture of measles vaccine.

A.4.1 *Source materials*

A.4.1.1 Strain of measles virus

The strain of measles virus used in the production of live measles vaccine shall be certified according to the specifications of section A.2. The vaccine strain shall be approved by the national control authority. The seed lot or five consistent lots of vaccine derived from the seed lot shall have been shown to be non-neuropathogenic in monkeys (see section A.4.2.1) and to yield live measles vaccine of adequate immunogenicity and safety in human beings.

A.4.1.2 Cell cultures

Measles virus used in the production of measles vaccine shall be propagated in cell cultures approved by the national control authority. All information on the source and method of preparation of the cell-culture system used shall be available to the national control authority.

> In some countries biochemical tests for the detection of RNA viruses are applied to production cell cultures.

A.4.1.3 Avian-embryo cell cultures

If avian-embryo cell cultures are used for the propagation of measles vaccine virus, the eggs used as a source of cells shall be derived from a closed, specific-pathogen-free, healthy flock. This flock shall be monitored at regular intervals for *Mycobacterium avium*, fowlpox, avian retroviruses, Newcastle disease virus and other avian parainfluenza viruses, avian encephalomyelitis virus, infectious laryngotracheitis virus, avian reticuloendotheliosis virus, Marek's disease virus, infectious bursal disease virus, avian reovirus, avian adenoviruses, avian influenza virus, *Haemophilus paragallinarum*, *Salmonella gallinarum*, *Salmonella pullorum*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and other agents pathogenic for birds.

> In some countries, all birds are bled when a colony is established, and thereafter 5% of the birds are bled each month. The serum samples are screened for antibodies to relevant pathogens. Any bird that dies is investigated to determine the cause of death.

A.4.1.4 Human diploid cells

If human diploid cells are used for the propagation of measles virus, a MWCB shall be established in conformity with the requirements of Appendix 2. The cell seed shall be derived from an early population doubling of the approved diploid cell strain, and the MWCB shall be prepared from it by serial subculture up to an approved population doubling level. Each manufacturer shall show to the satisfaction of the national control authority that the cell substrate propagated from the accepted cell strain and laid down as a MWCB conforms with the requirements of Appendix 2 concerning tests in cell cultures, animals and eggs for freedom from extraneous agents, lack of tumorigenicity, normal karyology at least up to the population doubling level at which the cells are used to propagate the measles virus (production cell cultures) and identity. The cells shall not be used beyond the highest population doubling level shown to be capable of meeting the requirements of Appendix 2.

A.4.1.5 Other cells

In some countries, dog kidney cells are used for production. They should be obtained from four- to six-week-old dogs (usually Beagles) from a specific-pathogen-free colony that is monitored at regular intervals and shown to be free from rabies, distemper, canine hepatitis, listeriosis, leptospirosis, brucellosis, salmonellosis, tuberculosis, toxoplasmosis and mycoplasmas. Animals whose kidneys have been removed should undergo autopsy. Supernatants from their minced kidney and liver tissue should be used to inoculate medium capable of detecting *Mycobacterium tuberculosis* as well as guinea-pigs to be tested after 21 days for anti-leptospira antibodies and after 42 days for tuberculosis. Kidneys derived from animals belonging to one litter may be processed together to prepare primary cell cultures.

A.4.1.6 Serum used in cell-culture medium

Serum used for the propagation of cells for measles vaccine production shall be tested to demonstrate freedom from bacteria, fungi and mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*11*) and to demonstrate freedom from viruses.

Suitable tests for detecting viruses in calf and newborn-calf serum are given in Appendix 1 of the revised Requirements for Biological Substances No. 7 (Requirements for Poliomyelitis Vaccine (Oral)) (*12*).

Serum of bovine origin must come from herds certified to be free of bovine spongiform encephalopathy and bovine leukosis.

Serum shall also be shown to be free from inhibitors of measles virus. Human serum shall not be used. If human albumin is used, it shall meet the revised Requirements for Biological Substances No. 27 (Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives) (*13*).

In some countries sera are also examined for freedom from certain phages.

A.4.1.7 Trypsin used for preparing cell cultures

Trypsin used for preparing cell cultures shall be bacteriologically sterile and free from mycoplasmas and viruses, especially porcine parvoviruses. The methods used to ensure this shall be approved by the national control authority.

A.4.2 ***Virus seed***

The production of vaccine shall be based on the virus seed lot system. Seed lots shall be prepared in cells homologous to those used for production of the final vaccine.

It is recommended that a large working seed lot be set aside for the preparation of batches of vaccine.

Each seed lot shall be identified as measles virus by appropriate serological methods (see section A.6.1).

Virus seed lots shall be stored lyophilized in a dedicated temperature-monitored refrigerator at a temperature lower than −20 °C or, if not lyophilized, at or below −60 °C.

The methods used to produce single harvests from the working seed lot shall be identical to those employed to produce the vaccine used in the clinical trials to establish the safety and efficacy of the virus strain and the suitability of the master seed lot.

Particular attention should be given to the need to maintain consistency with respect to such factors as multiplicity of infection and cell-culture conditions, for example the duration and temperature of incubation.

Some national control authorities require manufacturers to demonstrate consistency of the product for several consecutive (e.g. five) production lots.

A.4.2.1 Tests on virus seed lots

The seed lot used for the production of vaccine shall be free from detectable extraneous agents, and shall be produced in conditions that satisfy the requirements of sections A.4.3 and A.4.4 (with the exception of the tests for added substances and residual animal serum proteins in section A.4.4.5).

Tests for neurovirulence. Each master seed or working seed lot shall be shown to be free from neurovirulence by tests in measles-susceptible monkeys of a species approved by the national control authority. To avoid the unnecessary use of monkeys, virus seed lots should be prepared in large quantities.

Neurovirulence tests can be conducted as follows:

At least 10 monkeys should be employed in each test. Immediately before inoculation, all monkeys should be shown to be serologically negative for measles. The material under test should be given by injection of 0.5 ml into the thalamic region of each hemisphere. The total amount of measles virus given to each monkey should be not less than the amount contained in the

recommended single human dose of vaccine. The monkeys should be observed for 17–21 days for symptoms of paralysis and other evidence of neurological involvement. Animals that die within 48 hours of injection may be replaced. The test is invalid and should be repeated if more than 20% of the monkeys die, even from non-specific causes. At the end of the observation period each monkey is bled and its serum tested for anti-measles antibody. All test animals are anaesthetized and killed for autopsy; histopathological examinations of appropriate areas of the brain are made for evidence of central nervous system involvement.

As a check against the inadvertent introduction of wild measles virus, at least four measles-susceptible uninoculated monkeys should be maintained as a control, either as cage mates of, or within the same immediate area as, the inoculated test animals for the entire period of observation (17–21 days) plus an additional 10 days. Serum samples should be taken from the control monkeys at the time of inoculation of the test animals and again 10 days after the test animals are killed.

The virus seed lot passes the test if: (a) at least 80% of the inoculated monkeys are serologically positive for measles and all the serum samples from the control monkeys are shown to be free from anti-measles antibody; and (b) there is no clinical or histopathological evidence of involvement of the central nervous system attributable to the injected virus.

In some countries the seed lot is not tested, but vaccines are accepted provided that each of the first five undiluted clarified virus pools prepared from the same seed lot satisfies the requirements of the test for neurovirulence.

A 4.3 *Control cell cultures*

From the cells used to prepare the cell cultures for growing attenuated measles virus, an amount of processed cell suspension at least equivalent to 5% of the total volume or 500 ml, whichever is the greater, shall be used to prepare control cultures of uninfected cells. These control cultures shall be observed microscopically for changes atrributable to the presence of adventitious agents for at least 14 days after the day of inoculation of the production cultures or until the time of the last virus harvest, whichever is the later. At the end of the observation period, fluids collected from the control cultures shall be pooled and tested for the presence of adventitious agents as described below. Samples that are not tested immediately shall be stored at or below -60 °C.

In some countries, samples of fluid from each control vessel are collected at the same time as fluid is harvested from the corresponding production vessels. If several virus harvests are made from the same production cell batch, the control fluid taken at the time of each harvest is frozen and stored at or below -60 °C until the last virus harvest from that production cell batch is completed. The control fluids are then pooled in proportion to their amounts and submitted to the required tests.

If any tests show evidence of the presence of adventitious agents in control cultures, the corresponding virus harvest shall not be used for vaccine production.

For a test to be valid, no more than 20% of the culture vessels shall have been discarded for non-specific reasons by the end of the test period.

A.4.3.1 Test for haemadsorbing viruses

At the end of the observation period, 25% of the control cell cultures shall be tested for the presence of haemadsorbing viruses, using guinea-pig red cells. If the red cells have been stored, the duration of storage shall not have exceeded seven days, and the temperature of storage shall have been in the range of 2–8 °C.

In some countries, the national control authority requires that tests for haemadsorbing viruses should also be made on control cultures 3–5 days and 12 days after inoculation of the production cultures, and that other types of red cells, including cells from humans (blood group IV O), monkeys and chickens (or other avian species), should be used in addition to guinea-pig cells. In all tests readings should be taken after incubation for 30 minutes at 0–4 °C, and again after a further incubation for 30 minutes at 20–25 °C. For the test with monkey red cells readings should also be taken after a final incubation for 30 minutes at 34–37 °C.

A.4.3.2 Tests for non-haemadsorbing extraneous agents

Ten millilitres of the pooled cell-culture fluid collected at the end of the observation period shall be tested in the same cell substrate, but not the same batch, as that used for virus growth. Additional 10-ml samples of each pool shall be tested in both human and simian cells.

Cell monolayers shall be inoculated in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm^2 per ml of pooled fluid. At least one bottle of the cell cultures shall remain uninoculated as a control.

The inoculated cultures shall be incubated at a temperature of 35–37 °C and shall be examined for abnormal morphology for a period of at least 14 days.

Some national control authorities require that, at the end of this observation period, a subculture is made in the same culture system and observed for at least seven days. Furthermore, some national control authorities require that these cells should be tested for the presence of haemadsorbing viruses.

A.4.3.3 Additional tests if chick cell cultures are used for production

If chicken cell cultures are used, a sample of fluids pooled from the control cultures shall be tested for adenoviruses and for avian retroviruses such as avian leukosis virus, by a method approved by the national control authority.

Satisfactory procedures for testing for avian leukosis virus include tests for detecting the resistance-inducing factor (RIF), complement-fixation tests (CF) and enzyme-linked immunosorbent assays (ELISA).

The control cultures pass the test if there is no evidence of the presence of virus.

A certificate of freedom from avian leukosis virus and adenovirus provided by the supplier of the fertile eggs may satisfy the licensing authority.

A.4.3.4 Additional tests if human diploid cells are used for production

If human diploid cells are used for production, the cell cultures shall be identified as human by tests approved by the national control authority.

Suitable tests are isozyme analysis, HLA and other immunological tests, and karyotyping of at least one metaphase spread of chromosomes.

A.4.4 Production and harvest of vaccine virus

A.4.4.1 Cells used for vaccine production

On the day of inoculation with the seed lot virus, each production cell culture and control cell culture shall be examined for degeneration caused by infective agents. If such examination shows evidence of the presence in a cell culture of any adventitious agent, the whole group of cultures concerned shall not be used for vaccine production.

After virus inoculation, cultures for vaccine production shall be incubated under controlled temperature conditions approved by the national control authority.

If animal serum is used in the growth medium for the cell cultures, the serum shall be removed from the cell cultures either before or after inoculation with the seed virus. Before the virus is harvested, the cell cultures shall be rinsed and the growth medium replaced with serum-free maintenance medium.

Penicillin or other β-lactam antibiotics shall not be used at any stage of manufacture.

Minimal concentrations of other suitable antibiotics may be used if approved by the national control authority.

A.4.4.2 Single harvests

Virus fluids shall be harvested by a method approved by the national control authority. A single harvest may be a combination of several consecutive harvests from one production cell culture. Single harvests are usually partly or fully stabilized and stored at or below -60 °C until pooling. No antibiotics shall be added at the time of harvesting nor at any later stage of manufacturing. Samples of single harvests shall be taken for testing for sterility and virus content; if not tested immediately, they shall be kept at or below -60 °C until testing is done.

Sterility tests. A volume of at least 20 ml of each single harvest shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No.6 (General Requirements for the Sterility of Biological Substances) (*11*), or by a method approved by the national control authority.

In some countries, the sample of pooled fluid is ultracentrifuged and both the pellet and its supernatant fluid are tested for sterility.

Tests for mycoplasmas should be done in both solid and liquid media that have been shown to be capable of supporting the growth of sterol-requiring and non-sterol-requiring mycoplasmas. At least 10 ml of single harvests should be used for each group of tests. Certain nutritionally fastidious mycoplasmas are best detected by DNA fluorescent staining on the surface of cultured indicator cells. Approved non-culture methods including DNA probes may also be used.

Virus titration. The live virus content of each single harvest shall be determined by cell culture titration, using a reference preparation of live measles virus, the titre of which has been determined by comparison with the International Reference Reagent (see section A.1.3). Minimum acceptable titres should be established.

A.4.4.3 Virus pool

The virus pool shall be prepared from one or several single harvests and shall be submitted to the following tests, unless these tests have already been done on each single harvest; even in that event, the virus pool shall be tested for sterility. Samples that are not tested immediately shall be stored at or below -60 °C.

In tests that require prior neutralization of measles virus, the antiserum used shall not be of human, simian or avian origin. The immunizing antigen used to prepare the antiserum shall be produced in cell cultures from a species different from that used for vaccine production. These cell cultures shall be free from extraneous microbial agents that might elicit antibodies inhibitory to the growth of any extraneous agents that may be present in the measles virus pool. They shall be tested for extraneous agents as specified in paragraphs 2 and 3 of section A.4.3.2.

Sterility tests. Sterility tests shall be performed as indicated in section A.4.4.2.

Virus titration. The live virus content of each virus pool shall be determined by titration in cell culture against a reference preparation of live measles virus (see section A.1.3).

Tests of neutralized virus pool in cell cultures. A volume of each virus pool equivalent to at least 500 human doses or 50 ml, whichever represents the greater volume, shall be neutralized by specific antiserum and shall be tested for adventitious agents by inoculation of simian cell cultures. Similar volumes of the neutralized virus pool shall be tested likewise in human cell cultures and in cell cultures of the same type, but not of the same batch, as those used to prepare the virus pool. Uninoculated cell cultures shall be kept as a control. All cell cultures shall be observed for at least 14 days.

Some national control authorities require that, at the end of this observation period, a subculture is made in the same culture system.

The virus pool passes the test if there is no evidence of the presence of an adventitious agent, and no more than 20% of the culture vessels have been discarded for non-specific reasons by the end of the test period.

Additional tests if chick cell cultures are used for production. A volume of each virus pool at least equivalent to 100 human doses of vaccine or 10 ml, whichever represents the greater volume, shall be tested in a group of embryos of fertilized chickens' eggs by the allantoic route of inoculation, and a similar sample shall be tested in a separate group of eggs by the yolk-sac route of inoculation. In both cases 0.5 ml of inoculum shall be used per egg.

The virus pool passes the test if, at the end of a three- to seven-day observation period, there is no evidence of the presence of any adventitious agents. If an adventitious agent is detected in the uninoculated controls, the test may be repeated.

A.4.4.4 Clarification of the virus pool

The virus pool shall be clarified by a method that will maximize removal of cells and cell debris.

> The clarified virus pool may be stabilized and stored at or below −60 °C before being used to prepare final bulk for freeze-drying.

Samples of the clarified bulk suspension shall be taken immediately after clarification to ensure that no microscopically observable cells or cell particles remain. Samples shall also be taken from the pool for virus titration and sterility testing. If not tested immediately, the samples shall be kept at or below -60 °C until testing is done.

Virus titration. The live virus content of the clarified virus pool shall be determined by titration in cell culture against a reference preparation of live measles virus (see section A.1.3).

Sterility tests. Sterility tests shall be performed as indicated in section A.4.4.2.

A.4.4.5 Final bulk

The final bulk shall be prepared from one or more clarified virus pools obtained from substrates of which control cultures pass the tests specified in section A.4.3. The virus pools shall pass the tests specified in sections A.4.4.3 and A.4.4.4.

The operations necessary for preparing the final bulk shall be conducted in such a manner as to avoid contamination of the product.

> The final bulk may be assayed for virus content.

Added substances. Any substance such as diluent or stabilizer that is added to the product during preparation of the final bulk shall have been shown to the satisfaction of the national control authority not to impair the safety and efficacy of the vaccine in the concentration used.

Residual animal serum proteins. If serum has been used in the cell-culture system, a sample of the final bulk shall be tested to verify that the residual amount of serum albumin is less than 50 ng per single human dose.

> Alternatively the test may be performed on the clarified pools.

Sterility tests. Each final bulk shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No.6 (General Requirements for the Sterility of Biological Substances) (*11*), or by a method approved by the national control authority.

Storage. Until it is distributed into containers and lyophilized, the final bulk shall be stored in conditions shown by the manufacturer to retain the activity of the vaccine.

A.5 Filling and containers

The requirements concerning filling and containers given in Good Manufacturing Practices for Biological Products (*10*) shall apply.

Care shall be taken to ensure that the material of which the container and, if applicable, the closure are made does not adversely affect the virus content of the vaccine under the recommended conditions of storage.

> Single-dose containers are recommended, except for mass immunization campaigns.

A.6 Control tests on final product

Samples shall be taken from each final lot for the following tests.

A.6.1 *Identity tests*

The virus content of two or more individually labelled final containers, taken from the freeze-dried lot, shall be identified as measles virus by appropriate methods.

> Methods such as seroneutralization in cell culture with specific antiserum are suitable.

A.6.2 *Sterility tests*

Reconstituted vaccine shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (Requirements for the Sterility of Biological Substances) (*11*), or by acceptable methods approved by the national control authority.

A.6.3 *Virus concentration and thermostability*

The virus content in each of at least three containers selected at random from the freeze-dried lot shall be determined individually against a reference preparation of measles vaccine (see section A.1.3).

An additional three containers from the final freeze-dried vaccine lot shall be incubated at 37 °C for seven days. The geometric mean infectious virus titre of these vials shall be equal to or greater than the required minimum number of infective units per human dose, and the geometric mean virus titre of the vaccine shall not have decreased by more than 1.0 $\log_{10}$ infectious units during the period of incubation. Titration of non-exposed and exposed vials shall be made in parallel and results expressed in terms of PFU or $CCID_{50}$ per human dose. A reference reagent for measles virus, the titre of which has been determined by comparison with the International Reference Reagent (see section A.1.3), shall be included in each assay.

The detailed procedures for carrying out this test and for interpreting the results should be approved by the national control authority, which should also specify the acceptable confidence limits.

A suitable test for virus content is described in Appendix 3.

The national control authority shall determine the minimum and maximum amount of vaccine virus that one human dose should contain.

The minimum quantity is generally considered to be 1000 viral infective units. Epidemiological studies have linked the use of high-titre vaccine with adverse events. In at least one country, however, the minimum dose has been set at 5000 infective units.

A.6.4 *General safety tests*

Each final lot shall be tested for the absence of abnormal toxicity in mice and guinea-pigs by appropriate tests approved by the national control authority.

A.6.5 *Residual moisture*

The residual moisture in a representative sample of the freeze-dried lot shall be determined by a method approved by the national control authority. The upper limit of the moisture content shall be specified by the national control authority.

Generally, moisture levels of less than 2% are considered satisfactory.

A.6.6 *Inspection of final containers*

Each container shall be inspected visually and those showing abnormalities shall be discarded.

A.7 Records

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 27-28) shall apply.

A.8 Samples

The requirements of Good Manufacturing Practices for Biological Products (*10*, page 29, paragraph 9.5) shall apply.

A.9 Labelling

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 26-27) shall apply, with the addition of the following:

The label on the carton or the leaflet accompanying the container shall include:

- a statement that the vaccine fulfils Part A of these Requirements;
- a statement of the nature of the preparation, specifying the strain of measles virus in the vaccine, the minimum number of infective units per human dose, and the origin of the substrate used to prepare the vaccine;
- a statement of the nature and quantity of any antibiotic present in the vaccine;
- a statement concerning the photosensitivity of the vaccine, cautioning that both lyophilized and reconstituted vaccine should be protected from light;
- a statement indicating the volume and nature of diluent[1] to be added to reconstitute the vaccine, and specifying that the diluent should be supplied by the manufacturer;
- a statement that, after the vaccine is reconstituted, it should be used immediately or be stored between 0 °C and 8 °C in the dark for a period not exceeding eight hours.

A.10 Distribution and shipping

The requirements of Good Manufacturing Practices for Biological Products (*10*) shall apply.

Shipments should be at temperatures of 8°C or below and parcels should contain cold-chain monitors.

A.11 Storage and expiry date

The statements concerning storage temperature and expiry date appearing on the label and the leaflet, as required by Good Manufacturing Practices for Biological Products (*10*, pages 26-27), shall be based on experimental evidence and shall be submitted for approval to the national control authority.

A.11.1 *Storage conditions*

Before distribution, the manufacturer shall store lyophilized vaccines at a temperature shown by the manufacturer to be compatible with minimal

[1] No preservative or any substance that has a deleterious effect on the virus should be present in the diluent used to reconstitute the vaccine.

loss of virus titre. After distribution, live measles vaccine shall be stored at all times at a temperature below 8 °C. Storage below 0 °C is permitted.

A.11.2 ***Validity period***

The validity period is a defined period of time at prescribed conditions of storage. It shall be based upon the stability of the vaccine, as determined experimentally, and shall be approved by the national control authority.

Part B. National control requirements

B.1 General

The general requirements for control laboratories contained in the Guidelines for National Authorities on Quality Assurance for Biological Products (*14*) shall apply.

The national control authority shall give directions to manufacturers concerning the measles virus strains to be used in vaccine production and concerning the recommended human dose.

> The national control authority should take into consideration available information on strains before deciding on those permitted for vaccine production.

In addition, the national control authority shall provide, for virus titration (see sections A.4.4.2, A.4.4.3, A.4.4.4 and A.6.3), a reference preparation of live measles virus calibrated against the International Reference Reagent (see section A.1.3) and shall specify the virus content required to achieve adequate immunization of humans with the recommended human dose.

Protocols reporting the results of tests (Appendix 1) should be available for examination by the national control authority.

B.2 Release and certification

A vaccine lot shall be released only if it fulfils the national requirements and/or part A of the present Requirements. A protocol based on the model given in Appendix 1, signed by the responsible official of the manufacturing establishment, shall be prepared and submitted to the national control authority in support of a request for release of vaccine for use.

At the request of the manufacturing establishment, the national control authority shall provide a certificate that states whether the vaccine meets all national requirements and/or Part A of the present Requirements. The certificate shall be based on the model given in Appendix 4.

> The purpose of the certificate is to facilitate the exchange of live measles virus vaccines among countries.

II. Requirements for mumps vaccine

General considerations

Mumps vaccines have been shown to induce long-term protective immunity. In the United States of America, immunity induced by vaccines based on the Jeryl Lynn strain has apparently persisted for at least 20 years, while there is evidence from Japan that immunity of at least 10 years' duration is produced by vaccines containing the Urabe strain. In the United States of America widespread use of mumps vaccine since 1967 has dramatically reduced the reported incidence of mumps.

Several strains of attenuated mumps virus have been developed for use in vaccines. The Jeryl Lynn strain, which is grown in chick-embryo cell cultures, was licensed in the United States of America in 1967 and by 1992 had been given to approximately 135 million children and adults throughout the world. It induces seroconversion in at least 97% of children and at least 93% of adults, whether used singly or in combination with measles and rubella vaccines.

Vaccines based on the Leningrad-3 strain of attenuated mumps virus have been in use since 1974 in the former USSR, where they are produced in cell cultures of Japanese quail embryo, and have subsequently been introduced in other countries. The L-Zagreb vaccine produced in chick embryo cells has been used in the former area of Yugoslavia since 1976. About 8–11 million doses of vaccine based on the Leningrad-3 strain are produced annually as monovalent vaccine or in combination with measles and rubella vaccines.

The Urabe strain of attenuated live mumps vaccine was first licensed for production in 1979 in Japan and thereafter in Belgium, France and Italy. It is produced either in the amnion of embryonated hens' eggs or in chick-embryo cell cultures. By 1991, more than 60 million people had been immunized with the Urabe strain in Japan and other countries. Its immunogenic properties are similar to those of the Jeryl Lynn strain used as a monovalent product or in combination with measles and rubella vaccines.

The Rubini strain is the only mumps vaccine strain grown in human diploid cells. It was approved in Switzerland in 1985 and by 1990 more than four million people had been immunized with it throughout the world. Since the introduction of Rubini mumps vaccine in Switzerland, mumps cases have shown a downward trend.

Three additional strains of attenuated mumps vaccine have been approved for production in Japan: the Hoshino and Torii Miyahara strains, which are grown in chick-embryo cell culture, and the NK-M46 strain. These strains have been less extensively used than the Jeryl Lynn, Urabe, Leningrad-3, L-Zagreb and Rubini strains. Recent advances in molecular biology have permitted the characterization of different strains.

Lymphocytic meningitis has been associated with the administration of certain mumps vaccine strains (in particular the Urabe strain), as occasionally confirmed by sequencing of the genes of viral isolates from the cerebrospinal fluid. It is important to note that the prevalence is low and demonstrating the association of vaccination with meningitis is highly dependent on the numbers of subjects vaccinated, medical practices with regard to frequency of lumbar puncture, and the quality of follow-up observations. Lymphocytic meningitis after vaccination has often been difficult to detect and is benign in outcome. Active surveillance is necessary to establish the precise incidence of adverse reactions.

For the standardization of the infectious virus content of mumps vaccines, there is a need for an international reference preparation against which the titre can be assessed. A reference preparation of antibody to mumps virus is also needed.

Each of the following sections constitutes a recommendation. The parts of each section printed in normal type have been written in the form of requirements so that, if a health administration so desires, they may be adopted as they stand as definitive national requirements. The parts of each section printed in small type are comments or recommendations for guidance. To facilitate the international distribution of vaccine made in accordance with these Requirements, a summary protocol for recording the results of tests is included as Appendix 5.

Should individual countries wish to adopt these Requirements as the basis of their national regulations for mumps vaccine, it is recommended that modifications be made only on condition that the modified requirements ensure at least an equal degree of safety and potency of the vaccine. The World Health Organization should then be informed of the action taken.

Part A. Manufacturing requirements

A.1 Definitions

A.1.1 *International name and proper name*

The international name shall be "Vaccinum parotitidis vivum". The proper name in the country's language shall be the equivalent of the international name.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

A.1.2 *Descriptive definition*

"Vaccinum parotitidis vivum" is a preparation of live attenuated mumps virus grown in avian embryo cells or other suitable cells. The preparation shall satisfy all the requirements formulated below.

At present, live mumps vaccines are blended with an appropriate stabilizer and lyophilized. They are available as monovalent vaccines or in combination with live measles and live rubella vaccines.

A.1.3 *International reference materials*

Since no international reference materials have been established for live mumps vaccine, national control authorities should provide a reference preparation of live mumps virus for validating tests to determine virus content (see sections A.4.4.2, A.4.4.3, A.4.4.4 and A.6.3).

A.1.4 *Terminology*

The following definitions are given for the purpose of these Requirements only.

Original vaccine: A vaccine prepared according to the manufacturer's specifications and shown on administration to humans to be safe and immunogenic.

Master seed lot: A quantity of virus derived from, or used to prepare, an original vaccine; the virus suspension has been processed as a single lot to ensure a uniform composition and is fully characterized. The master seed lot is used for the preparation of working seed lots.

Working seed lot: A quantity of virus of uniform composition, fully characterized, derived from a master seed lot. The working seed lot is used for the production of vaccines.

Cell seed: A quantity of fully characterized cells of human, animal or other origin stored frozen at -70 °C or below in aliquots of uniform composition, one or more of which are used for the production of a manufacturer's working cell bank.

Manufacturer's working cell bank (MWCB): A quantity of cells of uniform composition derived from one or more ampoules of the cell seed and stored frozen at -70 °C or below in aliquots, one or more of which are used for production purposes.

In normal practice a cell seed is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer and approved by the national control authority. The cells are combined in a single pool, distributed into ampoules and preserved cryogenically to form the MWCB.

Production cell culture: A number of cell cultures derived from the same pool of cells and processed together.

Single harvest: A quantity of virus suspension derived from a batch of production cell cultures that were inoculated with the same working seed lot and processed together in a single production run.

Virus pool: A homogeneous pool of single harvests collected into a single vessel before clarification.

Final bulk: The homogeneous finished virus suspension prepared from one or more clarified virus pools in the vessel from which the final containers are filled.

Filling lot (final lot): A collection of sealed final containers of finished vaccine that are homogeneous with respect to the risk of contamination during filling and freeze-drying. All the final containers must, therefore, have been filled from a single vessel of final bulk in one working session and lyophilized under standardized conditions in a common chamber.

Cell culture infective dose 50% ($CCID_{50}$): The quantity of a virus suspension that is estimated to infect 50% of cell cultures.

Plaque-forming unit (PFU): The smallest quantity of a virus suspension that will produce a plaque in monolayer cell cultures.

A.2 Certification of the strain of virus for use in vaccine production

The strain of mumps virus used in the production of mumps vaccine shall be identified by historical records that include information on the origin of the strain, its method of attenuation and the passage level at which attenuation was demonstrated by clinical evaluation.

The strain of mumps virus used in the production of vaccine shall have been shown to be safe by appropriate laboratory tests (see section A.4 of these Requirements) and safe and immunogenic by tests in susceptible humans. Only strains that are certified by the national control authority shall be used.

The lowest immunizing dose of vaccine that induces seroconversion in susceptible individuals shall be established in a dose-response study. This dose shall serve as a basis for establishing parameters for stability and expiry of the vaccine.

A.3 General manufacturing requirements

The requirements of Good Manufacturing Practices for Pharmaceutical (*9*) and Biological (*10*) Products and the Requirements for Human Diploid Cells Used for the Production of Measles, Mumps and Rubella Vaccines (Live) (Appendix 2) shall apply to establishments manufacturing live mumps vaccine, with the addition of the following requirements.

Production areas shall be decontaminated before they are used for the manufacture of mumps vaccine.

Mumps vaccine shall be produced by staff who have not handled other infectious microorganisms or animals on the same working day. The staff shall consist of persons who are periodically examined medically and found to be healthy. Steps shall be taken to ensure that all personnel involved in the production areas are immune to mumps. Production and control shall be organized as two separate units of the manufacturing establishment with independent responsibilities.

Only the virus seed lot and substrates approved by the national control authority for the production of mumps vaccine shall be introduced or handled in the production area.

Persons not directly concerned with the production processes, other than official inspectors, shall not be permitted to enter the production area without valid reason and specific authorization.

Particular attention shall be given to the recommendations in Good Manufacturing Practices for Biological Products (*10*) regarding the training and experience of the persons in charge of production and testing, and of those assigned to various areas of responsibility in the manufacturing establishment, as well as to the registration of such personnel with the national control authority.

A.4 Production control

The general production precautions formulated in Good Manufacturing Practices for Biological Products (*10*) shall apply to the manufacture of mumps vaccine.

A.4.1 *Source materials*

A.4.1.1 Strain of mumps virus

The strain of mumps virus used in the production of live mumps vaccine shall be certified according to the specifications of section A.2. The vaccine strain shall be approved by the national control authority. The virus shall at no time have been passaged in a continuous cell line. The seed lot or five consistent lots of vaccine derived from the seed lot shall have been shown to be non-neuropathogenic in monkeys (see section A.4.2.1) and to yield live mumps vaccine of adequate immunogenicity and safety in human beings.

A.4.1.2 Cell cultures

Mumps virus used in the production of mumps vaccine shall be propagated in cell cultures approved by the national control authority. All information on the source and method of preparation of the cell-culture system used shall be available to the national control authority.

> In some countries biochemical tests for the detection of RNA viruses are applied to production cell cultures.

A.4.1.3 Avian embryos and cell cultures

If avian embryos or cell cultures are used for the propagation of mumps vaccine virus, the eggs used shall be derived from a closed, specific-pathogen-free, healthy flock. This flock shall be monitored at regular intervals for *Mycobacterium avium*, fowlpox, avian retroviruses, Newcastle disease virus and other avian parainfluenza viruses, avian encephalomyelitis virus, infectious laryngotracheitis virus, avian reticu-

loendoti sis virus, Marek's disease virus, infectious bursal disease virus, avian reovirus, avian adenoviruses, avian influenza virus, *Haemophilus paragallinarum*, *Salmonella gallinarum*, *Salmonella pullorum*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and other agents pathogenic for birds.

In some countries, all birds are bled when a colony is established, and thereafter 5% of the birds are bled each month. The serum samples are screened for antibodies to relevant pathogens. Any bird that dies is investigated to determine the cause of death.

A.4.1.4 Human diploid cells

If human diploid cells are used for the propagation of mumps virus, a MWCB shall be established in conformity with the requirements of Appendix 2. The cell seed shall be derived from an early population doubling of the approved diploid cell strain, and the MWCB shall be prepared from it by serial subculture up to an approved population doubling level. Each manufacturer shall show to the satisfaction of the national control authority that the cell substrate propagated from the accepted cell strain and laid down as a MWCB conforms with the requirements of Appendix 2 concerning tests in cell cultures, animals and eggs for freedom from extraneous agents, lack of tumorigenicity, normal karyology at least up to the population doubling level at which the cells are used to propagate the mumps virus (production cell cultures) and identity. The cells shall not be used beyond the highest population doubling level shown to be capable of meeting the requirements of Appendix 2.

A.4.1.5 Serum used in cell-culture medium

Serum used for the propagation of cells for mumps vaccine production shall be tested to demonstrate freedom from bacteria, fungi and mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*11*) and to demonstrate freedom from viruses.

Suitable tests for detecting viruses in calf and newborn-calf serum are given in Appendix 1 of the revised Requirements for Biological Substances No. 7 (Requirements for Poliomyelitis Vaccine (Oral)) (*12*).

Serum of bovine origin must come from herds certified to be free of bovine spongiform encephalopathy and bovine leukosis.

Serum shall also be shown to be free from inhibitors of mumps virus. Human serum shall not be used. If human albumin is used, it shall meet the revised Requirements for Biological Substances No. 27 (Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives) (*13*).

In some countries sera are also examined for freedom from certain phages.

A.4.1.6 Trypsin used for preparing cell cultures

Trypsin used for preparing cell cultures shall be bacteriologically sterile and free from mycoplasmas and viruses, especially porcine parvoviruses. The methods used to ensure this shall be approved by the national control authority.

A.4.2 ***Virus seed***

The production of vaccine shall be based on the virus seed lot system. Seed lots shall be prepared in cells homologous to those used for production of the final vaccine.

It is recommended that a large working seed lot be set aside for the preparation of batches of vaccine.

Each seed lot shall be identified as mumps virus by appropriate serological methods (see section A.6.1).

Virus seed lots shall be stored lyophilized in a dedicated temperature-monitored refrigerator at a temperature lower than -20 °C or, if not lyophilized, at or below -60 °C.

The methods used to produce single harvests from the working seed lot shall be identical to those employed to produce the vaccine used in the clinical trials to establish the safety and efficacy of the virus strain and the suitability of the master seed lot.

Particular attention should be given to the need to maintain consistency with respect to such factors as multiplicity of infection and cell-culture conditions, for example the duration and temperature of incubation.

Some national control authorities require manufacturers to demonstrate consistency of the product for several consecutive (e.g. five) production lots.

A.4.2.1 Tests on virus seed lots

The seed lot used for the production of vaccine shall be free from detectable extraneous agents, and shall be produced in conditions that satisfy the requirements of sections A.4.3. and A.4.4 (with the exception of the tests for added substances and residual animal serum proteins in section A.4.4.5).

Test for neurovirulence. Each master seed or working seed lot shall be shown to be free from neurovirulence by tests in mumps-susceptible monkeys of a species approved by the national control authority. To avoid the unnecessary use of monkeys, virus seed lots should be prepared in large quantities.

Neurovirulence tests can be conducted as follows:

At least 10 monkeys should be employed in each test. Immediately before inoculation, all monkeys should be shown to be serologically negative for mumps. The material under test should be given by injection of 0.5 ml into the thalamic region of each hemisphere. The total amount of mumps virus given to each monkey should be not less than the amount contained in the

recommended single human dose of vaccine. The monkeys should be observed for 17–21 days for symptoms of paralysis and other evidence of neurological involvement. Animals that die within 48 hours of injection may be replaced. The test is invalid and should be repeated if more than 20% of the monkeys die, even from non-specific causes. At the end of the observation period each monkey is bled and its serum tested for anti-mumps antibody. All test animals are anaesthetized and killed for autopsy; histopathological examinations of appropriate areas of the brain are made for evidence of central nervous system involvement.

The virus seed lot passes the test if: (a) at least 80% of the inoculated monkeys are serologically positive for mumps; and (b) there is no clinical or histopathological evidence of involvement of the central nervous system attributable to the injected virus.

In some countries the seed lot is not tested, but vaccines are accepted provided that each of the first five undiluted clarified virus pools prepared from the same seed lot satisfies the requirements of the test for neurovirulence.

A.4.3 *Control cell cultures*

From the cells used to prepare the cell cultures for growing attenuated mumps virus, an amount of processed cell suspension at least equivalent to 5% of the total volume or 500 ml, whichever is the greater, shall be used to prepare control cultures of uninfected cells. These control cultures shall be observed microscopically for changes attributable to the presence of adventitious agents for at least 14 days after the day of inoculation of the production cultures or until the time of the last virus harvest, whichever is the later. At the end of the observation period, fluids collected from the control cultures shall be pooled and tested for the presence of adventitious agents as described below. Samples that are not tested immediately shall be stored at or below -60 °C.

In some countries, samples of fluid from each control vessel are collected at the same time as fluid is harvested from the corresponding production vessels. If several virus harvests are made from the same production cell batch, the control fluid taken at the time of each harvest is frozen and stored at or below -60 °C until the last virus harvest from that production cell batch is completed. The control fluids are then pooled in proportion to their amounts and submitted to the required tests.

If any tests show evidence of the presence of any adventitious agents in control cultures, the corresponding virus harvest shall not be used for vaccine production.

For a test to be valid, no more than 20% of the culture vessels shall have been discarded for non-specific reasons by the end of the test period.

A.4.3.1 Test for haemadsorbing viruses

At the end of the observation period, 25% of the control cell cultures shall be tested for the presence of haemadsorbing viruses, using guinea-pig red cells. If the red cells have been stored, the duration of storage shall not have exceeded seven days, and the temperature of storage shall have been in the range of 2–8 °C.

In some countries, the national control authority requires that tests for haemadsorbing viruses should also be made on control cultures 3–5 days and 12 days after inoculation of the production cultures, and that other types of red cells, including cells from humans (blood group IV O), monkeys and chickens (or other avian species), should be used in addition to guinea-pig cells. In all tests readings should be taken after incubation for 30 minutes at 0–4 °C, and again after a further incubation for 30 minutes at 20–25 °C. For the test with monkey red cells readings should also be taken after a final incubation for 30 minutes at 34–37 °C.

A.4.3.2 Tests for non-haemadsorbing extraneous agents

Ten millilitres of the pooled cell-culture fluid collected at the end of the observation period shall be tested in the same cell substrate, but not the same batch, as that used for virus growth. Additional 10-ml samples of each pool shall be tested in both human and simian cells.

Cell monolayers shall be inoculated in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm^2 per ml of pooled fluid. At least one bottle of the cell cultures shall remain uninoculated as a control.

The inoculated cultures shall be incubated at a temperature of 35–37 °C and shall be examined for abnormal morphology for a period of at least 14 days.

Some national control authorities require that, at the end of this observation period, a subculture is made in the same culture system and observed for at least seven days. Furthermore, some national control authorities require that these cells should be tested for the presence of haemadsorbing viruses.

A.4.3.3 Additional test if avian-embryo cell cultures are used for production

If avian-embryo cell cultures are used, a sample of fluids pooled from the control cultures shall be tested for adenoviruses and for avian retroviruses such as avian leukosis virus, by a method approved by the national control authority.

Satisfactory procedures for testing for avian leukosis virus include tests for detecting the resistance-inducing factor (RIF), complement-fixation tests (CF) and enzyme-linked immunosorbent assays (ELISA).

The control cultures pass the test if there is no evidence of the presence of virus.

A certificate of freedom from avian leukosis virus and adenovirus provided by the supplier of the fertile eggs may satisfy the licensing authority.

A.4.3.4 Additional tests if human diploid cells are used for production

If human diploid cells are used for production, the cell cultures shall be identified as human by tests approved by the national control authority.

Suitable tests are isozyme analysis, HLA and other immunological tests, and karyotyping of at least one metaphase spread of chromosomes.

A.4.3.5 Embryonated eggs

Of each batch of eggs used for vaccine production, 2% (or 20 eggs, whichever is the larger quantity) shall be held as uninoculated controls and incubated for the same time and at the same temperature as the inoculated eggs. At the time of virus harvest, a sample of 0.25 ml of amniotic fluid taken from each control egg shall be tested for haemagglutinating agents by the addition of chick erythrocytes, both directly and after one passage through specific-pathogen-free eggs. The details of the test shall be approved by the national control authority. In addition, a pool of amniotic fluid shall be tested for adventitious agents, including avian leukosis virus, by the methods specified in section A.4.3.3.

A.4.4 ***Production and harvest of vaccine virus***

A.4.4.1 Cells used for vaccine production

On the day of inoculation with the seed lot virus, each production cell culture and control cell culture shall be examined for degeneration caused by infective agents. If such examination shows evidence of the presence in a cell culture of any adventitious agent, the whole group of cultures concerned shall not be used for vaccine production.

After virus inoculation, cultures for vaccine production shall be incubated under controlled temperature conditions approved by the national control authority.

If animal serum is used in the growth medium for the cell cultures, the serum shall be removed from the cell cultures either before or after inoculation with seed virus. Before the virus is harvested, the cell cultures shall be rinsed and the growth medium replaced with serum-free maintenance medium.

Penicillin or other β-lactam antibiotics shall not be used at any stage of manufacture.

Minimal concentrations of other suitable antibiotics may be used if approved by the national control authority.

A.4.4.2 Single harvests

Virus fluids shall be harvested by a method approved by the national control authority. A single harvest may be a combination of several consecutive harvests from one production cell culture. Single harvests are usually partly or fully stabilized and stored at or below -60 °C until pooling. No antibiotics shall be added at the time of harvesting nor at any later stage of manufacturing. Samples of single harvests shall be taken for testing for sterility and virus content; if not tested immediately, they shall be kept at or below -60 °C until testing is done.

Sterility tests. A volume of at least 20 ml of each single harvest shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological

Substances No.6, (General Requirements for the Sterility of Biological Substances) (*11*), or by a method approved by the national control authority.

In some countries, the sample of pooled fluid is ultracentrifuged and both the pellet and its supernatant fluid are tested for sterility.

Tests for mycoplasmas should be done in both solid and liquid media that have been shown to be capable of supporting the growth of sterol-requiring and non-sterol-requiring mycoplasmas. At least 10 ml of single harvests should be used for each group of tests. Certain nutritionally fastidious mycoplasmas are best detected by DNA fluorescent staining on the surface of cultured indicator cells. Approved non-culture methods including DNA probes may also be used.

Virus titration. The live virus content of each single harvest may be determined by cell culture titration, using a reference preparation of live mumps virus, the titre of which has been determined by comparison with a reference preparation approved by the national control authority (see section A.1.3). Minimum acceptable titres should be established.

A.4.4.3 Virus pool

The virus pool shall be prepared from one or several single harvests and shall be submitted to the following tests, unless these tests have already been done on each single harvest; even in that event, the virus pool shall be tested for sterility. Samples that are not tested immediately shall be stored at or below -60 °C.

In tests that require prior neutralization of mumps virus, the antiserum used shall not be of human, simian or avian origin. The immunizing antigen used to prepare the antiserum shall be produced in cell cultures from a species different from that used for vaccine production. These cell cultures shall be free from extraneous microbial agents that might elicit antibodies inhibitory to the growth of any extraneous agents that may be present in the mumps virus pool. The tests shall be carried out as specified in paragraphs 2 and 3 of section A.4.3.2.

Sterility tests. Sterility tests shall be performed as indicated in section A.4.4.2.

Virus titration. The live virus content of each virus pool shall be determined by titration in cell culture against a reference preparation of live mumps virus (see section A.1.3).

Tests of neutralized virus pool in cell cultures. A volume of each virus pool equivalent to at least 500 human doses or 50 ml, whichever represents the greater volume, shall be neutralized by specific antiserum and shall be tested for adventitious agents by inoculation of simian cell cultures. Similar volumes of the neutralized virus pool shall be tested likewise in human cell cultures and in cell cultures of the same type, but not of the same batch, as those used to prepare the virus pool. Uninoculated cell cultures shall be kept as a control. All cell cultures shall be observed for at least 14 days.

Some national control authorities require that, at the end of this observation period, a subculture is made in the same culture system.

The virus pool passes the test if there is no evidence of the presence of adventitious agents and no more than 20% of the culture vessels have been discarded for non-specific reasons by the end of the test period.

Additional tests if avian eggs or cell cultures are used for production. A volume of each virus pool at least equivalent to 100 human doses of vaccine or 10 ml, whichever is the greater volume, shall be tested in a group of embryos of fertilized avian eggs by the allantoic route of inoculation, and a similar sample shall be tested in a separate group of eggs by the yolk-sac route of inoculation. In both cases 0.5 ml of inoculum shall be used per egg.

The virus pool passes the test if, at the end of a three- to seven-day observation period, there is no evidence of the presence of any adventitious agents. If an adventitious agent is detected in the uninoculated controls, the test may be repeated.

A.4.4.4 Clarification of the virus pool

The virus pool shall be clarified by a method that will maximize removal of cells and cell debris.

The clarified virus pool may be stabilized and stored at or below −60 °C before being used to prepare final bulk for freeze-drying.

Samples of the clarified bulk suspension shall be taken immediately after clarification to ensure that no microscopically observable cells or cell particles remain. Samples shall also be taken for virus titration and sterility testing. If not tested immediately, the samples shall be kept at or below −60 °C until testing is done.

Virus titration. The live virus content of the clarified virus pool shall be determined by titration in cell culture against a reference preparation of live mumps virus (see section A.1.3).

Sterility tests. Sterility tests shall be performed as indicated in section A.4.4.2.

A.4.4.5 Final bulk

The final bulk shall be prepared from one or more clarified virus pools obtained from substrates of which controls pass the tests specified in section A.4.3. The virus pools shall pass the tests specified in sections A.4.4.3 and A.4.4.4.

The operations necessary for preparing the final bulk shall be conducted in such a manner as to avoid contamination of the product.

The final bulk may be assayed for virus content.

Added substances. Any substance such as diluent or stabilizer that is added to the product during preparation of the final bulk shall have been shown

to the satisfaction of the national control authority not to impair the safety and efficacy of the vaccine in the concentration used.

Residual animal serum proteins. If serum has been used in the cell-culture system, a sample of the final bulk shall be tested to verify that the residual amount of serum albumin is less than 50 ng per single human dose.

Alternatively the test may be performed on the clarified pools.

Sterility tests. Each final bulk shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*11*), or by a method approved by the national control authority.

Storage. Until it is distributed into containers and lyophilized, the final bulk shall be stored in conditions shown by the manufacturer to retain the activity of the vaccine.

A.5 Filling and containers

The requirements for filling and containers given in Good Manufacturing Practices for Biological Products (*10*) shall apply.

Care shall be taken to ensure that the material of which the container and, if applicable, the closure are made does not adversely affect the virus content of the vaccine under the recommended conditions of storage.

Single-dose containers are recommended, except for mass immunization campaigns.

A.6 Control tests on final product

Samples shall be taken from each final lot for the following tests.

A.6.1 *Identity tests*

The virus content of two or more individually labelled final containers, taken from the freeze-dried lot, shall be identified as mumps virus by appropriate methods.

Methods such as seroneutralization in cell culture with specific antiserum are suitable.

A.6.2 *Sterility tests*

Reconstituted vaccine shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (Requirements for the Sterility of Biological Substances) (*11*), or by acceptable methods appoved by the national control authority.

A.6.3 ***Virus concentration and thermostability***

The virus content in each of at least three containers selected at random from the freeze-dried lot shall be determined individually against a reference preparation of mumps vaccine (see section A.1.3).

An additional three containers from the final freeze-dried vaccine lot shall be incubated at 37 °C for seven days. The geometric mean infectious virus titre of these vials shall be equal to or greater than the required minimum number of infective units per human dose, and the geometric mean virus titre of the vaccine shall not have decreased by more than 1.0 $\log_{10}$ infectious units during the period of incubation. Titration of non-exposed and exposed vials shall be made in parallel and results expressed in terms of PFU or $CCID_{50}$ per human dose. A reference preparation of mumps virus (see section A.1.3) shall be included in each assay.

The detailed procedures for carrying out this test and for interpreting the results should be approved by the national control authority, which should also specify the acceptable confidence limits.

A suitable test for virus content is described in Appendix 6.

The national control authority shall determine the minimum amount of vaccine virus that one human dose should contain.

Most countries use at least 10^3 $CCID_{50}$ per dose.

A.6.4 ***General safety tests***

Each final lot shall be tested for the absence of abnormal toxicity in mice and guinea-pigs by appropriate tests approved by the national control authority.

A.6.5 ***Residual moisture***

The residual moisture in a representative sample of the freeze-dried lot shall be determined by a method approved by the national control authority. The upper limit of the moisture content shall be specified by the national control authority.

Generally, moisture levels of less than 2% are considered satisfactory.

A.6.6 ***Inspection of final containers***

Each container shall be inspected visually and those showing abnormalities shall be discarded.

A.7 Records

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 27-28) shall apply.

A.8 Samples

The requirements of Good Manufacturing Practices for Biological Products (*10*, page 29, paragraph 9.5) shall apply.

A.9 Labelling

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 26-27) shall apply, with the addition of the following:

The label on the carton or the leaflet accompanying the container shall include:

- a statement that the vaccine fulfils Part A of these Requirements;
- a statement of the nature of the preparation, specifying the strain of mumps virus in the vaccine, the minimum number of infective units per human dose, and the origin of the substrate used to prepare the vaccine;
- a statement of the nature and quantity of any antibiotic present in the vaccine;
- a statement concerning the photosensitivity of the vaccine, cautioning that both lyophilized and reconstituted vaccine should be protected from light;
- a statement indicating the volume and nature of the diluent[1] to be added to reconstitute the vaccine, and specifying that the diluent should be supplied by the manufacturer;
- the statement that, after the vaccine is reconstituted, it should be used immediately or be stored between 0 °C and 8 °C in the dark for a period not exceeding eight hours.

A.10 Distribution and shipping

The requirements of Good Manufacturing Practices for Biological Products (*10*) shall apply.

Shipments should be at temperatures of 8 °C or below and parcels should contain cold-chain monitors.

A.11 Storage and expiry date

The statements concerning storage temperature and expiry date appearing on the label and the leaflet, as required by Good Manufacturing Practices for Biological Products (*10*, pages 26-27), shall be based on experimental evidence and shall be submitted for approval to the national control authority.

A.11.1 ***Storage conditions***

Before distribution, the manufacturer shall store lyophilized vaccine at a temperature shown by the manufacturer to be compatible with minimal

[1] No preservative or any substance that has a deleterious effect on the virus should be present in the diluent used to reconstitute the vaccine.

loss of virus titre. After distribution, live mumps vaccine shall be stored at all times at a temperature below 8 °C. Storage below 0 °C is permitted.

A.11.2 ***Validity period***

The validity period is a defined period of time at prescribed conditions of storage. It shall be based upon the stability of the vaccine, as determined experimentally, and shall be approved by the national control authority.

Part B. National control requirements

B.1 General

The general requirements for control laboratories contained in the Guidelines for National Authorities on Quality Assurance for Biological Products (*14*) shall apply.

The national control authority shall give directions to manufacturers concerning the mumps virus strains to be used in vaccine production and concerning the recommended human dose.

> The national control authority should take into consideration available information on strains before deciding on those permitted for vaccine production.

In addition, the national control authority shall provide a reference preparation of live mumps virus (see section A.1.3) for virus titration (see sections A.4.4.2, A.4.4.3, A.4.4.4 and A.6.3), and shall specify the virus content required to achieve adequate immunization of humans with the recommended human dose.

Protocols reporting the results of tests (Appendix 5) should be available for examination by the national control authority.

B.2 Release and certification

A vaccine lot shall be released only if it fulfils the national requirements and/or Part A of the present Requirements. A protocol based on the model given in Appendix 5, signed by the responsible official of the manufacturing establishment, shall be prepared and submitted to the national control authority in support of a request for release of vaccine for use.

At the request of the manufacturing establishment, the national control authority shall provide a certificate that states whether the vaccine meets all national requirements and/or Part A of the present Requirements. The certificate shall be based on the model given in Appendix 4.

> The purpose of the certificate is to facilitate the exchange of live mumps virus vaccines among countries.

III. Requirements for rubella vaccine

General considerations

Three virus strains, attenuated by repeated passage in cell culture, have been widely used for rubella vaccine production: the HPV-77 strain, originally recovered from a young adult with typical rubella; the Cendehill strain isolated from the urine of a child with rubella; and the RA-27/3 strain recovered from the explanted tissues of a fetus obtained at therapeutic abortion because of maternal rubella virus infection. Of these, only the RA-27/3 strain grown in human diploid cells is currently in wide use. In addition, the Takahashi, Matsuura and TO-336 attenuated strains, grown in primary cultures of rabbit kidney ephithelial cells or Japanese quail embryo fibroblasts, have been used on a large scale in Japan.

All vaccines are administered by the subcutaneous route. The vaccine viruses produce infection and an immune response in approximately 95% of recipients. These infections differ from those produced by the wild virus in the limited duration and quantity of virus excretion by the pharynx, the low incidence of detectable viraemia and, as with other attenuated vaccines, the lower antibody response.

Although communicability of the vaccine-induced infections was considered a possibility, there is considerable evidence from programmes of mass vaccination that this does not occur. The clinical consequences of the natural disease (e.g. fever, rash, lymphadenopathy) are uncommon after vaccination. However, vaccine recipients may show transient joint and peripheral nervous system involvement, manifested clinically as arthralgia or arthritis, and paraesthesia of the extremities, which occur commonly in the natural disease. Such symptoms are related to age and sex and have been noted most commonly in women. The rates observed vary widely depending on the method used for observation and follow-up, but are usually less than 1% in children and less than 10% in adults. The immunity produced by the vaccine appears to be long-lasting, and antibodies have been shown to persist for at least seven years, but continued observations of vaccinated individuals will be necessary to determine the exact duration of the protective effect.

Vaccination of pregnant women with rubella vaccine is not recommended. Although to date no case of a congenital defect has been attributed to the vaccine viruses, the attenuated viruses can cross the placenta and have been recovered from the fetus. Thus, while the evidence suggests a lower risk than with the natural virus, no assurance of safety can be given to the pregnant woman. In addition, vaccination is not recommended in the two-month period before conception.

The extensive use of rubella vaccine has produced a significant decline in the number of reported cases of both rubella and the congenital rubella syndrome. With this evidence of efficacy, the use of attenuated vaccines is becoming increasingly common throughout the world. For this reason,

requirements for the production and testing of live rubella vaccine were published by WHO in 1977 (6).

The Requirements formulated below do not include genetic marker tests because there is no definitive laboratory test for attenuation of rubella virus analogous to the neurovirulence tests for poliomyelitis virus vaccine (oral) and yellow fever virus vaccines. Experience has shown that the maintenance of a standard operating procedure, including a standard temperature of incubation and the use of a seed lot system, results in a consistent product that shows a regular pattern of attenuation and immunogenicity. Nevertheless the search for suitable marker tests continues.

Each of following sections constitutes a recommendation. The parts of each section printed in normal type have been written in the form of requirements so that, if a health administration so desires, they may be adopted as they stand as definitive national requirements. The parts of each section printed in small type are comments or recommendations for guidance. To facilitate the international distribution of vaccine made in accordance with these Requirements, a summary protocol for recording the results of tests is included as Appendix 7.

Should individual countries wish to adopt these Requirements as the basis of their national regulations for rubella vaccine, it is recommended that modifications be made only on condition that the modified requirements ensure at least an equal degree of safety and potency of the vaccine. The World Health Organization should then be informed of the action taken.

Part A. Manufacturing requirements

A.1 Definitions

A.1.1 *International name and proper name*

The international name shall be "Vaccinum rubellae vivum". The proper name shall be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

A.1.2 *Descriptive definition*

"Vaccinum rubellae vivum" is a preparation of live attenuated rubella virus grown in a suitable cell culture. The preparation shall satisfy all the requirements formulated below.

At present, live rubella vaccines are blended with an appropriate stabilizer and lyophilized. They are available for distribution only in that form, either as monovalent vaccines or in combination with live measles and/or live mumps vaccines.

A.1.3 *International reference materials*

Since no international reference materials have been established for live rubella vaccine, national control authorities should provide a reference preparation of live rubella virus for use in tests to determine virus content (see sections A.4.4.2, A.4.4.3, A.4.4.4 and A.6.3).

The second International Reference Preparation of Human Anti-Rubella Serum is in the custody of the International Laboratory for Biological Standards, State Serum Institute, Copenhagen, Denmark. Samples are distributed free of charge to national control laboratories on request. The International Reference Preparation is intended for the calibration of national standards and reference preparations.

A.1.4 *Terminology*

The following definitions are given for the purpose of these Requirements only.

Original vaccine: A vaccine prepared according to the manufacturer's specifications and shown on administration to humans to be safe and immunogenic.

Master seed lot: A quantity of virus derived from, or used to prepare, an original vaccine; the virus suspension has been processed as a single lot to ensure a uniform composition and is fully characterized. The master seed lot is used for the preparation of working seed lots.

Working seed lot: A quantity of virus of uniform composition, fully characterized, derived from a master seed lot. The working seed lot is used for the production of vaccines.

Cell seed: A quantity of fully characterized cells of human, animal or other origin stored frozen at -70 °C or below in aliquots of uniform composition, one or more of which are used for the production of a manufacturer's working cell bank.

Manufacturer's working cell bank (MWCB): A quantity of cells of uniform composition derived from one or more ampoules of the cell seed and stored frozen at -70 °C or below in aliquots, one or more of which are used for production purposes.

In normal practice a cell seed is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer and approved by the national control authority. The cells are combined in a single pool, distributed into ampoules and preserved cryogenically to form the MWCB.

Production cell culture: A number of cell cultures derived from the same pool of cells and processed together.

Single harvest: A quantity of virus suspension derived from a batch of production cell cultures that were inoculated with the same working seed lot and processed together in a single production run.

Virus pool: A homogeneous pool of single harvests collected into a single vessel before clarification.

Final bulk: The homogeneous finished virus suspension prepared from one or more clarified virus pools in the vessel from which the final containers are filled.

Filling lot (final lot): A collection of sealed final containers of finished vaccine that are homogeneous with respect to the risk of contamination during filling and freeze-drying. All the final containers must, therefore, have been filled from a single vessel of final bulk in one working session and lyophilized under standardized conditions in a common chamber.

Cell culture infective dose 50% ($CCID_{50}$): The quantity of a virus suspension that is estimated to infect 50% of cell cultures.

Plaque-forming unit (PFU): The smallest quantity of a virus suspension that will produce a plaque in monolayer cell cultures.

A.2 Certification of the strain of virus for use in vaccine production

The strain of rubella virus used in the production of rubella vaccine shall be identified by historical records that include information on the origin of the strain, its method of attenuation and the passage level at which attenuation was demonstrated by clinical evaluation.

The strain of rubella virus used in the production of vaccine shall have been shown to be safe by appropriate laboratory tests (see section A.4 of these Requirements) and safe and immunogenic by tests in susceptible humans. Only strains that are certified by the national control authority shall be used.

The lowest dose of vaccine that induces seroconversion in susceptible individuals shall be established in a dose-response study. This dose shall serve as a basis for establishing parameters for stability and expiry of the vaccine.

A.3 General manufacturing requirements

The requirements of Good Manufacturing Practices for Pharmaceutical (*9*) and Biological (*10*) Products and the Requirements for Human Diploid Cells Used for the Production of Measles, Mumps and Rubella Vaccines (Live) (Appendix 2) shall apply to establishments manufacturing rubella vaccine with the addition of the following requirements.

Production areas shall be decontaminated before they are used for the manufacture of rubella vaccine.

Rubella vaccine shall be produced by staff who have not handled other infectious microorganisms or animals on the same working day. The staff shall consist of persons who are periodically examined medically and found to be healthy. Steps shall be taken to ensure that all personnel

involved in the production areas are immune to rubella. Production and control shall be organized as two separate units of the manufacturing establishment with independent responsibilities.

Only the virus seed lot and cell cultures approved by the national control authority for the production of rubella vaccine shall be introduced or handled in the production area.

Persons not directly concerned with the production processes, other than official inspectors, shall not be permitted to enter the production area without valid reason and specific authorization.

Particular attention shall be given to the recommendations in Good Manufacturing Practices for Biological Products (*10*) regarding the training and experience of the persons in charge of production and testing, and of those assigned to various areas of responsibility in the manufacturing establishment, as well as to the registration of such personnel with the national control authority.

A.4 Production control

The general production precautions formulated in Good Manufacturing Practices for Biological Products (*10*) shall apply to the manufacture of rubella vaccine.

A.4.1. *Source materials*

A.4.1.1 Strain of rubella virus

The strain of rubella virus used in the production of live rubella vaccine shall be certified according to the specifications of section A.2. The vaccine strain shall be approved by the national control authority. The virus shall at no time have been passaged in a continuous cell line. The seed lot or five consistent lots of vaccine derived from the seed lot shall have been shown to be non-neuropathogenic in monkeys (see section A.4.2.1) and to yield live rubella vaccine of adequate immunogenicity and safety in human beings.

A.4.1.2 Cell cultures

Rubella virus used in the production of rubella vaccine shall be propagated in cell cultures approved by the national control authority. All information on the source and method of preparation of the cell-culture system used shall be available to the national control authority.

In some countries, biochemical tests for the detection of RNA viruses are applied to production cell cultures.

A.4.1.3 Human diploid cells

If human diploid cells are used for the propagation of rubella virus, a MWCB shall be established in conformity with the requirements of Appendix 2. The cell seed shall be derived from an early population doubling of the approved diploid cell strain, and the MWCB shall be

prepared from it by serial subculture up to an approved population doubling level. Each manufacturer shall show to the satisfaction of the national control authority that the cell substrate propagated from the accepted cell strain and laid down as a MWCB conforms with the requirements of Appendix 2 concerning tests in cell cultures, animals and eggs for freedom from extraneous agents, lack of tumorigenicity, normal karyology at least up to the population doubling level at which the cells are used to propagate the rubella virus (production cell cultures) and identity. The cells shall not be used beyond the highest population doubling level shown to be capable of meeting the requirements of Appendix 2.

A.4.1.4 Rabbit kidney cell cultures

If rabbit kidney cell cultures are used for the propagation of rubella virus, the rabbits (*Oryctolagus cuniculus*) from which the kidneys are taken shall be from a closed colony continuously monitored for coccidiosis, myxomatosis, rabbitpox virus, fibromatosis, herpesvirus cuniculi, *Mycobacterium tuberculosis*, *Nosema cuniculi*, *Toxoplasma gondii*, rabbit kidney vacuolating virus, syncytium virus of rabbits, and other pathogenic microorganisms and viral agents naturally occurring in rabbits.

A.4.1.5 Serum used in cell-culture medium

Serum used for the propagation of cells for rubella vaccine production shall be tested to demonstrate freedom from bacteria, fungi and mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*11*) and to demonstrate freedom from viruses.

Suitable tests for detecting viruses in calf and newborn-calf serum are given in Appendix 1 of the revised Requirements for Biological Substances No. 7 (Requirements for Poliomyelitis Vaccine (Oral)) (*12*).

Serum of bovine origin must come from herds certified to be free from bovine spongiform encephalopathy and bovine leukosis.

Serum shall also be shown to be free from inhibitors of rubella virus. Human serum shall not be used. If human albumin is used, it shall meet the revised Requirements for Biological Substances No. 27 (Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives) (*13*).

In some countries sera are also examined for freedom from certain phages.

A.4.1.6 Trypsin used for preparing cell cultures

Trypsin used for preparing cell cultures shall be bacteriologically sterile and free from mycoplasmas and viruses, especially porcine parvoviruses. The method used to ensure this shall be approved by the national control authority.

A.4.2 *Virus seed*

The production of vaccine shall be based on the virus seed lot system. Seed lots shall be prepared in cells homologous to those used for production of the final vaccine.

It is recommended that a large working seed lot be set aside for the preparation of batches of vaccine.

Each seed lot shall be identified as rubella virus by appropriate serological methods (see section A.6.1).

Virus seed lots shall be stored lyophilized in a dedicated temperature-monitored refrigerator at a temperature lower than -20 °C or, if not lyophilized, at or below -60 °C.

The methods used to produce single harvests from the working seed lot shall be identical to those employed to produce the vaccine used in the clinical trials to establish the safety and efficacy of the virus strain and the suitability of the master seed lot.

Particular attention should be given to the need to maintain consistency with respect to such factors as multiplicity of infection and cell-culture conditions, for example the duration and temperature of incubation.

Some national control authorities require manufacturers to demonstrate consistency of the product for several consecutive (e.g. five) production lots.

A.4.2.1 Tests on virus seed lots

The seed lot used for the production of vaccine shall be free from detectable extraneous agents and shall be produced in conditions that satisfy the requirements of sections A.4.3 and A.4.4 (with the exception of the tests for added substances and residual animal proteins in section A.4.4.5).

Tests for neurovirulence. Each master seed or working seed lot shall be shown to be free from neurovirulence by tests in rubella-susceptible monkeys of a species approved by the national control authority. To avoid the unnecessary use of monkeys, virus seed lots should be prepared in large quantities.

Neurovirulence tests can be conducted as follows:

At least 10 monkeys should be employed in each test. Immediately before inoculation, all monkeys should be shown to be serologically negative for rubella. The material under test should be given by injection of 0.5 ml into the thalamic region of each hemisphere. The total amount of rubella virus given to each monkey should be not less than the amount contained in the recommended single human dose of vaccine. The monkeys should be observed for 17–21 days for symptoms of paralysis and other evidence of neurological involvement. Animals that die within 48 hours of injection may be replaced. The test is invalid and should be repeated if more than 20% of the monkeys die, even from non-specific causes. At the end of the observation period each monkey is bled and its serum tested for anti-rubella antibody. All

test animals are anaesthetized and killed for autopsy; histopathological examinations of appropriate areas of the brain are made for evidence of central nervous system involvement.

The virus seed lot passes the test if: (a) at least 80% of the inoculated monkeys are serologically positive for rubella; and (b) there is no clinical or histopathological evidence of involvement of the central nervous system attributable to the injected virus.

In some countries the seed lot is not tested, but vaccines are accepted provided that each of the first five undiluted clarified virus pools prepared from the same seed lot satisfies the requirements of the test for neurovirulence.

A.4.3 *Control cell cultures*

From the cells used to prepare the cell cultures for growing attenuated rubella virus, an amount of processed cell suspension at least equivalent to 5% of the total volume or 500 ml, whichever is the greater, shall be used to prepare control cultures of uninfected cells. These control cultures shall be observed microscopically for changes attributable to the presence of adventitious agents for at least 14 days after the day of inoculation of the production culture or until the time of the last virus harvest, whichever is the later. At the end of the observation period, fluids collected from the control cultures shall be pooled and tested for the presence of adventitious agents as described below. Samples that are not tested immediately shall be stored at or below -60 °C.

In some countries, samples of fluid from each control vessel are collected at the same time as fluid is harvested from the corresponding production vessels. If several virus harvests are made from the same production cell batch, the control fluid taken at the time of each harvest is frozen and stored at or below -60 °C until the last virus harvest from that production cell batch is completed. The control fluids are then pooled in proportion to their amounts and submitted to the required tests.

If any tests show evidence of the presence of any adventitious agents in control cultures, the corresponding virus harvest shall not be used for vaccine production.

For a test to be valid, no more than 20% of the culture vessels shall have been discarded for non-specific reasons by the end of the test period.

A.4.3.1 Test for haemadsorbing viruses

At the end of the observation period, 25% of the control cells shall be tested for the presence of haemadsorbing viruses, using guinea-pig red cells. If the red cells have been stored, the duration of storage shall not have exceeded seven days, and the temperature of storage shall have been in the range of 2-8 °C.

In some countries, the national control authority requires that tests for haemadsorbing viruses should also be made on control cultures 3-5 days and 12 days after inoculation of the production cultures, and that other types of red cells, including cells from humans (blood group IV O), monkeys and chickens (or other avian species), should be used in addition to guinea-pig cells. In all tests readings should be taken after incubation for 30 minutes at 0-4 °C, and again after a further incubation for 30 minutes at 20-25 °C. For the test with monkey red cells readings should also be taken after a final incubation for 30 minutes at 34-37 °C.

A.4.3.2 Tests for non-haemadsorbing extraneous agents

Ten millilitres of the pooled cell-culture fluid collected at the end of the observation period shall be tested in the same cell substrate, but not the same batch, as that used for virus growth. Additional 10-ml samples of each pool shall be tested in both human and simian cells.

Cell monolayers shall be inoculated in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm^2 per ml of pooled fluid. At least one bottle of the cell cultures shall remain uninoculated as a control.

The inoculated cultures shall be incubated at a temperature of 35-37 °C and shall be examined for abnormal morphology for a period of at least 14 days.

Some national control authorities require that, at the end of this observation period, a subculture is made in the same culture system and observed for at least seven days. Furthermore, some national control authorities require that these cells should be tested for the presence of haemadsorbing viruses.

A.4.3.3 Additional test if rabbit kidney cells are used for production

A sample of control culture taken at the time of the last virus harvest shall be stained by appropriate methods for the detection of *Nosema cuniculi*.

In some countries the Giemsa stain is used, but in others the immunofluorescent technique is preferred because it appears to be more sensitive for the detection of low levels of *Nosema cuniculi* contamination.

Only if the cells are shown to be free from contamination may the corresponding harvest be used for production.

A.4.3.4 Additional tests if human diploid cells are used for production

If human diploid cells are used for production, the cell cultures shall be identified as human by tests approved by the national control authority.

Suitable tests are isozyme analysis, HLA and other immunological tests, and karyotyping of at least one metaphase spread of chromosomes.

A.4.4 *Production and harvest of vaccine virus*

A.4.4.1 Cells used for vaccine production

On the day of inoculation with the seed lot virus, each production cell culture and control cell culture shall be examined for degeneration caused by infective agents. If such examination shows evidence of the presence in a cell culture of any adventitious agent, the whole group of cultures concerned shall not be used for vaccine production.

After virus inoculation, cell cultures for vaccine production shall be incubated under controlled temperature conditions approved by the national control authority.

If animal serum is used in the growth medium for cell cultures, the serum shall be removed from the cell cultures either before or after inoculation with seed virus. Before the virus is harvested, the cell cultures shall be rinsed and the growth medium replaced with serum-free maintenance medium.

Penicillin and other β-lactam antibiotics shall not be used at any stage of manufacture.

Minimal concentrations of other suitable antibiotics may be used if approved by the national control authority.

A.4.4.2 Single harvests

Virus fluids shall be harvested by a method approved by the national control authority. A single harvest may be a combination of several consecutive harvests from one production cell culture. Single harvests are usually partly or fully stabilized and stored at or below -60 °C until pooling. No antibiotics shall be added at the time of harvesting nor at any later stage of manufacturing. Samples of single harvests shall be taken for testing for sterility and virus content; if not tested immediately, they shall be kept at or below -60 °C until testing is done.

Sterility tests. A volume of at least 20 ml of each single harvest shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No.6 (General Requirements for the Sterility of Biological Substances) (*11*), or by a method approved by the national control authority.

In some countries, the sample of pooled fluid is ultracentrifuged and both the pellet and its supernatant fluid are tested for sterility.

Tests for mycoplasmas should be done in both solid and liquid media that have been shown to be capable of supporting the growth of sterol-requiring and non-sterol-requiring mycoplasmas. At least 10 ml of single harvests should be used for each group of tests. Certain nutritionally fastidious mycoplasmas are best detected by DNA fluorescent staining on the surface of cultured indicator cells. Approved non-culture methods including DNA probes may also be used.

Virus titration. The live virus content of each single harvest shall be determined by cell culture titration, using a reference preparation of live rubella virus, the titre of which has been determined by comparison with a reference preparation approved by the national control authority (see section A.1.3). Minimum acceptable titres should be established.

A.4.4.3 Virus pool

The virus pool shall be prepared from one or several single harvests and shall be submitted to the following tests, unless these tests have already

been done on each single harvest; even in that event, the virus pool shall be tested for sterility. Samples that are not tested immediately shall be stored at or below -60 °C.

In tests that require prior neutralization of rubella virus, the antiserum used shall not be of human, simian or avian origin. The immunizing antigen used to prepare the antiserum shall be produced in cell cultures from a species different from that used for vaccine production. These cell cultures shall be free from extraneous microbial agents that might elicit antibodies inhibitory to the growth of any extraneous agents that may be present in the rubella virus pool. The tests shall be carried out as specified in paragraphs 2 and 3 of section A.4.3.2.

Sterility tests. Sterility tests shall be performed as indicated in section A.4.4.2.

Virus titration. The live virus content of each virus pool shall be determined by titration in cell culture against a reference preparation of live rubella virus (see section A.1.3).

Tests of neutralized virus pool in cell cultures. A volume of each virus pool equivalent to at least 500 human doses or 50 ml, whichever represents the greater volume, shall be neutralized by specific antiserum and shall be tested for adventitious agents by inoculation into simian cell cultures. Similar volumes of the neutralized virus pool shall be tested likewise in human cell cultures and in cell cultures of the same type, but not of the same batch, as those used to prepare the virus pool. Uninoculated cell cultures shall be kept as a control. All cell cultures shall be observed for at least 14 days.

Some national control authorities require that, at the end of this observation period, a subculture is made in the same culture system.

The virus pool passes the test if there is no evidence of the presence of adventitious agents and no more than 20% of the culture vessels have been discarded for non-specific reasons by the end of the test period.

Additional test if rabbit kidney cell cultures are used for production. A minimum of 15 ml of each virus pool shall be tested by inoculation of at least five healthy rabbits, each weighing 1500-2500 g. Each rabbit shall receive intradermal injections in multiple sites of a total of 1.0 ml of the virus pool and a subcutaneous injection of 2.0 ml of the virus pool, and the animals shall be observed for at least 30 days. Any rabbit that dies after the first 24 hours of the test or is killed because of illness shall undergo autopsy and the brain and organs shall be removed and examined.

The virus pool is satisfactory if at least 80% of the rabbits remain healthy and survive the observation period, and provided that none of the rabbits used in the test show lesions of any kind at the sites of inoculation or evidence of any viral infection.

A.4.4.4 Clarification of the virus pool

The virus pool shall be clarified by a method that will maximize removal of cells and cell debris.

The clarified virus pool may be stabilized and stored at or below −60 °C before being used to prepare final bulk for freeze-drying.

Samples of the clarified bulk suspension shall be taken immediately after clarification to ensure that no microscopically observable cells or cell particles remain. Samples shall also be taken for virus titration and sterility testing. If not tested immediately, the samples shall be kept at or below −60 °C until testing is done.

Virus titration. The live virus content of the clarified virus pool shall be determined by titration in cell cultures against a reference preparation of live rubella virus (see section A.1.3).

Sterility tests. Sterility tests shall be performed as indicated in section A.4.4.2.

A.4.4.5 Final bulk

The final bulk shall be prepared from one or more clarified virus pools obtained from substrates of which control cultures pass the tests specified in section A.4.3. The virus pools shall pass the tests specified in sections A.4.4.3 and A.4.4.4.

The operations necessary for preparing the final bulk shall be conducted in such a manner as to avoid contamination of the product.

The final bulk may be assayed for virus content.

Added substances. Any substance such as diluent or stabilizer that is added to the product during preparation of the final bulk shall have been shown to the satisfaction of the national control authority not to impair the safety and efficacy of the vaccine in the concentration used.

Residual animal serum proteins. If serum has been used in the cell-culture system, a sample of the final bulk shall be tested to verify that the residual amount of serum albumin is less than 50 ng per single human dose.

Alternatively the test may be performed on the clarified pools.

Sterility tests. Each final bulk shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified in Part A, section 5.2 and 5.3 of the revised Requirements for Biological Substances No.6 (General Requirements for the Sterility of Biological Substances) (*11*), or by a method approved by the national control authority.

Storage. Until it is distributed into containers and lyophilized, the final bulk shall be stored in conditions shown by the manufacturer to retain the activity of the vaccine.

A.5 Filling and containers

The requirements concerning filling and containers given in Good Manufacturing Practices for Biological Products (*10*) shall apply.

Care shall be taken to ensure that the material of which the container and, if applicable, the closure are made does not adversely affect the virus content of the vaccine under the recommended conditions of storage.

Single-dose containers are recommended, except for mass immunization campaigns.

A.6 Control tests on final product

Samples shall be taken from each final lot for the following tests.

A.6.1 *Identity tests*

The virus content of two or more individually labelled final containers, taken from the freeze-dried lot, shall be identified individually as rubella virus by appropriate methods.

Methods such as seroneutralization in cell culture with specific antiserum are suitable.

A.6.2 *Sterility tests*

Reconstituted vaccine shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No.6 (General Requirements for the Sterility of Biological Substances) (*11*), or by methods approved by the national control authority.

A.6.3 *Virus concentration and thermostability*

The virus content in each of at least three ampoules selected at random from the freeze-dried lot shall be determined individually against a reference preparation of rubella vaccine (see section A.1.3).

An additional three containers from the final freeze-dried vaccine lot shall be incubated at 37 °C for seven days. The geometric mean infectious virus titre of these vials shall be equal to or greater than the required minimum number of infective units per human dose, and the geometric mean virus titre of the vaccine shall not have decreased by more than 1.0 $\log_{10}$ infectious units during the period of incubation. Titration of non-exposed and exposed vials shall be made in parallel and results expressed in terms of PFU or $CCID_{50}$ per human dose. A reference preparation of rubella virus (see section A.1.3) shall be included in each assay.

The detailed procedures for carrying out this test and for interpreting the results should be approved by the national control authority, which should specify the acceptable confidence limits.

A suitable test for virus content is described in Appendix 8.

The national control authority shall determine the minimum amount of vaccine virus that one human dose should contain.

Most countries use at least 10^3 $CCID_{50}$ per dose.

A.6.4 ***General safety tests***

Each final lot shall be tested for absence of abnormal toxicity in mice and guinea-pigs by appropriate tests approved by the national control authority.

A.6.5 ***Residual moisture***

The residual moisture in a representative sample of the freeze-dried lot shall be determined by a method approved by the national control authority. The upper limit of the moisture content shall be specified by the national control authority.

Generally, moisture levels of less than 2% are considered satisfactory.

A.6.6 ***Inspection of final containers***

Each container shall be inspected visually and those showing abnormalities shall be discarded.

A.7 Records

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 27-28) shall apply.

A.8 Samples

The requirements of Good Manufacturing Practices for Biological Products (*10*, page 29, paragraph 9.5) shall apply.

A.9 Labelling

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 26-27) shall apply, with the addition of the following:

The label on the carton or the leaflet accompanying the container shall include:

- a statement that the vaccine fulfils Part A of these Requirements;
- a statement of the nature of the preparation, specifying the strain of rubella virus in the vaccine, the minimum number of infective units per human dose, and the origin of the substrate used to prepare the vaccine;
- a statement of the nature and quantity of any antibiotic present in the vaccine;
- a statement concerning the photosensitivity of the vaccine, cautioning that both lyophilized and reconstituted vaccine should be protected from light;

- a statement indicating the volume and nature of diluent[1] to be added in order to reconstitute the vaccine, and specifying that the diluent should be supplied by the manufacturer;
- a statement that, after the vaccine is reconstituted, it should be used immediately or be stored between 0 °C and 8 °C in the dark for a period not exceeding eight hours;
- a statement that the vaccine must not be given to a pregnant woman and that a woman must not become pregnant within two months of receiving the vaccine.

A.10 Distribution and shipping

The requirements of Good Manufacturing Practices for Biological Products (*10*) shall apply.

Shipments should be at temperatures of 8 °C or below and parcels should contain cold-chain monitors.

A.11 Storage and expiry date

The statements concerning storage temperature and expiry date appearing on the label and the leaflet, as required by Good Manufacturing Practices for Biological Products (*10*, pages 26-27), shall be based on experimental evidence and shall be submitted for approval to the national control authority.

A.11.1 ***Storage conditions***

Before distribution, the manufacturer shall store lyophilized vaccines at a temperature shown by the manufacturer to be compatible with minimal loss of virus titre. After distribution, live rubella vaccine shall be stored at all times at a temperature below 8 °C. Storage below 0 °C is permitted.

A.11.2 ***Validity period***

The validity period is a defined period of time at prescribed conditions of storage. It shall be based upon the stability of the vaccine, as determined experimentally, and shall be approved by the national control authority.

Part B. National control requirements

B.1 General

The general requirements for control laboratories contained in the Guidelines for National Authorities on Quality Assurance for Biological Products (*14*) shall apply.

[1] No preservative or any substance that has a deleterious effect on the virus should be present in the diluent used to reconstitute the vaccine.

The national control authority shall give directions to manufacturers concerning the rubella virus strains to be used in vaccine production and concerning the recommended human dose.

The national control authority should take into consideration available information on strains before deciding on those permitted for vaccine production

In addition, the national control authority shall provide a reference preparation of live rubella virus (see section A.1.3) for virus titration (see sections A.4.4.2, A.4.4.3, A.4.4.4 and A.6.3), and shall specify the virus content required to achieve adequate immunization of humans with the recommended human dose.

Protocols reporting the results of tests (Appendix 7) should be available for approval by the national control authority.

B.2 Release and certification

A vaccine lot shall be released only if it fulfils the national requirements and/or part A of the present Requirements. A protocol based on the model given in Appendix 7, signed by the responsible official of the manufacturing establishment, shall be prepared and submitted to the national control authority in support of a request for release of vaccine for use.

At the request of the manufacturing establishment, the national control authority shall provide a certificate that states whether the vaccine meets all national requirements and/or Part A of the present Requirements. The certificate shall be based on the model given in Appendix 4.

The purpose of the certificate is to facilitate the exchange of live rubella virus vaccines among countries.

IV. Requirements for measles–mumps–rubella (MMR) combined vaccine

General considerations

Requirements for combined vaccines against measles, mumps and rubella (MMR) must include the tests applicable to the monovalent final bulks incorporated into the final product. However, further tests are required after blending, because of the possible effects that both diluents and stabilizers may have on the potency and stability of the final product. The tests to be carried out on combined vaccines are on the whole the same as those specified for the individual components, so that cross-references to the Requirements for these components would suffice in many cases.

No international reference materials exist for the combined vaccine. For each component, the live virus concentration is determined by titration

against a reference preparation in a suitable cell-culture system, after selective neutralization, as necessary, of the other two components with appropriate antisera.

The requirements for filling, sampling, labelling, transportation, distribution and storage are the same for the combined vaccine as for the individual (measles, mumps and rubella) vaccines. The validity period of combined vaccines is determined by the component with the shortest shelf-life.

Each of the following sections constitutes a recommendation. The parts of each section printed in normal type have been written in the form of requirements so that, if a health administration so desires, they may be adopted as they stand as definitive national requirements. The parts of each section printed in small type are comments or recommendations for guidance. To facilitate the international distribution of vaccine made in accordance with these Requirements, a summary protocol for recording the results of tests is included as Appendix 9.

Should individual countries wish to adopt these Requirements as the basis of their national regulations for combined vaccine, it is recommended that modifications be made only on condition that the modified requirements ensure at least an equal degree of safety and potency of the vaccine. The World Health Organization should then be informed of the action taken.

Part A. Manufacturing requirements

A.1 Descriptive definition

Measles, mumps and rubella combined vaccine (live) is a mixed preparation containing suitable attenuated strains of live measles, mumps and rubella viruses. The combined vaccine shall satisfy all the requirements formulated below.

A.2 Manufacturing requirements for MMR vaccine

A.2.1 *Final bulks of individual components*

Final bulks of the individual components shall be prepared and tested as follows: for measles components the Requirements for Measles Vaccine shall apply; for mumps components the Requirements for Mumps Vaccine shall apply; for rubella components, the Requirements for Rubella Vaccine shall apply.

A.2.2 *Final bulk of combined components*

The final bulk of combined virus suspension shall be prepared from final bulks of the individual components that satisfy the requirements of section A.4.4.5 of the Requirements for each of the individual vaccines.

Only sta zers, diluents and other substances approved by the national control authority shall be added to the vaccine. Any such substances shall have been shown by appropriate tests to have, in the amounts used, no deleterious effects on the product.

The final bulk may be assayed for virus content.

A.2.2.1 Residual animal serum proteins

If serum has been used in the cell-culture system, a sample of the final bulk shall be tested to verify that the residual amount of serum albumin is less than 50 ng per single human dose.

Alternatively the test may be performed on the clarified pools.

A.2.2.2 Sterility tests

Each final bulk shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No.6 (General Requirements for the Sterility of Biological Substances) (*11*), or by a method approved by the national control authority.

A.2.2.3 Storage

Until it is distributed into containers and lyophilized, the final bulk shall be stored in conditions shown by the manufacturer to retain the activity of the vaccine.

A.3 Filling and containers

The requirements concerning filling and containers given in Good Manufacturing Practices for Biological Products (*10*) shall apply.

Care shall be taken to ensure that the material of which the container and, if applicable, the closure are made does not adversely affect the virus content of the vaccine under the recommended conditions of storage.

Single-dose containers are recommended, except for mass immunization campaigns.

A.4 Control tests on final product

The following tests shall be carried out on the final product.

A.4.1 *Sterility test*

Each final lot shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No.6 (General Requirements for the Sterility of Biological Substances) (*11*), or by a method approved by the national control authority.

A.4.2 *Virus concentration, thermostability and identity*

The live virus concentration in each freeze-dried final lot shall be determined by titration in suitable cell-culture systems against appropriate reference preparations of individual vaccine components. The titration of each individual component shall be carried out after selective neutralization, as necessary, of the other two components with specific antisera. The virus content of at least three containers selected at random from each freeze-dried lot shall be determined individually. The minimum acceptable virus titre per human dose shall be approved by the national control authority.

The other virus vaccine components have been observed to interfere with mumps virus growth in individuals immunized with MMR vaccines who have shown a suboptimal immune response to mumps virus; the mumps virus concentration in the combined vaccine may therefore have to be higher than that in the monovalent mumps vaccine.

A suitable virus titration assay is described in Appendix 10.

The thermostability of MMR vaccine shall be tested by incubation of at least three containers of each final freeze-dried vaccine lot at 37 °C for seven days. At the end of this period, each individual component shall be titrated as outlined above after selective neutralization, as necessary, of the other two components.

The requirements for virus concentration and thermostability in section A.6.3 of the Requirements for each of the individual components shall apply.

The successful neutralization of individual vaccine components during the virus titration shall serve as an identity test.

A.4.3 *General safety tests*

Each final lot shall be tested for the absence of abnormal toxicity in mice and guinea-pigs by appropriate tests approved by the national control authority.

A.4.4 *Residual moisture*

The residual moisture in a representative sample of each freeze-dried lot shall be determined by a method approved by the national control authority. The upper limit of the moisture content shall be specified by the national control authority.

Moisture levels of less than 2% are usually considered satisfactory.

A.4.5 *Inspection of final containers*

Each container in each final lot shall be inspected visually and those showing abnormalities shall be discarded.

A.5 Records

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 27–28) shall apply.

A.6 Samples

The requirements of Good Manufacturing Practices for Biological Products (*10*, page 29, paragraph 9.5) shall apply.

A.7 Labelling

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 26–27) shall apply, with the addition of the following:

The label on the carton or the leaflet accompanying the container shall include:

- a statement that the vaccine fulfils Part A of these Requirements;
- a statement of the nature of the preparation, specifying the strains of measles, mumps and rubella viruses in the vaccine, the minimum number of infective units of each virus per human dose, and the origin of the substrates used to prepare the vaccine;
- a statement of the nature and quantity of any antibiotic present in the vaccine;
- a statement concerning the photosensitivity of the vaccine, cautioning that both lyophilized and reconstituted vaccine should be protected from light;
- a statement indicating the volume and nature of diluent[1] to be added in order to reconstitute the vaccine, and specifying that the diluent should be supplied by the manufacturer;
- a statement that, after the vaccine is reconstituted, it should be used immediately or be stored between 0 °C and 8 °C in the dark for a period not exceeding eight hours;
- a statement that the vaccine must not be given to a pregnant woman and that a woman must not become pregnant within two months of receiving the vaccine.

A.8 Distribution and shipping

The requirements of Good Manufacturing Practices for Biological Products (*10*) shall apply.

> Shipments should be at temperatures of 8 °C or below and parcels should contain cold-chain monitors.

[1] No preservative or any substance that has a deleterious effect on the virus should be present in the diluent used to reconstitute the vaccine.

A.9 Storage and expiry date

The statements concerning storage temperature and expiry date appearing on the label and the leaflet, as required by Good Manufacturing Practices for Biological Products (*10*, pages 26–27), shall be based on experimental evidence and shall be submitted for approval to the national control authority.

A.9.1 *Storage conditions*

Before distribution, the manufacturer shall store lyophilized vaccines at a temperature shown by the manufacturer to be compatible with minimal loss of virus titre. After distribution, live MMR vaccine shall be stored at all times at a temperature below 8 °C. Storage below 0 °C is permitted.

A.9.2 *Validity period*

The validity period is a defined period of time at prescribed conditions of storage. It shall be based upon the stability of the vaccine, as determined experimentally, and shall be approved by the national control authority.

Part B. National control requirements

B.1 General

The general requirements for control laboratories contained in the Guidelines for National Authorities on Quality Assurance for Biological Products (*14*) shall apply.

The national control authority shall give directions to manufacturers concerning the measles, mumps and rubella virus strains to be used in vaccine production and concerning the recommended human dose.

> The national control authority should take into consideration available information on strains before deciding on those permitted for vaccine production.

In addition, the national control authority shall provide a reference preparation of live measles, mumps and rubella vaccine for virus titration (see section A.4.2) and shall specify the virus content required to achieve adequate immunization of humans with the recommended human dose.

B.2 Release and certification

A vaccine lot shall be released only if it fulfils the national requirements and/or part A of the present Requirements. A protocol based on the model given in Appendix 9, signed by the responsible official of the manufacturing establishment, shall be prepared and submitted to the national control authority in support of a request for release of vaccine for use.

At the request of the manufacturing establishment, the national control authority shall provide a certificate that states whether the vaccine meets all national requirements and/or Part A of the present Requirements. The certificate shall be based on the model given in Appendix 4.

The purpose of the certificate is to facilitate the exchange of live MMR vaccines among countries.

Authors

The first draft of these Requirements was prepared by Dr V. P. Grachev, Scientist, and Dr D. Magrath, Chief, Biologicals, World Health Organization, Geneva, Switzerland.

Acknowledgements

Acknowledgements are due to the following experts for their comments and advice on the first and second revisions of the draft Requirements: Dr S. C. Arya, Centre for Logistical Research and Innovation, New Delhi, India; Dr C. R. Bennett, Director, Regulatory Liaison, Merck Research Laboratories, West Point, Pennsylvania, PA, USA; Dr G. Colinet, SmithKline Beecham Biologicals, Rixensart, Belgium; Dr A. Fenyves, Paul Ehrlich Institute, Langen, Germany; Dr L. B. Flugsrud, National Institute of Public Health, Oslo, Norway; Dr T. Forsey, National Institute for Biological Standards and Control, Potters Bar, Herts., England; Dr J. Furesz, formerly Director, Bureau of Biologics, Drugs Directorate, Ottawa, Ontario, Canada; Dr R. Gluck, Head, Virology, Swiss Serum and Vaccine Institute, Berne, Switzerland; Dr C. Guthrie, General Manager, Blood Products Division, CSL Ltd, Victoria, Australia; Dr K. J. Healy, CSL Ltd, Victoria, Australia; Mrs L. Jelstad, National Institute of Public Health, Oslo, Norway; Mr P. Lemoine, formerly Head, Biological Standardization, Institute of Hygiene and Epidemiology, Brussels, Belgium; Dr T. Matuhasi, Okinaka Memorial Institute for Medical Research, Toranomon Hospital, Tokyo, Japan; Dr H. Mirchamsy, Associate Director, Razi State Institute of Sera and Vaccines, Teheran, Islamic Republic of Iran; Dr R. Netter, Paris, France; Dr L. Palkonyay, Chief, Viral Products Division, Bureau of Biologics, Drugs Directorate, Ottawa, Ontario, Canada; Dr J. Peetermans, Director, Quality Assurance and Regulatory Affairs, SmithKline Beecham Pharmaceuticals, Rixensart, Belgium; Dr J. R. Vose, Vice President, Regulatory and Technical Affairs, Connaught Laboratories Ltd, Willowdale, Ontario, Canada; Dr N. White, Senior Virologist, Virology Section, Therapeutic Goods Administration Laboratories, Victoria, Australia.

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1. Requirements for Measles Vaccine (Live) and Measles Vaccine (Inactivated) (Requirements for Biological Substances No. 12). In: *WHO Expert Committee on Biological Standardization. Eighteenth Report.* Geneva, World Health Organization, 1966, Annex 2 (WHO Technical Report Series, No. 329).
2. Requirements for Measles Vaccine (Live) (Requirements for Biological Substances No. 12), Addendum 1981. In: *WHO Expert Committee on Biological Standardization. Thirty-second Report.* Geneva, World Health Organization, 1982, Annex 6 (WHO Technical Report Series, No. 673).

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4. Requirements for Mumps Vaccine (Live) (Requirements for Biological Substances No. 38). In: *WHO Expert Committee on Biological Standardization. Thirty-seventh Report.* Geneva, World Health Organization, 1987, Annex 7 (WHO Technical Report Series, No. 760).
5. *WHO Expert Committee on Biological Standardization. Twenty-third Report.* Geneva, World Health Organization, 1971: 18 (WHO Technical Report Series, No. 463).
6. Requirements for Rubella Vaccine (Live) (Requirements for Biological Substances No. 24). In: *WHO Expert Committee on Biological Standardization. Twenty-eighth Report.* Geneva, World Health Organization, 1977, Annex 3 (WHO Technical Report Series, No. 610).
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9. Good manufacturing practices for pharmaceutical products. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-second Report.* Geneva, World Health Organization, 1992, Annex 1 (WHO Technical Report Series, No. 823).
10. Good manufacturing practices for biological products. In: *WHO Expert Committee on Biological Standardization. Forty-second Report.* Geneva, World Health Organization, 1992, Annex 1 (WHO Technical Report Series, No. 822).
11. General Requirements for the Sterility of Biological Substances (Requirements for Biological Substances No. 6, revised 1973). In: *WHO Expert Committee on Biological Standardization, Twenty-fifth Report.* Geneva, World Health Organization, 1973, Annex 4 (WHO Technical Report Series, No. 530).
12. Requirements for Poliomyelitis Vaccine (Oral) (Requirements for Biological Substances No. 7, revised 1989). In: *WHO Expert Committee on Biological Standardization. Fortieth Report.* Geneva, World Health Organization, 1990, Annex 1 (WHO Technical Report Series, No. 800).
13. Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives (Requirements for Biological Substances No. 27, revised 1992). In: *WHO Expert Committee on Biological Standardization. Forty-third Report.* Geneva, World Health Organization, 1994, Annex 2 (WHO Technical Report Series, No. 840).
14. Guidelines for national authorities on quality assurance for biological products. In: *WHO Expert Committee on Biological Standardization. Forty-second Report.* Geneva, World Health Organization, 1992, Annex 2 (WHO Technical Report Series, No. 822).

Appendix 1

Summary protocol for production and testing of measles vaccine (live)[1]

The following protocol is intended *for guidance*, and indicates the information that should be provided as a minimum.

The section concerning the final product must be accompanied by a sample of the label, a copy of the leaflet that accompanies the vaccine container, and a certificate from the national control authority of the country in which the vaccine was produced stating that the product meets national requirements as well as the Requirements published by WHO (see Appendix 4).

Source materials (A.4.1)

Strain of measles virus (A.4.1.1) _____

Cell cultures (A.4.1.2)

Provide information on the source and method of preparation of the cell cultures.

Avian-embryo cell cultures (A.4.1.3)

Provide information on the source of the closed, specific-pathogen-free, healthy flock.

Types of test for infections _____ Results _____

Certified satisfactory _____ Date _____

Signature of head of laboratory _____

Human diploid cells (A.4.1.4)

Provide information on the source of the manufacturer's working cell bank (MWCB) (see Appendix 2).

Other cells (A.4.1.5) _____

Serum used in cell-culture medium (A.4.1.6)

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

[1] Based on Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live) (Requirements for Biological Substances No. 47). In: *WHO Expert Committee on Biological Standardization. Forty-third Report.* Geneva, World Health Organization, 1994, Annex 3 (WHO Technical Report Series, No. 840).

Tests for adventitious agents

Methods _____

Date of inoculation _____

Results _____

Trypsin used for preparing cell cultures (A.4.1.7)

Sterility tests

	bacteria	*mycoplasmas*
Date of inoculation		
Results		

Tests for adventitious agents (including porcine parvoviruses)

Methods _____

Date of inoculation _____

Results _____

Production of the working seed lot (A.4.2)

Summary Information

Name and address of manufacturer _____

_____

Virus strain _____

Reference no. of virus seed used to prepare manufacturer's original measles vaccine that was safe and immunogenic in humans _____

Reference no. of master seed lot _____

No. of passages between the two above seeds _____

Working seed lot

Date of preparation _____

No. of containers prepared _____

Reference no. _____

Conditions of storage _____

History of vaccine strain

Provide a brief account, indicating how the vaccine strain was acquired, outlining its history up to production of the master seed lot, and specifying the criteria on which acceptability for virus production is based.

Certificati... of working seed lot

Name (typed) and signature of head of production laboratory _____

_____

Certification by the head of the control laboratory of the manufacturer taking overall responsibility for production and control of the working seed lot:

I certify that the working seed lot of measles vaccine virus no. ____ satisfies Part A, sections 2 to 4.4.5, of the Requirements for Measles Vaccine in Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)).

Signature _____

Name (typed) _____

Date _____

Control cell cultures (A.4.3)

Provide information on the control cell cultures corresponding to each single harvest, using extra pages if necessary.

Cell substrate used for production of virus _____

Reference no. of control cell cultures _____

Quantity of cell cultures used as control cultures _____

Period of observation of control cells _____

Test for haemadsorbing viruses (A.4.3.1)

Type of red blood cells _____

Date of test _____

Results _____

Tests for non-haemadsorbing extraneous agents (A.4.3.2)

Cell substrate used for virus growth

Type of cells _____

Date of inoculation _____

Results _____

Simian cells

Type of cells _____

Date of inoculation _____

Results _____

Human cells

Type of cells _____

Date of inoculation _____

Results _____

Additional tests if chick cell cultures are used for production (A.4.3.3)

Test for avian adenoviruses

Method _____

Date _____

Results _____

Test for avian leukosis virus

Method _____

Date _____

Results _____

Additional tests if human diploid cells are used for production (A.4.3.4)

Identity test

Method _____

Date _____

Results _____

Production and harvest of vaccine virus (A.4.4)

Cells used for vaccine production (A.4.4.1)

Observation of cell cultures before inoculation

Methods _____

Results _____

Antibiotics added (if used) _____

Concentration _____

Single harvests (A.4.4.2)

Report the results of tests on each single harvest, using extra pages if necessary.

No. of passages from the primary seed _____

Reference no. of single harvest _____

Sterility tests	*bacteria*	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

Virus pool (A.4.4.3)

Reference no. of virus pool _____

If any test had to be repeated or any abnormal result was observed, this must be specified.

Tests for neurovirulence[1] (A.4.2.1)

No. of monkeys in test _____

Species _____

Volume injected _____

No. of monkeys surviving without specific symptoms _____

Results of serological tests _____

Results of histopathological examination (specify findings) _____

Sterility tests	*bacteria*	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

[1] Only for master seed or working seed lot

Tests of neutralized virus pool in cell cultures

Species in which neutralizing serum was prepared and cell substrate in which immunogen was produced _____

Cells used for virus growth

Type of cells _____

Date of inoculation _____

Results _____

Simian cells

Type of cells _____

Date of inoculation _____

Results _____

Human cells

Type of cells _____

Date of inoculation _____

Results _____

Additional tests if chick cell cultures are used for production

Test in embryonated eggs inoculated by allantoic route

No. and age of eggs inoculated _____

Date _____

Results _____

Test in embryonated eggs inoculated by yolk-sac route

No. and age of eggs inoculated _____

Date _____

Results _____

Clarification of the virus pool (A.4.4.4)

Date of clarification _____

Results of clarification _____

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

Sterility t

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Final bulk (A.4.4.5)

Reference no. of final bulk

Total volume of final bulk

Added substances (diluent, stabilizer) and final concentration

Residual animal serum proteins

Date

Method

Results (indicate amount and nature of serum protein(s) present per human dose)

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Filling and containers (A.5)

Name and address of manufacturer

Proprietary name of vaccine

Reference no. of final lot

Expiry date

No. of containers in the lot

No. of doses per container

Lot no. of final bulk

Date of filling of final containers

Control tests on final product (A.6)

Identity test (A.6.1)

Date

Method

Results

Sterility tests (A.6.2)

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Virus concentration and thermostability (A.6.3)

Date of inoculation

Type of cell cultures

	Control (unheated) samples			*Samples incubated at 37°C for 7 days*		
	1	*2*	*3*	*1*	*2*	*3*
Virus concentration in each container (in PFU or $CCID_{50}$)						
Mean virus titre per human dose, with 95% fiducial limits						
Mean loss in titre due to heat exposure (in $\log_{10}$ units)						

Reference preparation

Identification

Theoretical titre

Actual titre

General safety tests (A.6.4)

Test in mice

Date of inoculation

No. of mice tested

Volume and route of injection

Observation period

Results (give details of deaths)

Test in guinea-pigs

Date of inoculation

No. of guinea-pigs tested

Volume and route of injection

Observation period

Results (give details of deaths)

Residual moisture (A.6.5)

Date _____

Method _____

Size of sample _____

Moisture content (%) _____

Inspection of final containers (A.6.6)

Date and result _____

Submission addressed to national control authority for batch release

Name (typed) and signature of head of production laboratory _____

_____

Date _____

Certification by person taking overall responsibility for production and control of the vaccine:

I certify that lot no. _____ of measles vaccine (live) satisfies national requirements and/or Part A of the Requirements for Measles Vaccine in Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)).

Signature _____

Name (typed) _____

Date _____

Appendix 2

Requirements for human diploid cells used for the production of measles, mumps and rubella vaccines (live)

The following requirements concern the testing of the cell substrate for the production of measles, mumps and rubella vaccines if human diploid cells are used; they should therefore be added to or substituted for the corresponding sections in Part A of the individual Requirements, as appropriate. All the other sections in Parts A and B of the individual Requirements remain applicable.

1. Terminology

Production cell culture: A collection of cell cultures at the population doubling level used for virus growth that have been prepared together from one or more ampoules of the manufacturer's working cell bank (MWCB).

2. Production control

2.1 *Source materials*

The cell seed and MWCB shall be approved by and registered with the national control authority. The cells shall have been characterized with respect to their genealogy, growth characteristics, genetic markers (HLA), viability during storage and karyology, and have been shown to be free from bacteria, mycoplasmas, fungi and haemadsorbing and other viruses by the relevant tests in these Requirements. In addition, the cells of the MWCB shall have been shown to be diploid and stable with respect to karyology and morphology by the tests outlined in this section.

The MWCB shall also have been shown to yield cell cultures capable of producing vaccine that is both safe and immunogenic in humans.

Each production cell culture shall consist of cells at a passage level of up to two-thirds of the life span of the accepted cell strain and shall be tested for identity. It shall comply with the tests outlined in sections 2.1.1, 2.1.2 and 2.1.4 for normal karyology and freedom from adventitious agents.

2.1.1 *Tests in animals and eggs for extraneous agents*

The cells of the MWCB are suitable if at least 80% of the inoculated animals and eggs remain healthy and survive the observation period, and none of the animals or eggs shows evidence of the presence in the cells of any extraneous agent.

Tests in animals. The following groups of animals shall be inoculated with MWCB cells by the intramuscular route, at least 10^7 cells being divided equally between the animals in each group:

- two litters of suckling mice, totalling at least ten animals, less than 24 hours old

– ten a[illegible] nice weighing 15-20 g
– five guinea-pigs weighing 350-450 g
– five rabbits weighing 1.5-2.5 kg.

The animals shall be observed for at least four weeks. Any animals that are sick or show any abnormality shall be investigated to establish the cause of illness. The test is not valid unless at least 80% of the animals remain healthy and survive the observation period.

In some countries, the suckling and adult mice are also inoculated by the intracerebral route.

Tests in eggs. At least 10^6 viable MWCB cells shall be injected into the allantoic cavity of ten embryonated chickens' eggs, 9-11 days old, which shall be examined after not less than five days. The allantoic fluids of the fertile eggs shall be tested with erythrocytes from guinea-pigs and chickens (or other avian species) and human group O cells for the presence of haemagglutinins.

2.1.2 *Other tests for extraneous agents*

Suitable tests approved by the national control authority shall be performed to exclude the presence of retroviruses and the integration of nucleic acid of viral origin (hepatitis B virus and human immunodeficiency virus) in the genome of MWCB cells.

In some countries, the cells are also examined in ultra-thin sections and by negative staining under the electron microscope.

2.1.3 *Freedom from tumorigenicity*

The cells of the MWCB shall be shown to be free from potential tumorigenicity by appropriate animal tests, including positive controls, approved by the national control authority.

Suitable tests in immunosuppressed animals are as follows. Approximately 10^6 cells obtained from cultures at the same passage levels as those to be used for vaccine production are injected into: newborn mice or hamsters treated with antilymphocyte serum; or athymic mice (nude *nu/nu* genotype); or thymectomized, irradiated mice with reconstituted bone marrow (T–B+). Some of the same group of animals should be inoculated with a similar dose of HeLa or KB cells as positive controls. The animals should be observed for not less than three weeks. Other tests in animals treated with immunosuppressive agents and with equal sensitivity to neoplastic cells may also be used.

The test is valid if the positive control animals develop tumours.

The cells are suitable for vaccine production if at least 80% of inoculated animals remain healthy and survive the observation period, and none of the animals shows evidence of tumour formation attributable to the cells.

2.1.4 *Chromosomal characterization of the cell seed*

At least four samples from the cell seed shall be examined as described in section 2.1.5 at approximately equal intervals over the life span of the cell line during serial cultivation. Each sample shall consist of 1000 metaphase cells.

It is recommended that photographic reconstruction should be employed to prepare chromosome-banded karyotypes of 50 metaphase cells per 1000-cell sample, by either G-banding or Q-banding techniques. The incidence of karyotypic abnormalities (pseudodiploidy, inversions, translocations, etc.) that are detectable with the greater resolution provided by banding should not exceed that approved by the national control laboratory.

2.1.5 *Chromosomal characterization of the MWCB*

For the determination of the general character of the MWCB, a minimum of 500 cells in metaphase shall be examined at the production level, or at any passage thereafter, for frequency of polyploidy and for exact counts of chromosomes, frequency of breaks, structural abnormalities, and other abnormalities such as despiralization or marked attenuations of the primary or secondary constriction. The cells of the MWCB shall have normal karyology.

For vaccine production, examination of the cells is usually made between the 27th and 33rd population doubling. The national control authority should determine the permissible level of cell population doubling.

For WI-38 and MRC5 cells examined in metaphase, the generally accepted upper limits for abnormalities in 1000- and 500-cell samples are:[1]

Abnormality	*1000 cells*	*500 cells*
Chromatid and chromosome breaks	47/1000	26/500
Structural abnormalities	17/1000	10/500
Hyperploidy	8/1000	5/500
Hypoploidy	180/1000	90/500
Polyploidy	30/1000	17/500

All cells showing abnormalities shall be subjected to detailed examination and records shall be maintained of the detailed criteria applied to particular abnormalities in the karyotype analysis.

Stained slide preparations of the chromosomal monitoring of the working cell bank, or photographs of these, shall be maintained permanently as part of the record of the MWCB.

2.2 *Production precautions*

The general production precautions formulated in Good Manufacturing Practices for Pharmaceutical (*1*) and Biological (*2*) Products shall apply to the manufacture of virus vaccines with the addition of the following.

[1] These upper limits are based on extensive experience with the examination of WI-38 and MRC5 cells reported to and examined by the ad hoc Committee on Karyological Controls of Human Substrates, which met in 1978 at Lake Placid, NY, USA. These values will not necessarily be applicable if other human cell strains are used.

2.2.1 *Cell cultures used for vaccine production*

Only human diploid cell cultures derived from a MWCB approved by the national control authority shall be used for vaccine production. The production of each single harvest shall be initiated from one or more new ampoules of the MWCB. All processing of the MWCB and subsequent cell cultures shall be done in an area in which no other cells are handled during the entire period of vaccine production. The cell cultures shall be used only if: (a) no changes have occurred in their growth characteristics, and (b) no abnormal karyotypic changes have been found to occur up to a number of population doublings that corresponds to the average finite life of the cells, as determined under the particular conditions of the production establishment (see section 2.1.4).

It is advisable to ensure that both the trypsin and the animal serum used in the preparation or growth of the cell suspensions are free from extraneous agents.

2.2.2 *Identity test*

An identity test shall be performed on the control cell cultures by methods approved by the national control authority.

Suitable tests are isozyme analysis, HLA and other immunological tests, and karyotyping of at least one metaphase spread of chromosomes.

The cells shall be shown to be of human origin.

2.2.3 *Tests for bacteria, fungi and mycoplasmas*

A volume of 20 ml of the pooled supernatant fluids from the production cell cultures shall be tested for bacterial and mycotic sterility as specified in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*3*), as well as for mycoplasmas, by a method approved by the national control authority.

The tests for mycoplasmas should be done in both solid and liquid media that have been shown to be capable of supporting the growth of sterol-requiring and non-sterol-requiring mycoplasmas. At least 10 ml should be used for each group of tests. Approved non-culture methods may also be used.

References

1. Good manufacturing practices for pharmaceutical products. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-second Report.* Geneva, World Health Organization, 1992, Annex 1 (WHO Technical Report Series, No. 823).
2. Good manufacturing practices for biological products. In: *WHO Expert Committee on Biological Standardization. Forty-second Report.* Geneva, World Health Organization, 1992, Annex 1 (WHO Technical Report Series, No. 822).
3. General Requirements for the Sterility of Biological Substances (Requirements for Biological Substances No. 6, revised 1973). In: *WHO Expert Commitee on Biological Standardization. Twenty-fifth Report.* Geneva, World Health Organization, 1973, Annex 4 (WHO Technical Report Series, No. 530).

Appendix 3
Proposed assay method for determining the virus content of live measles vaccine

1. Cell-culture infective dose method

Three vials of the preparation to be assayed and at least one container of the reference preparation are reconstituted and diluted in an appropriate medium. It is convenient to use dilution steps of 0.5 $\log_{10}$ units selected so that the dilution range encompasses at least three dilutions that will infect between 10% and 90% of the cultures inoculated.

For each vaccine dilution, a volume of 0.1 ml is dispensed into each of 8-12 flat-bottomed wells in a microtitre plate, followed by 0.1 ml of a suitable cell suspension of the Vero cell line. The plates are incubated at the optimal growth temperature for the tested measles virus strain.

The presence or absence of a cytopathic effect is recorded after four days, with the final reading at 10 days. The titre is calculated as a multiple of the median cell-culture infective dose ($CCID_{50}$) by an appropriate method. If the potency is below the expected value, the assay can be repeated and the mean of all assays calculated. The assay is not considered valid when:

- the mean titre of the reference vaccine deviates by more than 0.5 $\log_{10}$ units from the established titre; and/or
- the difference in titre between any two vials of the test vaccine or between the vials of the reference vaccine is >0.5 $\log_{10}$ units.

Care must be taken to protect all virus samples from direct light.

2. Plaque method

Three vials of the preparation to be assayed and at least one of the reference preparation are reconstituted and diluted in an appropriate medium. It is convenient to use dilution steps selected to give at least 50% of wells with between 10 and 80 plaques per well.

For each vaccine dilution, a volume of 0.05 ml is dispensed into each of three wells in a 24-well tissue-culture plate. A separate plate is used for each of the three test samples and the reference preparation is included in each plate. The wells are seeded with 1.0 ml of an appropriate cell suspension of the Vero cell line and the plates are incubated for three hours, under 5% carbon dioxide, at the optimum growth temperature for the tested measles virus strain. The medium is then removed from each well and replaced with 1.0 ml of medium containing 0.8% carboxymethylcellulose or other appropriate overlay. The plates are then incubated as before for 7-10 days depending on the strain tested.

Plaques are visualized by fixing and staining the cell sheets. A convenient reagent for both purposes is 0.5% crystal violet in 20% ethanol. The medium is first removed from each well and the cell sheet gently washed

with buffe... crystal violet in ethanol is added and the plates are left at room temperature for 20 minutes. The stain is then removed, the cells are washed gently with water and the plates are dried. The plaques are counted and wells with numbers between 10 and 80 are considered valid. The titre is calculated in plaque-forming units (PFU) per dose from the direct counts.

If the potency is below the expected value, the assay can be repeated and the mean of all assays calculated. The assay is not considered valid when:

- the mean titre of the reference vaccine deviates by more than 0.5 $\log_{10}$ units from the established titre; and/or
- the difference in titre between any two vials of the test vaccine or between the vials of the reference vaccine is >0.5 $\log_{10}$ units.

Care must be taken to protect all virus samples from direct light.

3. Combined vaccines

For assays of the measles component in multivalent vaccines, the heterologous component(s) must first be neutralized. Reconstituted vaccine is incubated with appropriate polyclonal or monoclonal antibodies and the vaccine is then assayed in the normal way. Allowance must be made for the initial dilution effect of the neutralizing serum. (See also Appendix 10, page 201.)

Appendix 4
Model certificate for the release of measles, mumps, rubella or combined vaccines[1]

The following lots of _____[2] vaccine produced by _____[3] in _____,[4] whose numbers appear on the labels of the final containers, meet all national requirements[5] and comply with Part A[6] of Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live) [if applicable, revised 19__, addendum 19__]),[7] Good Manufacturing Practices for Pharmaceutical Products[8] and the Good Manufacturing Practices for Biological Products.[9]

Lot no.	Expiry date	Lot no.	Expiry date

As a minimum, this certificate is based on examination of the manufacturing protocol.

The Director of the National Control Laboratory (or Authority as appropriate):[10]

Signature _____

Name (typed) _____

Date _____

[1] To be completed by the national control authority of the country where the vaccines have been manufactured, and to be provided to importers.
[2] Indicate type of vaccine.
[3] Name of manufacturer.
[4] Country.
[5] If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the National Control Authority.
[6] With the exception of the provisions on shipping, which the national control authority may not be in a position to control.
[7] WHO Technical Report Series, No. 840, 1994, Annex 3.
[8] WHO Technical Report Series, No. 823, 1992, Annex 1.
[9] WHO Technical Report Series, No. 822, 1992, Annex 1.
[10] Or his or her representative.

Appendix 5

Summary protocol for production and testing of mumps vaccine (live)[1]

The following protocol is intended *for guidance*, and indicates the information that should be provided as a minimum.

The section concerning the final product must be accompanied by a sample of the label, a copy of the leaflet that accompanies the vaccine container, and a certificate from the national control authority of the country in which the vaccine was produced stating that the product meets national requirements as well as the requirements published by WHO (see Appendix 4).

Source materials (A.4.1)

Strain of mumps virus (A.4.1.1) _____

Cell cultures (A.4.1.2)

Provide information on the source and method of preparation of the cell cultures.

Avian embryos and cell cultures (A.4.1.3)

Provide information on the source of the closed, specific-pathogen-free, healthy flock.

Types of test for infections _____ Results _____

Certified satisfactory _____ Date _____

Signature of head of laboratory _____

Human diploid cells (A.4.1.4)

Provide information on the source of the manufacturer's working cell bank (MWCB) (see Appendix 2).

Serum used in cell-culture medium (A.4.1.5)

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

[1] Based on Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live) (Requirements for Biological Substances No 47). In: *WHO Expert Committee on Biological Standardization. Forty-third Report.* Geneva, World Health Organization, 1994, Annex 3 (WHO Technical Report Series, No 840).

Tests for adventitious agents

Methods _____

Date of inoculation _____

Results _____

Trypsin used for preparing cell cultures (A.4.1.6)

Sterility tests

	bacteria	*mycoplasmas*
Date of inoculation		
Results		

Tests for adventitious agents (including porcine parvoviruses)

Methods _____

Date of inoculation _____

Results _____

Production of the working seed lot (A.4.2)

Summary information

Name and adress of manufacturer _____

_____

Virus strain _____

Reference no. of virus seed used to prepare manufacturer's original mumps vaccine that was safe and immunogenic in humans _____

Reference no. of master seed lot _____

No. of passages between the two above seeds _____

Working seed lot

Date of preparation _____

No. of containers prepared _____

Reference no. _____

Conditions of storage _____

History of vaccine strain

Provide a brief account, indicating how the vaccine strain was acquired, outlining its history up to production of the master seed lot, and specifying the criteria on which acceptability for virus production is based.

Certificat... of working seed lot

Name (typed) and signature of head of production laboratory _____

_____

Certification by the head of the control laboratory of the manufacturer taking overall responsibility for production and control of the working seed lot:

I certify that the working seed lot of mumps vaccine virus no. ____ satisfies Part A, sections 2 to 4.4.5, of the Requirements for Mumps Vaccine in Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)).

Signature _____

Name (typed) _____

Date _____

Control cell cultures (A.4.3)

Provide information on the control cell cultures corresponding to each single harvest, using extra pages if necessary.

Cell substrate used for production of virus _____

Reference no. of control cell cultures _____

Quantity of cell cultures used as control cultures _____

Period of observation of control cells _____

Test for haemadsorbing viruses (A.4.3.1)

Type of red blood cells _____

Date of test _____

Results _____

Tests for non-haemadsorbing extraneous agents (A.4.3.2)

Cell substrate used for virus growth

Type of cells _____

Date of inoculation _____

Results _____

Simian cells

Type of cells _____

Date of inoculation _____

Results _____

Human cells

Type of cells _____

Date of inoculation _____

Results _____

Additional tests if avian-embryo cell cultures are used for production (A.4.3.3)

Test for avian adenoviruses

Method _____

Date _____

Results _____

Test for avian leukosis virus

Method _____

Date _____

Results _____

Additional tests if human diploid cells are used for production (A.4.3.4)

Identity test

Method _____

Date _____

Results _____

Embryonated eggs (A.4.3.5)

Provide details of test methods and results.

Production and harvest of vaccine virus (A.4.4)

Cells used for vaccine production (A.4.4.1)

Observation of cell cultures before inoculation

Methods _____

Results _____

Antibiotics added (if used) _____

Concentration _____

Single harvests (A.4.4.2)

Report the results of tests on each single harvest, using extra pages if necessary.

No. of passages from the primary seed _____

Reference no. of single harvest _____

Sterility tests	*bacteria*	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

Virus pool (A.4.4.3)

Reference no. of virus pool _____

If any test had to be repeated or any abnormal result was observed, this must be specified.

Tests for neurovirulence[1] (A.4.2.1)

No. of monkeys in test _____

Species _____

Volume injected _____

No. of monkeys surviving without specific symptoms _____

Results of serological tests _____

Results of histopathological examination (specify findings) _____

Sterility tests	*bacteria*	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

[1] Only for master seed or working seed lot

Tests of neutralized virus pool in cell cultures

Species in which neutralizing serum was prepared and cell substrate in which immunogen was produced _____

Cells used for virus growth

Type of cells _____

Date of inoculation _____

Results _____

Simian cells

Type of cells _____

Date of inoculation _____

Results _____

Human cells

Type of cells _____

Date of inoculation _____

Results _____

Additional tests if avian eggs or cell cultures are used for production

Test in embryonated eggs inoculated by allantoic route

No. and age of eggs inoculated _____

Date _____

Results _____

Test in embryonated eggs inoculated by yolk-sac route

No. and age of eggs inoculated _____

Date _____

Results _____

Clarification of the virus pool (A.4.4.4)

Date of clarification _____

Results of clarification _____

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Final bulk (A.4.4.5)

Reference no. of final bulk

Total volume of final bulk

Added substances (diluent, stabilizer) and final concentration

Residual animal serum proteins

Date

Method

Results (indicate amount and nature of serum protein(s) present per human dose)

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Filling and containers (A.5)

Name and address of manufacturer

Proprietary name of vaccine

Reference no. of final lot

Expiry date

No. of containers in the lot

No. of doses per container

Lot no. of final bulk

Date of filling of final containers

Control tests on final product (A.6)

Identity test (A.6.1)

Date

Method

Results

Sterility tests (A.6.2)

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Virus concentration and thermostability (A.6.3)

Date of inoculation

Type of cell cultures

	Control (unheated) samples			*Samples incubated at 37°C for 7 days*		
	1	*2*	*3*	*1*	*2*	*3*
Virus concentration in each container (in PFU or $CCID_{50}$)						
Mean virus titre per human dose, with 95% fiducial limits						
Mean loss in titre due to heat exposure (in $\log_{10}$ units)						
Reference preparation						
Identification						
Theoretical titre						
Actual titre						

General safety tests (A.6.4)

Test in mice

Date of inoculation

No. of mice tested

Volume and route of injection

Observation period

Results (give details of deaths)

Test in guinea-pigs

Date of inoculation

No. of guinea-pigs tested

Volume and route of injection

Observation period

Results (give details of deaths)

Residual moisture (A.6.5)

Date _____

Method _____

Size of sample _____

Moisture content (%) _____

Inspection of final containers (A.6.6)

Date and result _____

Submission addressed to national control authority for batch release

Name (typed) and signature of head of production laboratory _____

_____

Date _____

Certification by person taking overall responsibility for production and control of the vaccine:

I certify that lot no. _____ of mumps vaccine (live) satisfies national requirements and/or Part A of the Requirements for Mumps Vaccine in Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)).

Signature _____

Name (typed) _____

Date _____

Appendix 6

Proposed assay method for determining the virus content of live mumps vaccine

1. Cell-culture infective dose method

Three vials of the preparation to be assayed and at least one of the reference preparation are reconstituted and diluted in an appropriate medium. It is convenient to use dilution steps of 0.5 $\log_{10}$ units, selected so that the dilution range encompasses at least three dilutions that will infect between 10% and 90% of the cultures inoculated.

For each vaccine dilution, a volume of 0.1 ml is dispensed into each of 8–12 flat-bottomed wells in a microtitre plate, followed by 0.1 ml of a suitable cell suspension of the Vero cell line. The plates are incubated at the optimal growth temperature for the tested mumps virus strain.

The presence or absence of a cytopathic effect is recorded after four days, with the final reading at 7–10 days; if necessary, the medium should be changed after five days of incubation. The presence or absence of tissue-culture infection can also be determined by immunofluorescence. The titre is calculated as a multiple of the median cell-culture infective dose ($CCID_{50}$) by an appropriate method. If the potency is below the expected value, the assay can be repeated and the mean of all assays calculated. The assay is not considered valid when:

- the mean titre of the reference vaccine deviates by more than 0.5 $\log_{10}$ units from the established titre; and/or
- the difference in titre between any two vials of the test vaccine or between the vials of the reference vaccine is >0.5 $\log_{10}$ units.

Care must be taken to protect all virus samples from direct light.

2. Plaque method

Three vials of the preparation to be assayed and at least one of the reference preparation are reconstituted and diluted in an appropriate medium. It is convenient to use dilution steps selected to give at least 50% of wells with between 10 and 80 plaques per well.

For each vaccine dilution, a volume of 0.05 ml is dispensed into each of three wells in a 24-well tissue-culture plate. A separate plate is used for each of the three test samples and the reference preparation is included in each plate. The wells are seeded with 1.0 ml of an appropriate cell suspension of the Vero cell line and the plates are incubated for three hours, under 5% carbon dioxide, at the optimum growth temperature for the tested mumps virus strain. The medium is then removed from each well and replaced with 1.0 ml of medium containing 0.8% carboxymethylcellulose or other appropriate overlay. The plates are then incubated as before for 7–10 days depending on the strain tested.

Plaques visualized by fixing and staining the cell sheets. A convenient reagent for both purposes is 0.5% crystal violet in 20% ethanol. The medium is first removed from each well and the cell sheet gently washed with buffer. Crystal violet in ethanol is added and the plates are left at room temperature for 20 minutes. The stain is then removed, the cells are washed gently with water and the plates are dried. The plaques are counted and wells with numbers between 10 and 80 are considered valid. The titre is calculated in plaque-forming units (PFU) per dose from the direct counts.

If the potency is below the expected value, the assay can be repeated and the mean of all assays calculated. The assay is not considered valid when:

- the mean titre of the reference vaccine deviates by more than 0.5 $\log_{10}$ units from the established titre; and/or
- the difference in titre between any two vials of the test vaccine or between the vials of the reference vaccine is >0.5 $\log_{10}$ units.

Care must be taken to protect all virus samples from direct light.

3. Combined vaccines

For assays of the mumps component in multivalent vaccines, the heterologous component(s) must first be neutralized. Reconstituted vaccine is incubated with appropriate polyclonal or monoclonal antibodies and the vaccine is then assayed in the normal way. Allowance must be made for the initial dilution effect of the neutralizing serum. (See also Appendix 10, page 201.)

Appendix 7

Summary protocol for production and testing of rubella vaccine (live)[1]

The following protocol is intended *for guidance*, and indicates the information that should be provided as a minimum.

The section concerning the final product must be accompanied by a sample of the label, a copy of the leaflet that accompanies the vaccine container, and a certificate from the national control authority of the country in which the vaccine was produced stating that the product meets national requirements as well as the requirements published by WHO (see Appendix 4).

Source materials (A.4.1)

Strain of rubella virus (A.4.1.1) _____

Cell cultures (A.4.1.2)

Provide information on the source and method of preparation of the cell cultures.

Human diploid cells (A.4.1.3)

Provide information on the source of the manufacturer's working cell bank (MWCB) (see Appendix 2).

Rabbit kidney cell cultures (A.4.1.4)

Provide information on the source and method of preparation of the cell cultures.

Serum used in cell-culture medium (A.4.1.5)

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

[1] Based on Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live) (Requirements for Biological Substances No. 47). In: *WHO Expert Committee on Biological Standardization. Forty-third Report.* Geneva, World Health Organization, 1994, Annex 3 (WHO Technical Report Series, No. 840).

Tests for adventitious agents

Methods _____

Date of inoculation _____

Results _____

Trypsin used for preparing cell cultures (A.4.1.6)

Sterility tests

	bacteria	*mycoplasmas*
Date of inoculation	_____	_____
Results	_____	_____

Tests for adventitious agents (including porcine parvoviruses)

Methods _____

Date of inoculation _____

Results _____

Production of the working seed lot (A.4.2)

Summary information

Name and address of manufacturer _____

_____

Virus strain _____

Reference no. of virus seed used to prepare manufacturer's original rubella vaccine that was safe and immunogenic in humans _____

Reference no. of master seed lot _____

No. of passages between the two above seeds _____

Working seed lot

Date of preparation _____

No. of containers prepared _____

Reference no. _____

Conditions of storage _____

History of vaccine strain

Provide a brief account, indicating how the vaccine strain was acquired, outlining its history up to production of the master seed lot, and specifying the criteria on which acceptability for virus production is based.

Certification of working seed lot

Name (typed) and signature of head of production laboratory _____

_____

Certification by the head of the control laboratory of the manufacturer taking overall responsibility for production and control of the working seed lot:

I certify that the working seed lot of rubella vaccine virus no. _____ satisfies Part A, sections 2 to 4.4.5, of the Requirements for Rubella Vaccine in Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)).

Signature _____

Name (typed) _____

Date _____

Control cell cultures (A.4.3)

Provide information on the control cell cultures corresponding to each single harvest, using extra pages if necessary.

Cell substrate used for production of virus _____

Reference no. of control cell cultures _____

Quantity of cell cultures used as control cultures _____

Period of observation of control cells _____

Test for haemadsorbing viruses (A.4.3.1)

Type of red blood cells _____

Date of test _____

Results _____

Tests for non-haemadsorbing extraneous agents (A.4.3.2)

Cell substrate used for virus growth

Type of cells _____

Date of inoculation _____

Results _____

Simian cells

Type of cells _____

Date of inoculation _____

Results _____

Human cells

Type of cells _____

Date of inoculation _____

Results _____

Additional test if rabbit kidney cells are used for production (A.4.3.3)

Method for detection of *Nosema cuniculi* _____

Date _____

Results _____

Additional tests if human diploid cells are used for production (A.4.3.4)

Identity test

Method _____

Date _____

Results _____

Production and harvest of vaccine virus (A.4.4)

Cells used for vaccine production (A.4.4.1)

Observation of cell cultures before inoculation

Methods _____

Results _____

Antibiotics added (if used) _____

Concentration _____

Single harvests (A.4.4.2)

Report the results of tests on each single harvest, using extra pages if necessary.

No. of passages from the primary seed _____

Reference no. of single harvest _____

Sterility tests	*bacteria*	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

Virus pool (A.4.4.3)

Reference no. of virus pool _____

If any test had to be repeated or any abnormal result was observed, this must be specified.

Tests for neurovirulence[1] (A.4.2.1)

No. of monkeys in test _____

Species _____

Volume injected _____

No. of monkeys surviving without specific symptoms _____

Results of serological tests _____

Results of histopathological examination (specify findings) _____

Sterility tests	*bacteria*	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

[1] Only for master seed or working seed lot.

Tests of neutralized virus pool in cell cultures

Species in which neutralizing serum was prepared and cell substrate in which immunogen was produced _____

Cells used for virus growth
- Type of cells _____
- Date of inoculation _____
- Results _____

Simian cells
- Type of cells _____
- Date of inoculation _____
- Results _____

Human cells
- Type of cells _____
- Date of inoculation _____
- Results _____

Additional test if rabbit kidney cell cultures are used for production

Volume tested _____

No. of rabbits inoculated _____

No. that survived test _____

Results _____

Clarification of the virus pool (A.4.4.4)

Date of clarification _____

Results of clarification _____

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Final bulk (A.4.4.5)

Reference no. of final bulk _____

Total volume of final bulk _____

Added substances (diluent, stabilizer) and final concentration _____

Residual animal serum proteins

Date _____

Method _____

Results (indicate amount and nature of serum protein(s) present per human dose) _____

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Filling and containers (A.5)

Name and address of manufacturer _____

_____

Proprietary name of vaccine _____

Reference no. of final lot _____

Expiry date _____

No. of containers in the lot _____

No. of doses per container _____

Lot no. of final bulk _____

Date of filling of final containers _____

Control tests on final product (A.6)

Identity test (A.6.1)

Date _____

Method _____

Results _____

Sterility tests (A.6.2)

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Virus concentration and thermostability (A.6.3)

Date of inoculation _____

Type of cell cultures _____

	Control (unheated) samples			*Samples incubated at 37 °C for 7 days*		
	1	*2*	*3*	*1*	*2*	*3*
Virus concentration in each container (in PFU or $CCID_{50}$)	___	___	___	___	___	___
Mean virus titre per human dose, with 95% fiducial limits	_____			_____		

Mean loss in titre due to heat exposure (in log_{10} units) _____

Reference preparation

Identification _____

Theoretical titre _____

Actual titre _____

General safety tests (A.6.4)

Test in mice

Date of inoculation _____

No. of mice tested _____

Volume and route of injection _____

Observation period _____

Results (give details of deaths) _____

Test in guinea-pigs

Date of inoculation _____

No. of guinea-pigs tested _____

Volume and route of injection _____

Observation period _____

Results (give details of deaths) _____

Residual moisture (A.6.5)

Date _____

Method _____

Size of sample _____

Moisture content (%) _____

Inspection of final containers (A.6.6)

Date and result _____

Submission addressed to national control authority for batch release

Name (typed) and signature of head of production laboratory _____

_____

Date _____

Certification by person taking overall responsibility for production and control of the vaccine:

I certify that lot no. _____ of rubella vaccine (live) satisfies national requirements and/or Part A of the Requirements for Rubella Vaccine in Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)).

Signature _____

Name (typed) _____

Date _____

Appendix 8

Proposed assay method for determining the virus content of live rubella vaccine

1. Cell-culture infective dose method

Three vials of the preparation to be assayed and at least one of the reference preparation are reconstituted and diluted in an appropriate medium. It is convenient to use dilution steps of 0.5 $\log_{10}$ units, selected so that the dilution range encompasses at least three dilutions that will infect between 10% and 90% of the cultures inoculated.

For each vaccine dilution, a volume of 0.1 ml is dispensed into each of 8-12 flat-bottomed wells in a microtitre plate, followed by 0.1 ml of a suitable cell suspension of the RK-13 cell line. The plates are incubated at the optimal growth temperature for the tested rubella virus strain.

The presence or absence of a cytopathic effect is recorded after five days, with the final reading at 10-12 days. The titre is calculated as a multiple of the median cell-culture infective dose ($CCID_{50}$) by an appropriate method. If the potency is below the expected value, the assay can be repeated and the mean of all assays calculated. The assay is not considered valid when:

- the mean titre of the reference vaccine deviates by more than 0.5 $\log_{10}$ units from the established titre; and/or
- the difference in titre between any two vials of the test vaccine or between the vials of the reference vaccine is >0.5 $\log_{10}$ units.

Care must be taken to protect all virus samples from direct light.

2. Combined vaccines

For assays of the rubella component in multivalent vaccines, the mumps component must first be neutralized. Reconstituted vaccine is incubated with appropriate polyclonal or monoclonal antibodies and the vaccine is then assayed in the normal way. Allowance must be made for the initial dilution effect of the neutralizing serum. (See also Appendix 10, page 201.)

Appendix 9

Summary protocol for production and testing of measles–mumps–rubella combined vaccine (live)[1]

The following protocol is intended *for guidance*, and indicates the information that should be provided as a minimum.

The section concerning the final product must be accompanied by a sample of the label, a copy of the leaflet that accompanies the vaccine container, and a certificate from the national control authority of the country in which the vaccine was produced stating that the product meets national requirements as well as the Requirements published by WHO (see Appendix 4).

Information on blending

Measles component

Reference no. _____

$CCID_{50}$ or PFU/ml _____

Volume _____

Mumps component

Reference no. _____

$CCID_{50}$ or PFU/ml _____

Volume _____

Rubella component

Reference no. _____

$CCID_{50}$ or PFU/ml _____

Volume _____

Final bulk of combined components (A.2.2)

Reference no. of final bulk _____

Date of completion _____

Total volume of final bulk _____

Added substances (diluent, stabilizer) _____

[1] Based on Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live) (Requirements for Biological Substances No. 47). In: *WHO Expert Committee on Biological Standardization. Forty-third Report.* Geneva, World Health Organization, 1994, Annex 3 (WHO Technical Report Series, No. 840).

Residual [illegible] al serum proteins (A.2.2.1)

Date _____

Method _____

Results (indicate amount and nature of serum protein(s) present per human dose) _____

Sterility tests (A.2.2.2)

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

Filling and containers (A.3)

Name and address of manufacturer _____

_____

Proprietary name of vaccine _____

Reference no. of final lot _____

Expiry date _____

No. of containers in the lot _____

No. of doses per container _____

Lot no. of final bulk _____

Date of filling of final containers _____

Control tests on final product (A.4)

Sterility tests (A.4.1)

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

Virus concentration, thermostability and identity (A.4.2)

Record the results of virus titration and the thermostability test in the table opposite.

Identity test

	measles	*mumps*	*rubella*
Date of test	_____	_____	_____
Methods	_____	_____	_____
Results	_____	_____	_____

Virus concentration and thermostability test (A.4.2)

	Control (unheated) samples									Samples incubated at 37 °C for 7 days								
	Measles			Mumps			Rubella			Measles			Mumps			Rubella		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Virus concentration in each container (in PFU or $CCID_{50}$)																		
Mean virus titre per human dose, with 95% fiducial limits																		
Mean loss in titre due to heat exposure (in $\log_{10}$ units)																		
Reference preparation																		
Identification																		
Theoretical titre																		
Actual titre																		

General safety tests (A.4.3)

Test in mice

Date of inoculation _____

No. of mice tested _____

Volume and route of injection _____

Observation period _____

Results (give details of deaths) _____

Test in guinea-pigs

Date of inoculation _____

No. of guinea-pigs tested _____

Volume and route of injection _____

Observation period _____

Results (give details of deaths) _____

Residual moisture (A.4.4)

Date _____

Method _____

Size of sample _____

Moisture content (%) _____

Inspection of final containers (A.4.5)

Date and result _____

Submission addressed to national control authority for batch release

Name (typed) and signature of head of production laboratory _____

Date _____

Certification by person taking overall responsibility for production and control of the vaccine:

I certify that lot no. _____ of measles-mumps-rubella combined vaccine (live) satisfies national requirements and/or Part A of the Requirements for Measles-Mumps-Rubella Combined Vaccine in Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)).

Signature _____

Name (typed) _____

Date _____

Appendix 10

Proposed assay method for determining the virus content of measles-mumps-rubella combined live vaccine

Before titration of each individual virus in combined vaccine, the other components should be selectively neutralized, as necessary, with specific antisera: for measles virus determination, neutralize the mumps and rubella components; for rubella virus, neutralize the mumps component (it is not necessary to neutralize measles virus as it will not grow in RK-13 cells); and for mumps virus, neutralize the measles and rubella components.

Antisera

All antisera should be heated for 30 minutes at 56 °C before use. Antisera against measles, mumps and rubella viruses should have a titre of at least 1:320 against 500 $CCID_{50}$ of the respective viruses as determined in the test described in Appendices 3, 6 and 8.

The suitability of each antiserum must be demonstrated, at the appropriate concentration, and the antiserum must be shown to neutralize the homologous virus after 1.5 hours at 20 °C and not to reduce the titre of the heterologous viruses under the same conditions.

Procedures

Dilute the antisera as appropriate to the type of virus, the diluent and the titre required.

Reconstitute the vaccine samples and the references with an appropriate diluent and make dilutions with the antisera as required. Prepare the reference vaccines (if monovalent) by dilution both in diluent alone and in combination with antisera. Take precautions to protect all samples from direct light.

Incubate all dilutions at 20 °C for 1.5 hours, and titrate each virus according to the methods described for the monovalent vaccines (Appendices 3, 6 and 8).

E. ORAL POLIO VACCINE (OPV)

Documents provided in this section include:

1. Sample Procurement Requirements OPV Vaccine 407
2. OPV Vaccine Model Package Insert (UNICEF) 415
3. Poliovirus Vaccine Live Oral, *USP 23 - NF 18*, 1994 416
4. Poliovirus Vaccine Live Oral Trivalent, US Food and Drug Administration HHS 21 CFR, Part 630, Subpart B 417
5. Requirements for Poliomyelitis Vaccine (Oral) (Requirements for Biological Substances No. 7, revised 1989), WHO *Technical Report Series* No. 800, 1990 428

SAMPLE

PROCUREMENT REQUIREMENTS

OPV VACCINE

A. General Description of Intended Use

This vaccine is intended for use in a public-sector health programme for immunization of children (under one year of age) in _____ [*place*] beginning on _____ [*date*].

The total requirement for this solicitation is _____ [*quantity*] doses. _____ [name] expects to procure additional quantities of vaccine annually beginning in _____ [*month, year*] and subsequent years as follows: _____ [*quantity*], _____ [*quantity*] and _____ [*quantity*].

B. Specific Requirements

Item: Poliovirus Vaccine, live, oral.

Trivalent preparation of live, attenuated poliovirus Type 1, 2 and 3; tested for neurovirulence in monkeys, safety and immunogenicity; free from all demonstrable viable microbial agents except unavoidable bacteriophage; and found suitable for human immunization.

The vaccine is grown in *in vitro* cultures of suitable cells, containing any one type or any combination of the three types of the Sabin strains, prepared in a form suitable for oral administration.

Each dose shall contain not less than _____ [*specify*] infective units of type 1, and _____ of type 2 and _____ of type 3.

_____ [*specify*] is used as a stabilizer.

The vaccine shall meet _____ [*specify*] requirements for Poliomyelitis Vaccine (Oral) (WHO Requirements for Biological Substances No. 7, revised 1989 or equivalent requirements of the USFDA as detailed in 21 CFR, or another fully equivalent set of biological requirements). *Editors' note: It is recognised that equivalence does not mean identical, only that both provide the same level of potency, safety and efficacy.*

The vaccine shall be currently registered in the country of manufacture and shall meet all requirements of the licensing authority of the country of manufacture. The vaccine also shall be currently registered in the country of use and shall meet all requirements of the licensing authority of the country of use.

Quantity:	_____ doses from one production lot.
Dosage Size:	Two oral drops containing 0.1 ml (may be up to 0.5 ml depending on concentration).
Dose Package:	Vials of 10 doses (with dropper). Vials of 20 doses (with dropper).
Filling Volume:	Each 10-dose vial should contain 1 ml (may be up to 5 ml depending on concentration) plus 15% overfill. Each 20-dose vial should contain 2 ml (may be up to 10 ml depending on concentration) plus 15% overfill.
Tolerance:	Volume shall not vary by more than 5%. Weight of the antigen shall not vary by more than 5%.

Storage Temperature:	Below +8 degrees C; may be frozen.
Shelf Life:	6 months at -20 degrees C plus 6 months at a temperature not higher than +5 degrees C (from the last satisfactory test for potency); at least _____ months must remain after shipment.
Stability:	Stable for a minimum of _____ years at _____ to _____ degrees C; manufacturer to specify thermal stability characteristics.
Labelling:	The label on each vial shall conform to the requirements of the country of use and shall appear in the language of _____. All labelling shall withstand immersion in water and remain intact. All labels shall state the name of the vaccine, name of the manufacturer, place of manufacture, lot number, composition, concentration, dose and mode of administration, expiry date, storage temperature and any other marking that is appropriate. The label on each vial should include a Vaccine Vial Monitor (VVM) designed to meet the heat stability curve of the vaccine supplied (see Attachment One).
Optional Labelling:	If vaccine is destined for use in public-sector immunization programs, the statement "Not for Sale; For Use Only in Government-Approved Immunization Programmes" shall appear on each label in the language of the purchasing country.
Printed Materials:	Two (2) information sheets, printed in _____ [*language*], shall be included in each

box of ___ vials (see Model Package Insert, attached).[27]

C. Quality Assurance

Compliance: The Supplier shall guarantee that the products as packed for shipment (a) comply with all provisions of the specification and related documents; (b) meet internationally recognized standards for safety, efficacy and quality; (c) are fit for the purposes made known to the Seller and (d) are free from defects in workmanship and in materials.

Evidence: The Supplier shall provide objective evidence, acceptable to the Purchaser, of the satisfaction of the requirements of this document for which no specific inspection has been mentioned.

The Supplier shall provide a copy of the manufacturing record and procedures to the Purchaser for each lot intended for shipment.

The Supplier shall provide a copy of the batch record and all quality assurance documentation to the Purchaser for each lot being supplied.

The Supplier shall provide a copy of the Certificate of Analysis to the Purchaser for each lot intended for shipment.

The Supplier shall provide to the Purchaser a copy of the approval of each source material, constituent material and component for each lot intended for shipment.

The Supplier shall retain a sample of twenty (20) vials from each lot shipped for two years beyond the printed expiration date.

[27] See example, page 415.

Chemical, physical and microbiological test data for in-process and finished product testing must be on record for each lot shipped and must be available to Purchaser's representatives when requested.

Inspection: The Purchaser may inspect and sample, or cause to be sampled, the product at the Supplier's factory and/or warehouse at a mutually agreeable time prior to shipment of the product.

Testing: The Purchaser may cause independent laboratory testing to be performed as deemed necessary to assure that the goods conform to prescribed requirements. Said testing laboratory shall be of the Purchaser's choice and suitably equipped and qualified to conduct quality assurance tests on biological products.

D. Packing

Storage: Supplier shall state storage volume occupied per dose of vaccine (storage volume includes the vaccine vial, packet containing the vaccine vial and any intermediate packaging).

Inner Boxes: _____ [*number*] individual glass vials will be contained in sturdy white cardboard boxes outfitted with individual segments for protecting and separating each vial.

Cold Chain Monitor Cards: Cold chain monitor cards (description attached)[28] in _____ language shall be packed with each ___ doses of vaccine.

Overpacking: Boxes shall be overpacked so that the vaccine remains refrigerated at below +8 degrees C. The containers must be suitable for export handling

[28] See example in Supplement VI.G.1.a., page 783.

in accordance with WHO/EPI Guidelines on International Packaging and Shipping of Vaccines. They must have adequate insulation and/or sufficient refrigerant to ensure that the warmest storage temperature of the vaccine does not rise above +8 degrees C in continuous outside ambient temperature of +43 degrees C during transit and for a period of at least 48 hours after arrival at the airport of destination.

Additional cushioning shall be provided, sufficient to protect the vials from breakage during transit and handling.

Exterior Shipping Cartons: Product and printed materials, packaged as specified above, shall be packed in weather-resistant, triple-wall corrugated fibreboard cartons with a bursting test strength of not less than 1,900 kPa.

The overall dimensions of the exterior shipping cartons should be such that the product does not become damaged during transportation and storage.

E. Markings

All containers and invoices must bear the name of the vaccine, expiry dates of the vaccine and appropriate storage temperature.

Inner Boxes: The inner boxes containing vaccine vials shall be marked with the following information in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Manufacturer's name and registered address
- Manufacturer's national registration number
- Lot or batch number
- Composition and concentration

- Number of vials contained in box
- Expiration date (month and year)
- Instructions for storage and handling†
- Place of manufacture (Made in _____)

Exterior Shipping Cartons: The following information shall be stencilled or labelled on the exterior shipping cartons on two opposing sides in bold letters at least __ mm high with waterproof ink in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Lot or batch number
- Expiration date (month and year)
- Manufacturer's name and registered address
- Manufacturer's national registration number
- Destination country license or registration number
- Consignee's address and emergency phone number
- Destination airport
- Contract number
- Number of vials contained in the carton
- Gross weight of each carton (in kg)
- Carton #___ of ___
- Instructions for storage and handling†
- Place of manufacture (Made in _____)

F. Documentation

Supplier shall provide to Purchaser a copy of the batch record, including all quality assurance documentation for the vaccine being supplied.

† To be provided by Purchaser.

Attachment One

Vaccine Vial Monitor (VVM)

A circle of colour, minimum diameter 7.0 mm, with a square of colour, minimum dimensions 2.0 x 2.0 mm, positioned in the center of the circle; used to warn health workers when the cumulative heat exposure of a vial of vaccine has exceeded a pre-set limit, beyond which the vaccine should not be used.

The circle of the VVM acts as a static, reference colour and the square is an active colour change device. At the start point, the colour of the square is lighter than the circle. The end point is indictaed when the colour of the square matches the circle. The end point is exceeded when the colour of the square is darker than the circle. The indicator does not revert to a lighter colour at any point in its life when exposed to conditions likely to be found during normal use. Reaction rates are specific to different vaccines according to their heat stability.

ORAL POLIO VACCINE

DESCRIPTION
The live oral poliomyelitis vaccine (OPV) is a trivalent vaccine containing suspensions of types 1, 2 and 3 attenuated poliomyelitis viruses (Sabin strains). Each dose contains not less than _____ (specify) infective units of type 1, and _____ of type 2, and _____ of type 3. _____ (specify) is used as a stabilizer.

ADMINISTRATION
OPV must only be administered orally. Two drops are delivered directly into the mouth from the multi-dose vial through dropper or dispenser. For older children it may be preferred to avoid the possible bitter taste by first placing the drops on a sugar lump or in syrup. Care should be taken not to contaminate a multi-dose dropper with saliva of the vaccinee. Opened vials of vaccine may be used in subsequent immunization sessions until a new shipment of vaccine arrives providing that the conditions described in WHO/EPI/LH15/95.1 are met.

IMMUNIZATION SCHEDULE
Infants should receive at least three doses of OPV at minimum intervals of 4 weeks. In non-endemic areas the first dose can be given from 6 weeks with the first dose of DTP. In endemic areas, an extra dose should be given as soon as possible after birth.

OPV can be given safely and effectively at the same time as measles, DTP, DT, Td, TT, BCG, Hepatitis B and Yellow Fever vaccine.

SIDE EFFECTS
In the vast majority of cases there are no side effects. Very rarely, there may be vaccine-associated paralysis (less than one case per 3 million doses administered).

CONTRAINDICATIONS
The vaccine should not be administered to those with an immune deficiency. No adverse effects are produced by giving OPV to a sick child. However, if in doubt, for instance during diarrhoea, a repeat dose can be given after recovery.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED INFANTS: HIV infected infants, both asymptomatic and symptomatic, should be immunized with OPV according to standard schedules.

STORAGE
Vaccine is potent if stored at not higher than -20°C until the expiry date indicated on the vial. It should not be stored for a period of longer than six months at a temperature not higher than +5°C. Protect from light.

PRESENTATION
The vaccine comes in vial of _____ doses.

the thaw is not more than 24 hours. Label the Vaccine to state the type of tissue in which it was prepared and to state that it is not for injection.

Poliovirus Vaccine Live Oral

» Poliovirus Vaccine Live Oral conforms to the regulations of the federal Food and Drug Administration concerning biologics (630.10 to 630.18) (see *Biologics* ⟨1041⟩). It is a preparation of a combination of the three types of live, attenuated polioviruses derived from strains of virus tested for neurovirulence in monkeys in comparison with the U. S. Reference Attenuated Poliovirus, Type 1, for such tests, and for immunogenicity, free from all demonstrable viable microbial agents except unavoidable bacteriophage, and found suitable for human immunization. The strains are grown, for purposes of vaccine production, separately in primary cell cultures of monkey renal tissue. Monovalent lots produced with each strain meet the requirements of the specific safety tests for absence of adventitious and other infective agents including polioviruses of other types, SV_{40}, SV_5 and foamy viruses, *Mycobacterium tuberculosis*, pox virus, lymphocytic choriomeningitis virus, Echo viruses, Coxsackie viruses, and B virus. These tests are made in rabbits, adult and suckling mice, and guinea pigs, as well as in monkey kidney, human tissue, and rabbit kidney cell cultures. The lots also meet the requirements of the specific monkey neurovirulence test in comparison with the Reference Attenuated Poliovirus, and the requirements of the specific in-vitro marker tests. The Vaccine meets the requirements of the specific tissue culture tests for live virus titer, in a single immunizing dose, of not less than $10^{5.4}$ to $10^{6.4}$ for Type 1, $10^{4.5}$ to $10^{5.5}$ for Type 2, and $10^{5.2}$ to $10^{6.2}$ for Type 3, using the U. S. Reference Poliovirus, Live, Attenuated of the corresponding type for correlation of such titers. The Vaccine is filtered to prevent possible inclusion of bacteria in the final product.

Packaging and storage—Preserve in single-dose or in multiple-dose containers at a temperature that will maintain ice continuously in a solid state. Preserve thawed Vaccine at a temperature between 2° and 8°.

Expiration date—The expiration date is not later than 1 year after date of issue from manufacturer's cold storage (−10°, 1 year).

Labeling—Label the Vaccine to state that it may be thawed and refrozen not more than 10 times, provided that the thawed material is kept refrigerated and the total cumulative duration of

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Subpart B—Poliovirus Vaccine Live Oral Trivalent

SOURCE: 56 FR 21432, May 8, 1991, unless otherwise noted.

§ 630.10 Poliovirus Vaccine Live Oral Trivalent.

(a) *Proper name and definition.* The proper name of this product shall be Poliovirus Vaccine Live Oral Trivalent. The vaccine shall be a preparation containing the three types of live, attenuated polioviruses grown in monkey kidney cell cultures, or in a cell line found by the Director, Center for Biologics Evaluation and Research, to meet the requirements of § 610.18(c) of this chapter. The vaccine shall be prepared in a form suitable for oral administration.

(b) *Criteria for acceptable strains.* (1) The Sabin strains of attenuated poliovirus, Type 1 (LS-c, 2ab/KP_2), Type 2 (P712, Ch, 2ab/KP_2), and Type 3 (Leon 12a_1b/KP_3), or derivatives from them, may be used in the manufacture of vaccine.

(2)(i) Other poliovirus strains may be used in the manufacture of Poliovirus Vaccine Live Oral Trivalent provided that they are identified by historical records including:

(A) Origin,

(B) Techniques of attenuation,

(C) Antigenic properties,

(D) Neurovirulence for monkeys,

(E) Pathogenicity for tissue cultures of various cell types, and

(F) Established virus markers, including rct/40, and d.

(ii) The data shall be submitted to the Director, Center for Biologics Evaluation and Research, along with other data that establish:

(A) That each such strain is at least as safe as the Sabin strain of the corresponding type,

(B) That each such strain demonstrates results comparable to the Sabin srain when inoculated into monkeys by the intrathalamic and intramuscular routes, and

(C) That each such strain has been used to produce vaccines meeting the safety and potency requirements of §§ 630.11, 630.15, 630.16 or 630.17, and 630.18.

(3) The Director, Center for Biologics Evaluation and Research, may prohibit the use of a specified strain whenever the Director finds that it is practicable to use another strain of the same type that will produce a vaccine of greater safety and of at least equivalent potency.

(4) If vaccine lots have been produced directly from strain materials (e.g., Sabin Original, Sabin Original Merck, or Sabin Original Rederived), the strain material is not required to be tested in accordance with the provisions of § 630.10(c).

(c) *Criteria for qualification of the seed virus.* (1) Each seed virus used in vaccine manufacture shall be prepared from an acceptable strain in monkey kidney cell cultures, derived from animals which have met all of the requirements of § 630.12(a), or in a cell culture of a type determined to be suitable by the Director, Center for Biologics Evaluation and Research. The seed virus used in vaccine manufactures shall be demonstrated to be free of extraneous microbial agents except for unavoidable bacteriophage.

(2) Seed virus used for the manufacture of oral poliovirus vaccine shall meet the requirements of §§ 630.13, 630.16 or 630.17, and 630.18. In addition, the neurovirulence of each of the first five consecutive monovalent virus pools prepared from the seed virus shall meet the neurovirulence requirements prescribed in §§ 630.16(b)(2) or 630.17 (b)(3).

(3) A new seed virus may be used for production provided data are submitted in the form of a product license a supplement that show the new seed virus and each of the first five consecutive monovalent virus pools prepared from it meet the safety requirements of §§ 630.13 and 630.16 or 630.17 and 630.18 and approval for the use of the seed virus is received in writing from the Director, Center for Biologics Evaluation and Research.

(4) Seed virus in vaccine manufacture shall be prepared in a seed lot system from a master virus seed lot at a passage level consistent with § 630.13(a).

(5) For monovalent virus pools tested in accordance with § 630.16(b), the use of the seed virus may continue provided that the frequency of monovalent virus pools produced with it which fail to meet the criteria of neurovirulence for monkeys prescribed in § 630.16(b)(2) is not greater than predicted on the basis of comparison with the corresponding reference preparation. If the frequency of monovalent virus pools produced with the same seed virus which fail to meet the criteria of neurovirulence for monkeys prescribed in §§ 630.16(b)(2) is greater than the predicted 1 percent on the basis of the 99-percent fiduciary one-sided upper limit, that seed virus shall be disqualified for further use in vaccine production.

(6) For monovalent virus pools tested in accordance with § 630.17, subsequent and identical neurovirulence tests of the seed virus shall be performed in monkeys whenever there is evidence of a significant increase in the neurovirulence of the seed virus, upon introduction of a new production seed lot, and as often as is necessary to otherwise establish, to the satisfaction of the Director, Center for Biologics Evaluation and Research, that the seed virus for vaccine manufacture has maintained its neurovirulence properties as set forth in § 630.17 (b)(3).

[56 FR 21432, May 8, 1991, as amended at 59 FR 49351, Sept. 28, 1994]

§ 630.11 Clinical trials to qualify for license.

To qualify for license, the antigenicity of the vaccine shall have been determined by clinical trials of adequate statistical design conducted in compliance with part 56 of this chapter, unless exempted under § 56.104 or granted a waiver under § 56.105, and with part 50 of this chapter. Such clinical trials shall be conducted with five lots of oral poliovirus vaccine that have been manufactured by the same methods. Type specific neutralizing antibody for each type of poliovirus in the vaccine shall be induced in 90 percent or more of susceptibles after a series of doses.

§ 630.12 Animal source and quarantine; personnel.

(a) *Monkeys*—(1) *Species permissible as source of kidney tissue.* Only Macaca monkeys, Cercopithecus monkeys, or other species found by the Director, Center for Biologics Evaluation and Research, to be equally suitable, which meet the requirements of § 600.11 (f)(2) and (f)(8) of this chapter, shall be used as the source of kidney tissue for the manufacture of Poliovirus Vaccine Live Oral Trivalent.

(2) *Experimental and test monkeys.* Monkeys that have been used previously for experimental or test purposes shall not be used as a source of kidney tissue in the processing of vaccine.

(3) *Quarantine; additional requirements.* Excluding deaths from accidents or causes not due to infectious diseases, if the death rate of any group of monkeys being conditioned in accordance with § 600.11(f)(2) of this chapter exceeds 5 percent per month, the remaining monkeys may be used for the manufacture of Poliovirus Vaccine Live Oral Trivalent only if all of the monkeys survive a new quarantine period.

(b) *Personnel.* All reasonably possible steps shall be taken to ensure that personnel involved in processing the vaccine are immune to all three types of poliovirus and do not excrete poliovirus.

[56 FR 21432, May 8, 1991; 56 FR 27787, June 17, 1991]

§ 630.13 Manufacture of Poliovirus Vaccine Live Oral Trivalent.

(a) *Virus passages.* Virus in the final vaccine shall represent no more than five tissue culture passages from the original strain or no more than five tissue culture passages from a virus clone derived from one of the first five tissue culture passages of the original strain.

(b) *Virus propagated in primary monkey kidney cell cultures*—(1) *Continuous cell lines.* When primary monkeys kidney cell cultures are used in the manufacture of poliovirus vaccine, continuous cell lines shall not be introduced or propagated in vaccine manufacturing areas.

(2) *Identification of processed kidneys.* The kidneys from each monkey shall be processed separately. The resulting viral fluid shall be identified as a separate monovalent harvest and kept separately from other monovalent harvests until all samples for the tests prescribed in paragraphs (b)(3) and (b)(4) of this section relating to that pair of kidneys have been withdrawn from the harvest.

(3) *Monkey kidney tissue production vessels prior to virus inoculation.* Prior to inoculation with the seed virus and at least 3 days after complete formation of the tissue sheet, the tissue culture growth in vessels derived from each pair of kidneys shall be examined microscopically for evidence of cell degeneration. If such evidence is observed, the tissue cultures from that pair of kidneys shall not be used for poliovirus vaccine manufacture. To test the tissue found free of cell degeneration for further evidence of freedom from demonstrable viable microbial agents, the fluid shall be removed from the cell cultures immediately prior to virus inoculation and tested in each of four culture systems:

(i) Macaca monkey kidney cells,

(ii) Cercopithecus monkey kidney cells,

(iii) Primary rabbit kidney cells, and

(iv) Cells from one of the systems described in § 630.18(a)(6).

The fluid shall be tested in the following manner: Aliquots of fluid from each vessel derived from the same pair of kidneys shall be pooled and at least 10 milliliters of the pool inoculated into each system. The dilution of the

pool with medium shall be no greater than 1:4 and the area of surface growth of cells shall be at least 3 square centimeters per milliliter of test inoculum. The cultures shall be observed for at least 14 days. At the end of the observation period, at least one subculture of fluid from the Cercopithecus monkey kidney cell cultures shall be made in the same tissue culture system and the subculture shall be observed for at least 14 days. If these tests indicate the presence in the monkey kidney tissue culture production vessels of any viable microbial agent, the viral harvest from these tissue cultures so implicated shall not be used for poliovirus vaccine manufacture.

(4) *Control vessels.* At least 25 percent of the cell suspension from each pair of kidneys shall be set aside and used to establish control cultures. The control cultures shall be examined microscopically for cell degeneration for an additional 14 days. The culture fluids from such control cells shall be tested, both at the time of virus harvest and at the end of the additional observation period, by the method prescribed for testing of fluids in paragraph (b)(3) of this section. In addition, the control cell sheet shall be examined for presence of hemadsorbing viruses by the addition of guinea pig red blood cells.

(5) *Interpretation of test results.* At least 80 percent of the control vessels shall be free of cell degeneration at the end of the observation period to qualify the kidneys for poliovirus vaccine manufacture. If the test results of the control cells indicate the presence of any extraneous agent at the time of virus harvest, the virus harvest from that tissue culture preparation shall not be used for poliovirus vaccine manufacture. If any of the tests or observations described in paragraph (b)(3) or (b)(4) of this section demonstrate the presence in the tissue culture preparation of any microbial agent known to be capable of producing human disease, the virus grown in each tissue culture preparation shall not be used for poliovirus vaccine manufacture.

(6) *Temperature of kidney tissue production vessels after virus inoculation.* After virus inoculation, production vessels shall be maintained at 33.0 to 35.0 °C during the course of virus propagation.

(7) *Kidney tissue virus harvests.* Virus shall be harvested not later than 72 hours after virus inoculation. Virus harvested from vessels containing the kidney tissue from one monkey may be tested separately, or samples of viral harvests from more than one pair of kidneys may be combined, identified, and tested as a monovalent virus pool. Each pool shall be mixed thoroughly and samples withdrawn for testing as prescribed in §630.18(a). The samples shall be withdrawn immediately after harvesting and prior to further processing, except that samples of test materials frozen immediately after harvesting and maintained at −60 °C or below, may be tested upon thawing, provided no more than one freeze-thaw cycle is employed.

(8) *Filtration.* After harvesting and removal of samples for the safety tests prescribed in §630.18(a), the pool shall be passed through sterile filters having a sufficiently small porosity to assure bacteriologically sterile filtrates.

[56 FR 21432, May 8, 1991, as amended at 58 FR 19609, Apr. 15, 1993]

§630.14 Reference virus preparations.

(a) *Titration test controls.* The following reference viruses may be obtained from the Center for Biologics Evaluation and Research:

(1) Reference Poliovirus, Live, Attenuated, Type 1, as a control for correlation of virus titers in tissue cultures.

(2) Reference Poliovirus, Live, Attenuated, Type 2, as a control for correlation of virus titers in tissue cultures.

(3) Reference Poliovirus, Live, Attenuated, Type 3, as a control for correlation of virus titers in tissue cultures.

(4) Reference Poliovirus, Live, Attenuated, Trivalent, as a control for correlation of virus titers in tissue cultures.

(b) *Neurovirulence test controls.* (1) Except as provided in paragraph (b)(2) of this section, the following reference virus may be obtained from the Center for Biologics Evaluation and Research:

(i) Reference Attenuated Poliovirus, Type 1, as a control for evaluation of monkey neurovirulence tests.

(ii) Reference Attenuated Poliovirus, Type 2, as a control for evaluation of monkey neurovirulence tests.

(iii) Reference Attenuated Poliovirus, Type 3, as a control for evaluation of monkey neurovirulence tests.

(2) Alternatively, upon FDA approval, World Health Organization (WHO) reference standards of the corresponding type, WHO/I, WHO/II, and WHO/III, may be used as controls for evaluation of monkey neurovirulence tests.

§ 630.15 Potency test.

(a) *Test for virus titer.* The concentration of living virus in each monovalent virus pool and in each trivalent vaccine, expressed as infectivity titer per milliliter for cell cultures, shall be determined using the Reference Poliovirus, Live, Attenuated of the same type as a control or using another reference preparation of the same type that has been calibrated against the appropriate reference preparation listed in § 630.14(a). A titration of the monovalent virus pool or the trivalent vaccine shall not constitute a valid test unless the titration of the reference virus when tested in parallel is within $\pm 0.5 \log_{10}$ of its established titer. The titration of the parallel reference is intended to validate the test system and shall not be used to adjust the titer of the pool or lot under test.

(b) *Dose.* The human dose of trivalent vaccines shall be constituted to have infectivity titers in the final container material of $10^{6.0}$ to $10^{7.0}$ for type 1, $10^{5.1}$ to $10^{6.1}$ for type 2, and $10^{5.8}$ to $10^{6.8}$ for type 3, when assayed in HEp–2 cells, or the equivalent when titrated by a different method.

§ 630.16 Test for neurovirulence.

(a) Except as provided in § 630.17, the following test relating to safety prescribed in paragraph (b) of this section shall be performed on each monovalent virus pool after the filtration process.

(b) *Neurovirulence in monkeys.* Except as provided in paragraph (b)(5) of this section, each monovalent virus pool shall be tested concurrently with the corresponding type Reference Attenuated Poliovirus for neurovirulence by the intraspinal route of injection in Macaca monkeys. Whenever possible the monkeys should be of comparable age and weight and from the same quarantine group. The monkeys shall be distributed randomly between the two test groups. If the number of monkeys included in both groups precludes completion during a single workday, approximately equal numbers of monkeys shall be inoculated with the monovalent virus pool and the reference preparation during each of the testing days. A preinjection serum sample obtained from each monkey shall be shown to contain no neutralizing antibody in a dilution of 1:4 when tested against no more than 1,000 $TCID_{50}$ (mean tissue culture infectious doses) of each of the three types of poliovirus. The neurovirulence test is not valid unless the inoculation sample is shown to contain the equivalent of $10^{6.5}$ to $10^{7.5}$ $TCID_{50}$ per milliliter when a representative sample of the monovalent virus pool is titrated in HEp-2 cells in comparison with the Reference Poliovirus, Live, Attenuated of the appropriate type. All monkeys shall be observed for 17 to 21 days and any evidence of physical abnormalities indicative of poliomyelitis or other viral infections shall be recorded.

(1) *Intraspinal inoculation.* For tests with type 1 and type 2 monovalent virus pools and the Reference Attenuated Poliovirus of the corresponding types, each of a group of at least 12 monkeys after being suitably anesthetized shall be injected intraspinally into the enlargement of the lumbar cord with 0.1 milliliter of the inoculation sample. For tests with type 3 poliovirus materials, groups of at least 20 monkeys shall be injected as above after being suitably anesthetized. A test of a virus pool shall include at least one group of monkeys, and no more than three groups shall be inoculated, with the results from testing one, two, or three groups of monkeys being evaluated as prescribed in § 630.16(b)(2). In addition, if on examination there is no evidence of correct inoculation, additional animals may be inoculated in order to reestablish the minimum number of 11 positive monkeys for tests of types 1 and 2 virus

pools and the minimum number of 18 positive monkeys for tests of Type 3 virus pools. A positive monkey is an animal which either survives for 11 or more days or succumbs or is sacrificed due to a severe poliovirus infection at any time before the 11th day of the observation period and in which neural lesions specific for poliovirus are seen in the central nervous system. If at least 60 percent of the animals of a group survive 48 hours after inoculation, those animals that did not survive may be replaced by additional animals. If less than 60 percent of the animals in a group survive 48 hours after inoculation, the test shall be considered invalid and shall be repeated.

(2) *Determination of neurovirulence.* At the conclusion of the observation period, the animals are sacrificed and a comparative evaluation shall be made of the evidence of neurovirulence of the monovalent virus pool under test and the Reference Attenuated Poliovirus of the corresponding type with respect to the histopathology of lesions caused by poliovirus. Animals dying or sacrificed when severely paralyzed or moribund during the test period, should be included in the evaluation, except that these examinations of these monkeys shall be made immediately after death. Histopathological examinations by a qualified pathologist shall be made of at least the lumbar and cervical enlargements, the medulla, the mesencephalon, the thalamus, and motor cortex of each monkey in the groups injected with the monovalent virus pool or with the reference under test. The magnitude of the neuropathology exhibited in the lumbar and cervical areas, the medulla, and mesencephalon of all positive monkeys inoculated with the monovalent virus pool shall be quantified and compared to the magnitude of the neuropathology determined based on the same type of evaluation of monkeys in the current test and all previous tests of the Reference Attenuated Poliovirus of the corresponding type. The monovalent virus pool may be used for poliovirus vaccine if a comparative analysis of the test results demonstrates that the numerical value assigned for neurovirulence of the monovalent virus pool is equal to or less than that of the Reference Attenuated Poliovirus of the corresponding type. If the numerical value assigned for neurovirulence of the monovalent virus pool is greater than that of the Reference Atteneuated Poliovirus, the monovalent virus pool is acceptable if the difference is not greater than that calculated by a mathematical method that is expected to reject vaccines with neurovirulence identical to the reference at a frequency of not less than 1 in 100 when 1 group of monkeys is inoculated. If 2 groups are injected with the same monovalent virus pool under test, the frequency of rejection shall be not less than 5 in 100 and for 3 groups, not less than 10 in 100. If the difference in numerical values is greater than that calculated, irrespective of which reference preparation was used in the test, the monovalent virus pool shall be considered unacceptable and shall not be used for vaccine manufacture.

(3) *Outlier scores.* In the event that one or more monkeys inoculated with virus from the monovalent virus pool have individual mean lesion scores higher than that previously or concurrently associated with the Reference Attenuated Poliovirus of the corresponding type, but the monovalent virus pool meets the criteria for acceptable neurovirulence given in § 630.16(b)(2), the significance of the outlier scores shall be evaluated by a method approved by the Director, Center for Biologics Evaluation and Research before the vaccine may be released for use.

(4) *Test with Reference Attenuated Poliovirus.* Except as provided in paragraph (b)(5) of this section, the Reference Attenuated Poliovirus of the appropriate type shall be tested as prescribed in paragraph (b)(1)(i) of this section concurrently with the monovalent virus pool. More than one monovalent virus pool of the same type may be tested with the same corresponding Reference Attenuated Poliovirus. Initially, a minimum of four tests by the testing laboratory of each Reference Attenuated Poliovirus is required. These tests must be such as to provide sufficient experience to define the performance of the Reference Attenuated Poliovirus and establish the variability of the assay. Each test of

the Reference Attenuated Poliovirus shall be considered acceptable and added to the previous testing experience only if the magnitude of its poliovirus neuropathology is statistically compatible with the results of all previous tests with the same reference preparations of the same type performed by the testing laboratory.

(5) *Alternative procedures in case of monkey shortage.* In the event of a shortage of test monkeys and upon approval of the Director, Center for Biologics Evaluation and Research, a monovalent virus pool may be tested without concurrent testing of the corresponding type Reference Attenuated Poliovirus. In such a case, the magnitude of the neuropathology of the monovalent virus pool shall be compared with the magnitude of the neuropathology exhibited in all previous tests of the corresponding Reference Attenuated Poliovirus.

§ 630.17 Alternative test for neurovirulence.

(a) In lieu of the neurovirulence test in § 630.16, the following test may be performed after the filtration process, on each monovalent virus pool or on each multiple thereof (monovalent lot).

(b) *Neurovirulence in monkeys.* Each monovalent virus pool or monovalent lot shall be tested in comparison with the Reference Attenuated Poliovirus, Type 1, for neurovirulence in Macaca monkeys by both the intrathalamic and intraspinal routes of injection. A preinjection serum sample obtained from each monkey must be shown to contain no neutralizing antibody in a dilution of 1:4 when tested against no more than 1,000 $TCID_{50}$ (mean tissue culture infectious dose) of each of the three types of poliovirus. The neurovirulence tests are not valid unless the sample contains at least $10^{7.6}$ $TCID_{50}$ per milliliter when titrated in HEp-2 cells in comparison with the Reference Poliovirus, Live, Attenuated of the appropriate type. All monkeys shall be observed for 17 to 21 days and any evidence of physical abnormalities indicative of poliomyelitis or other viral infections shall be recorded.

(1) *Intrathalamic inoculation.* Each of at least 30 monkeys shall be injected intracerebrally by placing 0.5 milliliter of virus pool material into the thalamic region of each hemisphere. Comparative evaluations shall be made with the virus pool under test and the Reference Attenuated Poliovirus, Type 1. Only monkeys that show evidence of inoculation into the thalamus shall be considered as having been injected satisfactorily. With respect to inoculation, a test is deemed valid if at least 24 monkeys are considered as having been injected satisfactorily. If on examination there is evidence of failure to inoculate virus pool material into the thalamus, additional monkeys may be inoculated in order to reestablish the minimum number of monkeys for the test.

(2) *Intraspinal inoculation.* Each of a group of at least five monkeys shall be injected intraspinally with 0.2 milliliter of virus pool material containing at least $10^{7.6}$ $TCID_{50}$ per milliliter when titrated in HEp-2 cells, and each monkey in additional groups of at least five monkeys shall be injected intraspinally with 0.2 milliliter of a 1:1,000 and 1:10,000 dilution, respectively, of the same virus pool material. Comparative evaluations shall be made with the virus pool under test and the reference material. Only monkeys that show microscopic evidence of inoculation into the gray matter of the lumbar cord shall be considered as having been injected satisfactorily. With respect to inoculation, a test is deemed valid if at least four monkeys per group are considered as having been injected satisfactorily. If on examination there is evidence of failure to inoculate intraspinally, additional animals may be inoculated in order to reestablish the minimum number of animals per group.

(3) *Determination of neurovirulence.* At the conclusion of the observation period comparative histopathological examinations by a qualified pathologist shall be made of the lumbar cord, cervical cord, lower medulla, upper medulla, mesencephalon and motor cortex of each monkey in the groups injected with virus under test and those injected with the Reference Attenuated Poliovirus, Type 1, except that for animals dying during the test period, these examinations shall be made immediately after death. If at least 60

percent of the animals of a group survive 48 hours after inoculation, those animals which did not survive may be replaced by an equal number of animals tested as prescribed in paragraph (b) of this section. If less than 60 percent of the animals of a group survive 48 hours after inoculation, the test must be repeated. At the conclusion of the observation the animals shall be examined to ascertain whether the distribution and histological nature of the lesions are characteristics of poliovirus infection. A comparative evaluation shall be made of the evidence of neurovirulence of the virus under test and the Reference Attenuated Poliovirus, Type 1, with respect to:

(i) The number of animals showing lesions characteristic of poliovirus infection;

(ii) The number of animals showing lesions other than those characteristic of poliovirus infection;

(iii) The severity of the lesions;

(iv) The degree of dissemination of the lesions; and

(v) The rate of occurrence of paralysis not attributable to the mechanical injury resulting from inoculation trauma. These five factors may be weighted and interpreted as the Director, Center for Biologics Evaluation and Research, or the Director's delegatees deem appropriate. Among permissible interpretations, the factors may be considered in different ways for monkeys inoculated intraspinally and for monkeys inoculated intrathalamically. Other relevant factors in addition to those listed in paragraph (b)(3)(i) through (b)(3)(v) of this section, such as public health consequences, may be considered in evaluating neurovirulence test results. The virus pool under test is satisfactory for poliovirus vaccine only if at least 80 percent of the animals in each group survive the observation period and if a comparative analysis of the test results demonstrates that the neurovirulence of the test virus pool does not exceed that of the Reference Attenuated Poliovirus, Type 1.

(4) *Test with Reference Attenuated Poliovirus.* The Reference Attenuated Poliovirus, Type 1, shall be tested as prescribed in paragraphs (b)(1) and (b)(2) of this section at least once for every 10 production lots of vaccine, except that the interval between the test of the reference and the test of any lot of vaccine shall not be greater than 3 months. The test procedure shall be considered acceptable only if lesions of poliomyelitis are seen in monkeys inoculated with the reference material at a frequency statistically compatible with all previous tests with this preparation.

§ 630.18 Additional tests for safety.

(a) *Tests prior to filtration.* Monovalent virus pools shall contain no demonstrable viable microbial agent, except for unavoidable bacteriophage and the intended attenuated live poliovirus. The vaccine shall be tested for the absence of other infectious agents, including polioviruses of other types or strains. Testing of each monovalent pool shall include the following procedures:

(1) *Inoculation of rabbits.* A minimum of 100 milliliters of each monovalent virus pool shall be tested by inoculation into at least 10 healthy rabbits, each weighing 1,500 to 2,500 grams. Each rabbit shall be injected with a total of 1.0 milliliter intradermally in multiple sites, and subcutaneously with 9.0 milliliters, of the monovalent virus pool and the animals observed for at least 3 weeks. Each rabbit that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and the brain and organs removed and examined. The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of the rabbits remain healthy and survive the entire period and if all the rabbits used in the test fail to show lesions of any kind at the sites of inoculation and fail to show evidence of cercopithecid herpesvirus 1 or any other viral infection.

(2) *Inoculation of adult mice.* Each of at least 20 adult mice, each weighing 15 to 20 grams, shall be inoculated intraperitoneally with 0.5 milliliter and intracerebrally with 0.03 milliliter of each monovalent virus pool. The mice shall be observed for 21 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and

examined for evidence of viral infection by direct observation and subinoculation of appropriate tissue into at least five additional mice which shall be observed for 21 days. The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of the mice remain healthy and survive the entire period and if all the mice used in the test fail to show evidence of lymphocytic choriomeningitis virus or other viral infection.

(3) *Inoculation of suckling mice.* Each of at least 20 suckling mice less than 24 hours old shall be inoculated intracerebrally with 0.01 milliliter and intraperitonally with 0.1 milliliter of the monovalent virus pool. The mice shall be observed daily for at least 14 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection. Such examination shall include subinoculation of appropriate tissue suspensions into an additional group of at least five suckling mice by the intracerebral and intraperitoneal routes and observed daily for 14 days. In addition, a blind passage shall be made of a single pool of the emulsified tissue (minus skin and viscera) of all mice surviving the original 14-day test. The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of the mice remain healthy and survive the entire period and if all the mice used in the test fail to show evidence of Coxsackie or other viral infection.

(4) *Inoculation of guinea pigs.* Each of at least five guinea pigs, each weighting 350 to 450 grams, shall be inoculated intracerebrally with 0.1 milliliter and intraperitoneally with 5.0 milliliters on the monovalent virus pool to be tested. The animals shall be observed for at least 42 days and rectal temperatures recorded daily for the last 3 weeks of the test. Each animal that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and its tissues shall be examined both microscopically and culturally for evidence of tubercle bacilli, and by passage of tissue suspensions into at least three other guinea pigs by the intracerebral and intraperitoneal routes of inoculation for evidence of viral infection. If clinical signs suggest infection with lymphocytic choriomeningitis virus, serological tests shall be performed on blood samples of the test guinea pigs to confirm the clinical observations. Animals that die or are sacrificed during the first 3 weeks after inoculation with the monovalent virus pools shall be examined for infection with lymphocytic choriomeningitis virus. Animals that die in the final 3 weeks shall be examined both microscopically and culturally for *Mycobacterium tuberculosis.* The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of all animals remain healthy and survive the observation period and if all the animals used in the test fail to show evidence of infection with *Mycobacterium tuberculosis* or any viral infection.

(5) *Inoculation of monkey kidney tissue cultures.* At least 500 doses or 50 milliliters, whichever is a greater volume of virus, taken either from each undiluted monovalent virus pool or, in equal proportions from individual harvests or subpools, shall be tested for simian viruses in Macaca monkey kidney tissue cultures and, in the same volume, in Cercopithecus monkey kidney tissue cultures. A dilution of the virus pool in medium not to exceed 1:4 shall be used. The area of surface growth of the cells shall be at least 3 square centimeters per milliliter of test inoculum. The test poliovirus shall be neutralized by high-titer specific antiserum of nonprimate origin. The immunizing antigens used for the preparation of antisera shall be grown in a cell line other than the cell line used for testing the vaccine. The cultures shall be observed for at least 14 days. At the end of the observation period at least one subculture of fluid from the Cercopithecus kidney cell culture shall be made in the same tissue culture system and the subculture shall be observed for at least 14 days. The monovalent virus pool may be used for poliovirus vaccine only if all the tissue cultures fail to show evidence of the presence of simian viruses or any other viral infection.

(6) *Inoculation of human cell cultures.* At least 500 doses or 50 milliliters, whichever represents a greater volume of virus, taken from either a single

monovalent pool or, in equal proportions from individual harvests or subpools, shall be tested for the presence of measles virus in either:

(i) Primary human amnion cells,

(ii) Primary human kidney cells, or

(iii) Any other human or nonhuman cell system of comparable suspectibility to unmodified measles virus.

The virus pool shall be diluted with medium not to exceed 1:4. The area of surface growth of cells shall be at least 3 square centimeters per milliliter of test inoculum. The test material shall be neutralized with poliovirus antiserum of other than primate origin if the tissue culture cell system used is susceptible to poliovirus. The immunizing antigens used for the preparation of antiserum shall be grown in a cell line other than the cell line used for testing the vaccine. The culture shall be observed for at least 14 days. The monovalent virus pool may be used for poliovirus vaccine only if all tissue cultures fail to show evidence of the presence of measles virus or any other viral infection.

(7) *Inoculation of a rabbit kidney tissue culture.* At least 500 milliliters of virus pool, taken from either a single monovalent pool or in equal proportions from individual harvests or subpools, shall be tested in primary rabbit kidney tissue culure preparations for evidence of cercopithecid herpesvirus 1. The virus pool shall be diluted with medium not to exceed 1:4. The area of surface growth of cells shall be at least 3 square centimeters per milliliter of test inoculum. The culture shall be observed for at least 14 days. The monovalent virus pool may be used for poliovirus vaccine only if all tissue cultures fail to show evidence of the presence of herpesvirus.

(b) *Tests for in vitro markers.* In addition to the neurovirulence test required by §§ 630.16 or 630.17, the following tests relating to safety shall be performed on each monovalent virus pools after the filtration process. Tests shall be performed on each monovalent virus pool using the marker tests described below or other methods shown to be of comparable value in indentification of the attenuated strain. The test results shall demonstrate that the monovalent virus pool under test and the seed virus have substantially the same marker characteristics.

(1) *rct/40 Marker.* Attenuated strains which grow readily at 40 °C (±0.5 °C) are classified as rct/40 positive (+) in contrast to the rct/40 negative (−) strains, which show an increased growth of at least 100,000 fold at 36 °C over that obtained at 40 °C. Comparative determinations shall be made in suitable culture vessels. ≤

(2) *d Marker.* Attenuated strains which grow readily at low concentrations of bicarbonate under agar are classified as d positive (+) in contrast to the d negative (−) strains, which exhibit delayed growth under the same conditions. The cultures shall be grown in a 36 °C incubator, in suitable culture vessels in an environment of 5 percent CO_2 in air.

(c) *Final container sterility test.* The final container sterility test need not be performed provided aseptic techniques are used in the filling process.

[56 FR 21432, May 8, 1991; 56 FR 27787, June 17, 1991]

§ 630.19 General requirements.

(a) *Vaccine release.* No lot of trivalent vaccine shall be released by the manufacturer unless each monovalent virus pool contained therein:

(1) Has been manufactured by the same procedures;

(2) Has met the criteria of neurovirulence for monkeys prescribed in §§ 630.16(b) or 630.17(b);

(3) Has met the criteria of in vitro markers prescribed in § 630.18(b); and

(4) Has been released for further manufacturing by the Director, Center for Biologics Evaluation and Research unless, at the Director's discretion, the Director determines that lot release by the Center for Biologics Evaluation and Research is not required. The protocols for all monovalent virus pools produced sequentially from the same seed and tested, in whole or in part, in accordance with §§ 630.16(b) or 630.17(b) shall be submitted to the Director, Center for Biologics Evaluation and Research, whether or not release of the pool for further manufacturing is requested. For monovalent virus pools not tested under §§ 630.16(b) or 630.17(b),

the manufacturer shall report the reasons for partial manufacture to the Director, Center for Biologics Evaluation and Research.

(b) *Labeling.* In addition to the items required by other applicable labeling provisions of this chapter, the final container label shall bear a statement indicating that liquid vaccine may not be used for more than 7 days after opening the container. Labeling may include a statement indicating that, for frozen vaccine, a maximum of 10 freeze-thaw cycles is permissible provided the total cumulative duration of thaw does not exceed 24 hours, and provided the temperature does not exceed 8 °C during the periods of thaw.

(c) *Samples and protocols.* For each trivalent lot of vaccine and for each monovalent virus pool, the following materials shall be submitted in accordance with instructions received from the Director, Center for Biologics Evaluation and Research, 8800 Rockville Pike, Bethesda, MD 20892.

(1) A protocol that consists of a summary of the history of manufacture of each trivalent lot or monovalent virus pool, including any test results requested by the Director, Center for Biologics Evaluation and Research.

(2) Twenty milliliters of monovalent virus pool before filtration.

(3) Forty milliliters of monovalent virus pool after filtration. The titer of the sample shall be no less than the equivalent of $10^{7.5}$ $TCID_{50}$ per milliliter when titrated in HEp-2 cells; if the titer is greater than $10^{7.5}$ $TCID_{50}$ per milliliter, a correspondingly smaller volume may be submitted.

(4) A total of at least 50 single doses or the equivalent thereof of the trivalent vaccine.

(5) When deemed appropriate, the Director, Center for Biologics Evaluation and Research, may require submission of samples or sample volumes other than those specified in paragraphs (c)(2), (c)(3), and (c)(4) of this section.

(d) *Public health implications.* In interpreting any provision of the regulations governing oral poliovirus vaccine, the agency may consider any potential effect on individual or public health, including effects related to vaccine supply.

(e) *Alternative procedures.* (1) The Director, Center for Biologics Evaluation and Research, may approve an exception or alternative to any requirement in subpart B of part 630 regarding Poliovirus Vaccine Live Oral. Requests for such exceptions or alternatives should ordinarily be made in writing. However, in limited circumstances such requests may be made orally and permission may be given orally by the Director, Center for Biologics Evaluation and Research. Oral requests and approvals must be followed by written requests and written approvals.

(2) FDA will publish a list of approved alternative procedures and exceptions periodically in the FEDERAL REGISTER.

(f) *Status of vaccine in distribution.* Poliovirus Vaccine Live Oral released or in distribution prior to May 8, 1991, is deemed to meet the requirements of supart B of part 630.

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World Health Organization Technical Report Series, No. 800, 1990

Annex 1

REQUIREMENTS FOR POLIOMYELITIS VACCINE (ORAL)

(Requirements for Biological Substances No. 7) (Revised 1989)

	Page
Introduction	31
General considerations	31
Part A. Manufacturing requirements	35
1. Definitions	35
2. General manufacturing requirements	37
3. Control of source materials	38
4. Control of vaccine production	40
5. Filling and containers	51
6. Control tests on final product	51
7. Records	52
8. Samples	52
9. Labelling	53
10. Distribution and shipping	53
11. Storage and expiry date	53
Part B. National control requirements	54
1. General	54
2. Release and certification	55
Part C. Requirements for Poliomyelitis Vaccine (Oral) Prepared in Primary Cultures of Monkey Kidney Cells	56
4. Control of vaccine production	56
Authors	63
Acknowledgements	64
References	65
Appendix 1. Tests for bovine viruses in serum	66
Appendix 2. Assay method for the determination of the virus content of poliomyelitis vaccine (oral)	67
Appendix 3. Criteria for the acceptance of vaccines after neurovirulence testing	68
Appendix 4. Form on which to report the score of virus activity for each histological section from all monkeys included in the neurovirulence test	73
Appendix 5. Preparation of poliomyelitis vaccine (oral) using cell banks	76
Appendix 6. Preparation of poliomyelitis vaccine (oral) using monkey kidney-cell cultures	77
Appendix 7. Summary protocol for poliomyelitis vaccine (oral)	78

INTRODUCTION

The Requirements for Poliomyelitis Vaccine (Oral) were last revised in 1982 (*1*). Since then, general requirements for the characterization of continuous cell lines for the preparation of biologicals were adopted in 1985 at the thirty-sixth meeting of the WHO Expert Committee on Biological Standardization (*2*). Safety issues relating to the use of continuous cell lines for the production of biologicals were reviewed by a WHO Study Group in 1986, which concluded that, in principle, such cell lines are acceptable as substrates for the production of biologicals, but that differences in the nature of the products and in the characteristics of the manufacturing processes must be taken into account in deciding whether a given product is acceptable (*3*).

The Expert Committee on Biological Standardization, at its meeting in October 1988, suggested that the modified draft requirements for poliomyelitis vaccine (oral) prepared in continuous cell lines should be combined with the existing Requirements for Poliomyelitis Vaccine (Oral) (Requirements for Biological Substances No. 7, revised 1982 (*1*) and addendum 1987 (*4*)).

The WHO Secretariat has taken the amendments suggested by various consultants and incorporated them in this reformulated document.

GENERAL CONSIDERATIONS

The strains of poliovirus used in the production of poliomyelitis vaccine (oral) (OPV) must have been shown to yield vaccines that are both immunogenic and highly attenuated when administered orally to susceptible children and adults. At present, vaccine production is based mainly on the use of the Sabin strains, and great practical problems could be expected to arise in ensuring that new strains are suitable for the manufacture of vaccine for use on the large scale. No provision has been made in these requirements for vaccines that might be prepared from genetically engineered variants of the Sabin strains.

Live poliomyelitis vaccines prepared from the Sabin strains of Types 1, 2, and 3 poliomyelitis viruses were introduced for large-scale immunization in 1957. From then until 1972, Sabin himself authorized laboratories to produce vaccine from his strains, if he

considered them capable of doing so, and controlled the methods of production in collaboration with the control authorities of the countries concerned. In 1972, Sabin proposed that WHO should accept the responsibility hitherto exercised by himself of approving laboratories that wished to produce poliovirus vaccines from the seed materials of the Sabin strains of the Types 1, 2 and 3 viruses. The Director-General agreed to assume the responsibility for ensuring the proper use of the strains, and established a scientific committee, the Consultative Group on Poliomyelitis Vaccines, to advise him on all matters pertaining to their use.

As a few ampoules of the original viruses produced by Sabin were still available, it was decided that they should be used to generate stocks at the SO + 1 (Sabin Original plus one passage) level and that these stocks would be the "master seeds". Behringwerke AG, Marburg/Lahn, Federal Republic of Germany, generously agreed to produce the seeds for WHO, free of charge. The new seeds were produced directly from the original viruses.

All the tests were satisfactory, and the Consultative Group was satisfied that the working seed lots (SO + 2) prepared by Behringwerke from the WHO master seeds satisfied the requirements for neurovirulence. Approximately 1000 1-ml ampoules of each type (SO + 2) were sent for storage to each of three national control laboratories (in Italy, the United Kingdom, and the USA). Although WHO has taken every possible precaution to ensure that these seeds meet the requirements for poliomyelitis vaccine (oral) made from the Sabin strains, it should be emphasized that, in each country, the national health authority must accept responsibility for the quality of the vaccine produced from the seeds and used in that country.

In the meantime, some producers, who had found it difficult to meet the neurovirulence requirements for Type 3 vaccines made from their established working seeds, began to use a Sabin Type 3 seed prepared by Pfizer Ltd from a plaque prepared from viral RNA extracted from material at the SO + 2 level. This seed has inhibition-of-growth properties at 40 °C (rct/40) similar to those of the Type 1 and Type 2 attenuated strains, and gives satisfactory results in the intraspinal test for neurovirulence. The seed is designated as RSO1, so that the working seed passage level is RSO2 and the vaccine RSO3. It has been used safely and effectively in trivalent vaccines in national immunization programmes involving millions of children.

More detailed information on stocks of seed viruses and the preparation of the WHO master seeds and working seed has been published by Cockburn (*5*). National control authorities should decide on the detailed procedures applicable to the preparation and use of virus seed lots.

It has been found that the intraspinal test described in these Requirements, in which the distribution of virus-specific lesions within the central nervous system is observed, provides the information necessary for a precise comparison of vaccines with the appropriate reference preparation.

Neurovirulence reference materials are distributed to both control authorities and manufacturers. It is recommended that, before the first vaccine lots are licensed, both the national control authorities and the manufacturer should perform the test on the working reference preparation and homotypic vaccine lots on at least four occasions for all types to establish baseline data for future comparison with the production lots.

In some countries, a manufacturing establishment may limit vaccine production to filling final containers with vaccine obtained in the bulk form from another manufacturing establishment. In such cases, ensuring conformity with all the requirements applicable to the final vaccine (Part A, sections 5 et seq.) must be the responsibility of the manufacturer of the final product. In other countries, with the approval of the national control authority, certain production and control tests that have not been performed in the manufacturing establishment may be carried out in an independent laboratory. In such cases, the manufacturer must nevertheless assume responsibility for the safety and efficacy of the product.

The safety of OPV depends on several factors, of which the most important are strict adherence to the seed virus system and the period and temperature of incubation of the production cultures. Tests on the working seed viruses and vaccine lots derived from them for consistency of virus characteristics, including tests in monkeys for neurovirulence and other tests *in vitro*, are of great importance in the production of a safe vaccine. If the quality of consecutive lots of OPV consistently meets these requirements, there is a high level of assurance that the vaccines will be both safe and efficacious.

Oral poliomyelitis vaccines have been in worldwide use for more than 25 years and, although those produced in human diploid cells have been used to a lesser extent than those produced in primary monkey kidney-cell cultures, experience has indicated that both are safe and effective.

It is gene y accepted that monkey kidney-cell cultures can be contaminated with one or more adventitious agents, including simian viruses. The frequency of contaminated cell cultures can be significantly reduced both by the use of animals derived from closed colonies, and by careful screening of the animals to be used in production for the absence of antibodies to the relevant simian viruses. However, nonhuman primates are becoming increasingly difficult to obtain, and this could affect the supply of OPV worldwide. Although production of oral poliomyelitis vaccines using human diploid cells was introduced in the 1960s, some manufacturers have found it difficult, using these cells, to prepare large quantities of vaccine meeting the requirements published by WHO. Continuous cell lines have therefore been of interest to manufacturers as an alternative to primary monkey kidney and human diploid cells as a substrate for the production of biological substances. In one country, more than 250 000 doses of OPV produced in Vero cells were administered to children during the year following licensing in 1988. No serious adverse reactions have been observed.

OPV produced in properly defined and characterized continuous cell lines (*2*) is a live attenuated vaccine which can safely be administered to healthy infants. Contamination of the vaccine with cell-derived DNA is regarded as posing a negligible risk since the preparation is given orally (*3*). However, contamination with unwanted cell-derived material has been reduced by purification of the product following filtration of the bulk suspension. It has been suggested that purification procedures may concentrate neurovirulent subpopulations of the harvest and may also affect the response of monkeys in tests for neurovirulence, but this requires further study.

The effects of new cell substrates and culture technologies on the properties of the virus call for careful examination, including the monitoring of phenotypic and molecular characteristics following multiple passages of each virus type on the substrate of choice in comparison with those following such passages on currently accepted cell substrates. Similarly, it is necessary to monitor the phenotypic and molecular characteristics of stool isolates from samples obtained during initial clinical trials.

A particular concern in the use of OPV has been its low thermal stability under actual conditions of use as compared with that of other vaccines. These Requirements therefore include a provision for

an accelerated degradation test to indicate those vac lots which may not be adequately stable.

In these Requirements, Part A describes the general provisions for the production of OPV, including the use of a cell bank, Part B describes national control requirements, and Part C describes additional or alternative requirements applicable to production from primary monkey kidney-cell cultures.

Each of the following sections constitutes a recommendation. Those parts of each section printed in large type have been written in the form of requirements so that, if a health administration so desires, they may be adopted as they stand as definitive national requirements. Those parts of each section printed in small type are comments and/or recommendations for guidance.

Individual countries may wish to adopt these Requirements as the basis of their national regulations on OPV. If national requirements differ from these requirements, it is recommended that the former should be shown to ensure that the vaccine is at least as safe and as potent as that prepared in accordance with the requirements formulated below. It is desirable that the World Health Organization should be kept informed of any such differences.

PART A. MANUFACTURING REQUIREMENTS

1. Definitions

1.1 International name and proper name

The international name shall be *Vaccinum poliomyelitidis perorale typus I, II, III* (whichever type or types apply). The proper name shall be the equivalent of the international name in the language of the country of use.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

1.2 Descriptive definition

Vaccinum poliomyelitidis perorale is a preparation of live attenuated poliovirus Type 1, 2 or 3 grown in *in vitro* cultures of suitable cells, containing any one type or any combination of the three types of the Sabin strains, prepared in a form suitable for oral administration, and satisfying all the requirements formulated in this document.

1.3 International reference materials

The WHO Consultative Group on Poliomyelitis Vaccines has selected three monotypic virus suspensions of Types 1, 2 and 3 and one trivalent virus mixture as international reference materials for the determination of virus titre. These preparations are dispensed in ampoules and held in the frozen state in the gas phase over liquid nitrogen and are in the custody of the National Institute for Biological Standards and Control, Potters Bar, England. They are distributed to national control authorities for the purpose of establishing national reference materials for the determination of virus titre in the vaccine.

Reference preparations WHO/I for Type 1 virus, WHO/II for Type 2 virus and WHO/III for Type 3 virus at the SO + 2 passage level are available for the comparison of neurovirulence with that of homotypic vaccines. The relevant reference materials should be included in each test of vaccine. They are available on application to Biologicals, WHO, Geneva, Switzerland.

1.4 Terminology

Cell seed: A quantity of cells of human or animal origin stored frozen at −70 C or below in aliquots of uniform composition, one or more of which may be used for the production of a manufacturer's working cell bank.

Manufacturer's working cell bank (MWCB): A quantity of cells of uniform composition derived from one or more ampoules of the cell seed, which may be used for the production cell culture.

In normal practice, a cell bank is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer, at which point the cells are combined to give a single pool and preserved cryogenically to form the MWCB. One or more of the ampoules from such a pool may be used for the production cell culture.

Production cell culture: A cell culture derived from one or more ampoules of the MWCB or primary tissue used for the production of OPV.

Adventitious agents: Contaminating microorganisms of the cell substrate or materials used in its culture, including bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses.

Original vaccine: A monovalent vaccine, prepared according to the author's stated specification from the original seed virus, and shown on oral administration to humans in field trials to be immunogenic and highly attenuated.

Virus master seed lot: A quantity of virus of uniform composition derived from an original vaccine processed at one time and passaged for a number of times that does not exceed the maximum approved by the national control authority.

Virus working seed lot: A quantity of virus of uniform composition derived from the master seed by a single passage by a method approved by the national control authority.

Single harvest: A virus suspension of one virus type harvested from cell cultures prepared from a single production run.

Bulk suspension: A pool of a number of single harvests of the same virus type.

Final bulk: The finished biological preparation present in the container from which the final containers are filled. The final bulk may be prepared from one or more filtered bulk suspensions, and may contain more than one virus type.

Filling lot (final lot): A collection of sealed, final containers of liquid vaccine that are homogeneous with respect to the risk of contamination during the filling process or the preparation of the finished vaccine. A filling lot must therefore have been filled or prepared in one working session.

Plaque-forming unit (PFU): The smallest quantity of a virus suspension that will produce a plaque in monolayer cell cultures.

Cell culture infective dose 50% ($CCID_{50}$): The quantity of a virus suspension that will infect 50% of cell cultures.

2. General manufacturing requirements

The general manufacturing requirements contained in the revised Requirements for Biological Substances No. 1 (*6*) shall apply to

establishments manufacturing oral poliomyelitis vaccine, with the addition of the following:

Production areas shall be decontaminated before they are used for the manufacture of oral poliomyelitis vaccine.

The production of oral poliomyelitis vaccine shall be conducted by a separate staff, which shall consist of healthy persons, who shall be examined medically at regular intervals. Steps shall be taken to ensure that all such persons in the production areas are immune to poliomyelitis and do not excrete poliovirus or other microorganisms of significance from the point of view of the safety of the vaccine. Personnel working in monkey quarters shall also be examined for tuberculosis as outlined in Part A, section 2 of the Requirements for Biological Substances No. 11 (*7*).

Visitors and persons not directly concerned with the production processes shall not be permitted to enter the production areas, except with the permission of the national control authority.

3. Control of source materials

The general production precautions as formulated in the requirements of Part A, section 3, of the revised Requirements for Biological Substances No. 1 (*6*, p. 15) shall apply to the manufacture of OPV, except that, during production, only one type of cell shall be introduced or handled in the production area at any one time.

3.1 Cell lines

3.1.1 *Cell seed and manufacturer's working cell bank*

The use of a cell line for the manufacture of polio vaccines shall be based on the cell seed system. The cell seed shall be approved by the national control authority. The maximum number of passages (or population doublings) by which the MWCB is derived from the cell seed shall be established by the national control authority.

WHO has established a cell bank of Vero cells characterized in accordance with the requirements in the thirty-sixth report of the WHO Expert Committee on Biological Standardization (*2*), which is available to manufacturers on application to Biologicals, WHO, Geneva, Switzerland.

3.1.2 *Identity test*

Cell seed shall be characterized according to the Requirements for Continuous Cell Lines Used for Biologicals Production (*2*), or those relating to human diploid cells (*1*), as appropriate.

The MWCB shall be identified by means, *inter alia*, of biochemical (e.g., isoenzyme analysis), immunological, and cytogenetic marker tests, approved by the national control authority.

3.1.3 *Cell culture medium*

Serum used for the propagation of cells shall be tested to demonstrate freedom from bacteria, fungi and mycoplasmas, according to the requirements given in Part A, sections 5.2 and 5.3 of the revised Requirements for Biological Substances No. 6 (*8*, p. 49), as well as freedom from pathogens of the species of origin of the serum, by methods approved by the national control authority (see also Appendix 1).

In some countries the national control authority may require that the serum be obtained from herds certified healthy and therefore free from infectious agents for which serological and virological tests are currently not available. In some countries, to monitor the quality of the source, sera are examined for freedom from phage and endotoxin.

Penicillin and other beta-lactams shall not be used at any stage of the manufacture.

Other antibiotics may be used at any stage in the manufacture provided that the quantity present in the final product is acceptable to the national control authority. Nontoxic pH indicators may be added, e.g., phenol red in a concentration of 0.002%. Only substances that have been approved by the national control authority may be added.

It is advisable to ensure that the trypsin used in the preparation of the cell suspension is free from adventitious agents.

3.2 Virus strains

Strains of poliovirus used in the production of oral poliomyelitis vaccine shall be identified by historical records, which should include information on their origin. Only virus strains that are approved by the national control authority shall be used (see General Considerations, p. 31).

3.2.1 *Virus seed lot system*

Vaccine production shall be based on the virus seed lot system. The virus working seed lot used for the production of vaccine batches shall be prepared by a single passage from a master seed lot by a method and at a passage level from the original seed virus approved by the national control authority.

A large working seed lot should be set aside as the basic material which the manufacturer will use for the preparation of batches of vaccine.

All virus seed lots shall be stored at a temperature of −60 °C or below.

3.2.2 *Tests on virus seed lots*

The virus working seed lot used for the production of vaccine batches shall be free from detectable extraneous viruses and shall satisfy the requirements specified in Part A, sections 4.3 and 4.4. It shall not be purified. Each virus working seed lot shall have been derived from material tested according to the requirements of Part A, section 4.4.5.1. In addition, the neurovirulence of consecutive monovalent virus batches prepared from the seed virus shall meet the criteria for acceptability given in Appendix 3.

The seed virus may continue to be used provided that the frequency with which monovalent virus pools produced with it fail to meet the neurovirulence criteria for monkeys is not greater than that predicted from a comparison with the corresponding reference preparation. If the frequency of failure of the monovalent virus pools is greater than predicted, the seed virus shall cease to be used in vaccine production.

4. Control of vaccine production

4.1 Control cell cultures

At least 5% or 1000 ml of the cell suspension at the concentration and cell passage level employed for seeding vaccine production cultures shall be used to prepare control cultures. (See Appendix 5 for an example of a flowsheet of tests in cell cultures.)

In countries in which large-scale production technology has been developed, the national control authority should determine the size and treatment of the cell sample to be examined.

4.1.1 *Tests of control cell cultures*

The treatment of the cells set aside as control material shall be similar to that of the production cell cultures, but they shall remain uninoculated for use as control cultures for the detection of extraneous viruses.

These control cell cultures shall be incubated under similar conditions to the inoculated cultures for at least two weeks, and shall be examined during this period for evidence of cytopathic changes. For the test to be valid, not more than 20% of the control cell cultures shall have been discarded for nonspecific, accidental reasons.

At the end of the observation period, the control cell cultures shall be examined for degeneration caused by an extraneous agent. If this examination, or any of the tests specified in this section, shows evidence of the presence in a control culture of any adventitious agent, the poliovirus grown in the corresponding inoculated cultures shall not be used for vaccine production.

4.1.2 *Tests for haemadsorbing viruses*

At the end of the observation period, 25% of the control cells shall be tested for the presence of haemadsorbing viruses using guinea-pig red blood cells. If the latter have been stored, the duration of storage shall not exceed seven days and the storage temperature shall have been in the range 2–8 °C.

In tests for haemadsorbing viruses, calcium and magnesium ions should be absent from the medium.

A reading should be taken after incubation for 45 minutes at 0–4 °C and again after a further incubation for 30 minutes at 20–25 °C.

In some countries cell cultures grown on coverslips are stained with haematoxylin and eosin as a check for these extraneous agents.

4.1.3 *Tests for other adventitious agents*

At the end of the observation period, a sample of the pooled fluid from each group of control cultures shall be tested for adventitious agents. For this purpose, 10 ml of each pool shall be tested in the same cells, but not the same batch of cells, as those used for the production of virus, and additional 10-ml samples of each pool shall

be tested in human cells sensitive to measles and at least one other sensitive cell system.

The pooled fluid shall be inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. At least one bottle of each kind of cell culture shall remain uninoculated and shall serve as a control.

The inoculated cultures shall be incubated at a temperature of 35–37 C and shall be observed for a period of at least 14 days.

For the tests to be valid, not more than 20% of the culture vessels shall have been discarded for nonspecific accidental reasons by the end of the test period.

If any cytopathic changes due to adventitious agents occur in any of the cultures, the virus harvests produced from the batch of cells from which the control cells were taken shall be discarded.

If these tests are not performed immediately, the samples shall be kept at a temperature of −60 °C or below.

4.1.4 *Identity test*

At the production level, the cells shall be identified by means of tests approved by the national control authority.

Suitable tests are isoenzyme analysis, immunological tests and cytogenetic marker tests.

4.2 Cell cultures for vaccine production

4.2.1 *Tests for adventitious agents*

On the day of inoculation with the virus working seed lot, each cell culture or a sample from each culture vessel shall be examined for degeneration caused by infective agents. If such examination shows evidence of the presence in a cell culture of any adventitious agent, the culture shall not be used for vaccine production.

If animal serum is used for cell cultures before the inoculation of virus, the medium shall be removed and replaced with serum-free maintenance medium, after the cells have been washed with serum-free medium, if appropriate.

4.2.2 *Tests for bacteria, fungi and mycoplasmas*

A volume of at least 20 ml of the pooled supernatant fluids from the production cell cultures shall be tested for bacterial and mycotic sterility and for mycoplasmas. The tests for bacterial and mycotic sterility shall be performed as described in Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*8*, p. 48).

In some countries, the volume of pooled supernatant fluids is ultracentrifuged and both the pellet and its supernatant fluid are tested for sterility.

The tests for mycoplasmas should be performed with solid and liquid media that have been shown to be capable of supporting the growth of sterol-requiring and non-sterol-requiring mycoplasmas, 10 ml being used for each group of tests. Nonculture methods for detecting mycoplasmas may also be used.

4.3 Control of single harvests

4.3.1 *Single harvest*

After inoculation of the production cells with the virus working seed lot, both inoculated and control cell cultures shall at no time be at a temperature outside the range 33–35 °C for the relevant incubation periods. The temperature should not vary by more than ±0.5 °C within that range. The optimal range for pH, multiplicity of infection, cell density and time of incubation shall be established for each manufacturer, and be approved by the national control authority.

The virus suspension shall be harvested not later than four days after virus inoculation.

The inoculated cell cultures should be processed in such a manner that each virus suspension harvested remains identifiable as a single harvest and is kept separate from other harvests until the results of all the tests prescribed in Part A sections 4.1.2, 4.1.3, 4.2.2, 4.3.3 and 4.3.4 have been obtained.

4.3.2 *Sampling*

Samples required for the testing of single harvests shall be taken immediately on harvesting. If the tests for adventitious agents as described in Part A, section 4.3.3 are not performed immediately, the samples taken for these tests shall be kept at a temperature of

−60 °C or lower, and subjected to no more than one freeze-thaw cycle.

4.3.3 *Tests of neutralized single harvests for adventitious agents*

For the purposes of the requirements specified in this section of Part A, the volume of each single harvest taken for neutralization and testing shall be at least 10 ml and shall be such that at least a total of 50 ml or the equivalent of 500 doses of final vaccine, whichever is the greater, has been withheld from the corresponding bulk suspension.

The antisera used for neutralization shall be of nonhuman origin and shall have been prepared in animals other than monkeys, using virus cultured in cells from a species different from that used in the production of the vaccine. Samples of each virus harvest shall be tested in human cells sensitive to measles and at least one other sensitive cell system.

The neutralized suspensions shall be inoculated into bottles of these cell cultures in such a way that the dilution of the suspension in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm^2 per ml of neutralized suspension. At least one bottle of each kind of cell culture shall remain uninoculated and shall serve as a control and shall be maintained by nutrient medium containing the same concentration of the specific antiserum used for neutralization.

Animal serum may be used in the propagation of the cells, but the maintenance medium used after inoculation of the test material should contain no added serum other than the poliovirus neutralizing antiserum.

The inoculated cultures shall be incubated at a temperature of 35–37 °C and shall be observed for a period of at least 14 days.

In some countries, cultures may be incubated at lower temperatures.

For the tests to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific accidental reasons by the end of the test period.

If any cytopathic changes due to adventitious agents occur in any of the cultures, the virus harvest shall be discarded.

4.3.4 *Sterility tests*

A volume of at least 10 ml of each single harvest shall be tested for bacterial and mycotic sterility according to the requirements given in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (*8*), as well as for mycoplasmas, by a method approved by the national control authority.

Tests for mycoplasmas should be performed with solid and liquid media that have been shown to be capable of supporting the growth of sterol-requiring and non-sterol-requiring mycoplasmas, at least 10 ml of each single harvest being used for each group of tests. Nonculture methods for detecting mycoplasmas may also be used.

4.4 Control of bulk suspension

4.4.1 *Preparation of bulk suspension*

The bulk suspension shall be filtered through a filter able to retain bacteria and cell debris.

The national control authority may require the further purification of harvests derived from continuous cell lines. If the harvests are derived from human diploid or monkey kidney cells, further purification is not required.

In one country, oral polio vaccine grown on continuous cell lines is purified so as to give a product containing less than 100 pg per dose of residual DNA.

4.4.2 *Sampling*

Samples of the bulk suspension prepared as described in section 4.4.1 shall be taken immediately and, if not tested immediately, shall be kept at a temperature of −60 °C or below until the tests described in the following sections are performed.

4.4.3 *Identity test*

The poliovirus type in the bulk suspension shall be serologically identified.

Care should be taken to ensure that the sera used are monospecific by titrating them against homotypic and heterotypic viruses of known virus titre. The use of monoclonal antibodies may be useful in this test.

4.4.4 *Virus concentration*

The amount of infective poliovirus per ml of filtered bulk suspension shall be determined in cell cultures in comparison with an existing reference preparation.

The virus concentration as determined by this test shall be the basis for the quantity of virus used in the neurovirulence tests in monkeys (Part A, section 4.4.5.1) and for preparing the final bulk (Part A, section 4.5).

The detailed procedures for carrying out this test and for interpreting the results shall be those approved by the national control authority.

A suitable test is described in Appendix 2.

4.4.5 *Test for consistency of virus characteristics*

The poliovirus in the filtered bulk suspension prepared as described in section 4.4.1 shall be tested in comparison with the seed lot or a reference virus preparation (see Part A, section 1.3) with regard to certain characteristics, as described in the following subsections, to ensure that the vaccine virus has not undergone changes during its multiplication in the production cell culture.

From the results of these tests for successive batches of vaccine a critical assessment may be made of the consistency of vaccine quality (see Part B, section 2).

4.4.5.1 *Neurovirulence tests in monkeys.* Monkeys used for neurovirulence tests must satisfy the relevant requirements in Part C, section 4.1.1, and weigh not less than 1.5 kg. The pathogenicity of the filtered bulk suspension for *Macaca* or *Cercopithecus* monkeys shall be tested in comparison with that of a reference virus preparation for neurovirulence testing (see Part A, section 1.3) by inoculation into the lumbar region of the central nervous system. A pre-injection serum sample obtained from each monkey shall be shown not to contain any neutralizing antibody in a dilution of 1:4 when tested against no more than 1000 $CCID_{50}$ of each of the three types of poliovirus.

If the neurovirulence test is performed only by the manufacturer, the histological sections must be made available to the national control authority for evaluation.

(1) *Number of monkeys*

It is recommended that a vaccine and the appropriate homotypic reference virus should, whenever possible, be tested concurrently in a single group of monkeys. Equal numbers of animals should be inoculated with the reference virus and the vaccine being tested. Monkeys should be allocated to vaccine or reference virus and to particular cages using a randomization procedure.

The number of monkeys inoculated shall be such that at least 11 positive monkeys shall be included in the evaluation of the vaccine and at least 11 positive monkeys shall be included for the reference preparation for Types 1 and 2 virus (a "positive" monkey is one in which neuronal lesions characteristic of poliovirus are seen in the central nervous system). For Type 3 virus, there shall be at least 18 positive monkeys for the reference and a further 18 positive monkeys for the vaccine. More than one vaccine lot may be tested with the same homotypic reference. The monkeys shall, when possible, be from the same quarantine group and shall be allocated randomly to the preparations. If it is not possible to use monkeys for both the homotypic reference and the test vaccine from the same quarantine group, an equal number of monkeys from two quarantine groups shall undergo tests with each of the preparations. If a test is done on two working days, equal numbers of monkeys shall be inoculated with the vaccine and the homotypic reference on each working day.

In order to obtain 11 and 18 positive monkeys, it is usual to inoculate 12 and 20 monkeys, respectively.

The monkeys are sedated with ketamine hydrochloride or any other substance that has been shown to be suitable.

(2) *Virus content of vaccines and reference preparations inoculated*

The virus contents of the vaccine and homotypic reference preparation shall be adjusted so as to be as close to one another as possible and shall be between $10^{5.5}$ and $10^{6.5}$ $CCID_{50}$/0.1 ml, based on the virus concentration determined as described in Part A, section 4.4.4. Virus at one concentration only shall be inoculated into the monkeys.

(3) *Observation of monkeys*

All monkeys shall be observed for 17–22 days for symptoms suggestive of poliomyelitis or other virus infection. Monkeys that survive the first 24 hours but die before the 11th day after inoculation

shall be autopsied to determine whether poliomyelitis has been the cause of death. Those that have died from causes other than poliomyelitis shall be excluded from the evaluation.

Animals that become moribund or are severely paralysed should be killed and autopsied.

All monkeys that survive the observation period shall be autopsied.

For the test to be valid, no more than 20% of the animals in each group shall show signs of an intercurrent infection during the observation period.

(4) *Number of sections examined*

The lumbar cord, the cervical cord, the lower and upper medulla oblongata, the mesencephalon, the thalamus and the motor cortex of each monkey, as a minimum, shall be subjected to histological examination.

Sections shall be cut at a thickness of 15 µm and stained with gallocyanin.

In some countries, sections cut at a thickness of 8–15 µm are permitted, as is Nissl staining.

The minimum number of sections examined shall be as follows:

- 12 sections representative of the whole of the lumbar enlargement,
- 10 sections representative of the whole of the cervical enlargement,
- 2 sections from the medulla oblongata,
- 1 section from the pons and cerebellum,
- 1 section from the midbrain, and
- 1 section each from the left and the right of the thalamus and cerebral cortex.

(5) *Scoring of virus activity*

In the evaluation of virus activity in the hemisections of the spinal cord and brain-stem, a method of scoring the severity of the lesions shall be used. Since the type of damage, whether cellular infiltration or destruction of neurons, is important, the lesions shall be scored as follows:

1. Cellular infiltration only (this is not sufficient for the monkey to be considered as positive).
2. Cellular infiltration with minimal neuronal damage.
3. Cellular infiltration with extensive neuronal damage.
4. Massive neuronal damage with or without cellular infiltration.

The scores obtained shall be recorded on a standard form (see Appendix 4).

A monkey with neuronal lesions in the sections, but which shows no needle tract, shall be regarded as positive.

A monkey showing a needle tract in the sections, but no neuronal lesions, shall not be regarded as positive.

A section that shows damage due to trauma, but no specific virus lesions, is not included in the score.

Severity scores are based on hemisection readings of the lumbar (L), cervical (C) and brain (B) histological sections. The Lesion Score (LS) for each positive monkey is calculated as follows:

$$LS = \left[\frac{\Sigma \text{ of L score}}{\text{No. of hemisections}}\right] + \left[\frac{\Sigma \text{ of C score}}{\text{No. of hemisections}}\right] + \left[\frac{\Sigma \text{ of B score}}{\text{No. of hemisections}}\right] \div 3$$

A mean Lesion Score is calculated for each group of positive monkeys.

(6) *Evaluation of neurovirulence test*

The comparison of the virus activity in the vaccine and reference preparation shall be based on the activity in the lumbar enlargement of the cord and the degree of spread of activity from this region to the cervical enlargement and the brain.

The acceptance or rejection of the vaccine shall be based on the total score of all the test animals. Individual animals showing unusually high activity, either in the lumbar region or as the result of spread from this region, shall also be taken into consideration in the final evaluation.

The filtered bulk passes the test if the required number of animals is positive and if none of the clinical and histopathological examinations shows a significant difference in pathogenicity between the vaccine virus and the reference material.

Criteria for the acceptance of vaccines after neurovirulence testing are given in Appendix 3.

4.4.5.2 *Tests in vitro*. The virus in the filtered bulk suspension shall be tested for the property of reproducing at temperatures of 36 °C and 40 °C in comparison with the seed lot or a reference virus preparation for the marker tests and with appropriate rct/40− and rct/40+ strains of poliovirus of the same type. The incubation temperatures used in this test shall be controlled to within ±0.1 °C.

The filtered bulk suspension passes the test if, for both the virus in the bulk suspension and that in the appropriate reference material, the titre determined at 36 °C is at least 5.0 $\log_{10}$ greater than that determined at 40 °C. Unless the titres obtained for all the reference viruses are in line with the expected values, the test shall be repeated.

In some countries, the virus titre must not exceed 10 $CCID_{50}$/ml or 10 PFU/ml at the higher temperature.

It is desirable that the temperatures used in the test should also include one in the region of 39.0–39.5 °C, at which the titre of the reference material should be reduced by a factor in the range 3.0–5.0 $\log_{10}$ of its value at 36 °C. In one laboratory, a temperature of 39.2 °C has been found suitable. The aim is to obtain values of the ratio of the reproductive capacities of the bulk suspension and the reference material over a range of temperatures so that a more accurate comparison can be made.

The national control authorities should provide reference virus preparations and appropriate rct/40+ and rct/40− virus strains for this test.

It is recommended that the manufacturer should perform at least one other test for a genetic marker, since a genetic change might occur that might not be detected by the rct/40 marker test only. Other tests currently used are based on the study of the antigenic character of the strain, or tests for the sensitivity of reproduction to different concentrations of sodium bicarbonate (*d*-marker).

4.5 Final bulk

The operations necessary for preparing the final bulk shall be conducted in such a manner as to avoid contamination of the product.

The dilution and mixing procedures involved in preparing the final bulk should be those approved by the national control authority.

4.5.1 *Stabilizers*

Any stabilizers that may be added to the bulk suspension shall have been shown to the satisfaction of the national control authority

not to impair the safety and to improve the stability of the vaccine in the concentrations used.

All the tests described in Part A, sections 4.3 and 4.4, shall be performed on samples taken before any stabilizers are added.

4.5.2 *Tests for bacteria and fungi*

The final bulk shall be tested for bacterial and mycotic sterility in accordance with the requirements given in Part A, section 5, of the revised Requirements for Biological Substances No. 6 (Requirements for the Sterility of Biological Substances) (*8*, p. 48).

5. Filling and containers

The requirements concerning filling and containers given in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*6*, p. 16) shall apply to vaccine filled in the final form.

Care should be taken to ensure that the material of which the container is made does not adversely affect the virus content of the vaccine under the recommended storage conditions.

6. Control tests on final product

Samples shall be taken from each filling lot for the tests described in the following sections.

6.1 Identity test

The poliovirus type or types shall be serologically identified.

Care should be taken to ensure that the sera used are monospecific by titrating them against homotypic and heterotypic viruses of known virus titre.

6.2 Tests for bacteria and fungi

Liquid vaccine shall be tested for bacterial and mycotic sterility according to the requirements given in Part A, section 5, of the revised Requirements for Biological Substances No. 6 (Requirements for the Sterility of Biological Substances) (*8*, p. 48).

6.3 Virus titration

The poliovirus titre shall be determined as described in Part A, section 4.4.4, of these Requirements in assays including a reference preparation. If the vaccine contains more than one poliovirus type, each type shall be titrated separately by using appropriate type-specific antiserum for neutralizing each of the other types present. The national control authorities should specify the minimum virus titres per human dose.

> It is recommended that, when the Sabin strains are used, the estimated mean virus titres for a single human dose of trivalent OPV should be not less than $10^{6.0}$ infectious units for Type 1, $10^{5.0}$ infectious units for Type 2, and $10^{5.5}$ infectious units for Type 3, as determined by the procedure described in Appendix 2. The 95% confidence intervals of the assays should not differ by a factor of more than $10^{0.5}$ from the estimated number of infectious units in the vaccine.
>
> In some countries, the national control authorities recommend both an upper and a lower limit for the virus titre per human dose.

6.4 Accelerated degradation test

Representative final containers of the vaccine shall be incubated at 37 C for 48 hours. The total virus content in both exposed and unexposed vials shall be determined concurrently with that of a trivalent reference preparation. The vaccine passes the test when the loss on exposure is not greater than a factor of $10^{0.5}$ infectious units per human dose.

The titre of the vaccine after exposure shall be not less than that specified by the national control authority.

7. Records

The requirements given in Part A, section 6, of the revised Requirements for Biological Substances No. 1 (*6*, p. 17) shall apply.

8. Samples

The requirements given in Part A, section 7, of the revised Requirements for Biological Substances No. 1 (*6*, p. 18) shall apply.

9. Labelling

The requirements given in Part A, section 8, of the revised Requirements for Biological Substances No. 1 (*6*, p. 18) shall apply with the addition of the following:

The label on the container or package shall include the following information:

- the designation(s) of the strain(s) of poliovirus contained in the vaccine,
- the minimum amount of virus of each type contained in one recommended human dose,
- the cell substrate used for the preparation of the vaccine,
- the nature and amount of any stabilizer present in the vaccine,
- the fact that the vaccine is not to be injected.

> It is desirable for the label to carry the name both of the producer and of the source of the bulk material, if it was not prepared by the producer of the final vaccine. The nature and amount of the antibiotics present in the vaccine, if any, may be included.

10. Distribution and shipping

The requirements given in Part A, section 9, of the revised Requirements for Biological Substances No. 1 (*6*, p. 18) shall apply.

11. Storage and expiry date

The statements concerning storage temperature and expiry date appearing on the label and the leaflet, as required in Part A, section 10, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*6*), shall be based on experimental evidence and shall be submitted for approval to the national control authority.

11.1 Storage conditions

Before being released by the manufacturing establishment, all vaccines in final containers shall be kept continuously in the frozen state at a temperature below −20 °C.

The maximum duration of storage shall be fixed with the approval of the national control authority and shall be such as to ensure that the minimum titre of each virus type specified on the label of the container (or package) will still be maintained after release by the manufacturing establishment, if the conditions under which the vaccine is stored are in accordance with what is stated on the label. The maximum duration of storage at 2–8 °C or below −20 °C may be specified.

11.2 Expiry date

The expiry date shall be fixed with the approval of the national control authority and shall relate to the date of the last satisfactory determination, performed in accordance with Part A, section 4.4.4, of virus concentration, i.e., the date on which the test system was inoculated.

Provided that the vaccine has been stored continuously at a temperature below −20 °C, the expiry date shall be not more than two years after the last titration.

The label shall specify only one storage temperature and expiry date.

PART B. NATIONAL CONTROL REQUIREMENTS

1. General

The general requirements for control laboratories given in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*6*, p. 19), which specify that no new biological substance shall be released until consistency of production has been established, shall apply.

In some countries, consistency of production has to be established for the first five production batches.

The detailed production and control procedures and any significant changes in them shall be discussed with and approved by the national control authority. The national control authority shall obtain the international reference preparations for virus titre and establish national working reference preparations by comparison with them.

The national control authority shall obtain the international working reference preparations for neurovirulence. The control authority and the manufacturers should each carry out at least four tests on these preparations to obtain the necessary baseline data for comparison with the neurovirulence of test vaccines.

Unless the national control authority itself performs the neurovirulence tests, it should carry out a second reading of the histological sections provided by the manufacturer for each monovalent bulk.

The national control authority should encourage the use of the standard form for the reporting of data on virus activity in the sections taken for histopathological examination.

Neurovirulence test data obtained in a given laboratory and in different laboratories are of considerable interest and, since all national control authorities will be using the same neurovirulence reference preparations, accumulated data on the results obtained with these preparations would be of value to all laboratories. In order to build up such a data bank, national control authorities are requested to report their data to Biologicals, WHO, Geneva, Switzerland, for coordination.

2. Release and certification

A vaccine lot shall be released only if it satisfies Part A of the present Requirements. Before any vaccine lot is released from a manufacturing establishment, the requirements for consistency of production given in Part A, section 9.1, of the revised Requirements for Manufacturing Establishments and Control Laboratories (*6*, p. 18) shall be met.

A statement signed by the appropriate official of the national control authority shall be provided if requested by a manufacturing establishment and shall certify whether or not the lot of vaccine in question meets all national requirements as well as Part A of the present Requirements. The certificate shall further state the date of the last satisfactory determination of virus concentration, the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, a copy of the official national release document shall be attached.

The purpose of the certificate is to facilitate the exchange of poliomyelitis vaccine (oral) between countries.

he monkeys are used. After the last monkey of a group has taken, the room that housed the group shall be thoroughly d and decontaminated before being used for a fresh group.

In countries in which the kidneys from near-term monkeys are used, the mother should be quarantined for the term of pregnancy.

actions taken by working personnel shall be based on the ıption that a great potential hazard exists at all times in the ntine area. Personnel shall be provided with protective ng, including gloves, footwear and masks or visors. Street s shall not be permitted in the animal rooms. Smoking, eating rinking shall be forbidden while personnel are in the animal s.

supervisor shall be made responsible for reporting unusual s among employees and for ensuring that all injuries are rly treated. No worker who has cuts or abrasions on exposed of the body shall enter the animal area. Any unexplained e illness while off duty shall be considered as potentially related e employee's occupation.

onkeys from which kidneys are to be removed shall be sthetized and thoroughly examined, particularly for evidence of culosis and cercopithecid herpesvirus 1 (B virus) infection.

a monkey shows any pathological lesion relevant to the use of dneys in the preparation of a seed lot or vaccine, it shall not be nor shall any of the remaining monkeys of the quarantine p concerned be used unless it is evident that their use will not ir the safety of the product.

ll the operations described in this section shall be conducted de the areas were vaccine is made.

ıe monkeys used shall be shown to be free from antibodies to) virus and simian immunodeficiency virus.

In some countries, monkeys are tested for antibodies to cercopithecid herpesvirus 1 (B virus).

Production precautions

he general production precautions called for by the require- ts of Part A, section 3, of the revised Requirements for ogical Substances No. 1 (General Requirements for Manu- ıring Establishments and Control Laboratories) (*6*, p. 11) shall

apply to the .nufacture of oral poliomyelitis vaccine, with the addition of the following:

4.2.1 *Monkey kidney-cell cultures for vaccine production*

Cultures of monkey kidney cells that have not been propagated in series shall be prepared from kidneys that shown no pathological signs. Virus for the preparation of vaccine shall be grown by aseptic methods in such cultures. If animal serum is used in the propagation of the cells, the maintenance medium after virus inoculation shall contain no added serum.

In some countries, the virus may be grown in serially passaged monkey kidney-cell cultures from primary monkey kidney cells.

Each group of cell cultures derived from a single monkey or from no more than 10 near-term monkeys shall be prepared and tested as an individual group.

4.2.2 *Tests of cell cultures used for vaccine production (see Appendix 6)*

On the day of inoculation with virus working seed lot, each cell culture shall be examined for degeneration caused by an infective agent. If, in this examination, evidence is found of the presence in a cell culture of any adventitious agent, the entire group of cultures concerned shall not be used for vaccine production.

On the day of inoculation with the virus working seed lot, a sample of at least 30 ml of the pooled fluid removed from the cell cultures of the kidneys of each single monkey or from no more than 10 near-term monkeys shall be divided into two equal portions. One portion of the pooled fluid shall be tested in monkey kidney-cell cultures prepared from the same species, but not the same animal, as that used for vaccine production. The other portion of the pooled fluid shall be tested in monkey kidney-cell cultures from another species, provided that the tests on the pooled fluids shall be done in cell cultures from at least one species known to be sensitive to SV40 virus. The pooled fluid shall be inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm^2 per ml of pooled fluid. At least one bottle of each kind of cell culture shall remain uninoculated and shall serve as a control.

In some countries in which a monkey specie for vaccine production is known to be sensitive to SV40, a test in a second species is not required.

Animal serum may be used in the propagation of the cells, provided that it does not contain SV40 antibody or other inhibitors, but the maintenance medium after inoculation of test material should contain no added serum except as described below.

The cultures shall be incubated at a temperature of 35–37 °C and shall be observed for a total period of at least four weeks. During this observation period and after not less than two weeks' incubation, from each of these cultures at least one subculture of fluid shall be made in the same tissue culture system. The subculture shall also be observed for at least two weeks.

Serum may be added to the original culture at the time of subculturing, provided that the serum does not contain SV40 antibody or other inhibitors.

Fluorescent antibody techniques may be useful for detecting SV40 virus and other viruses in the cells.

A further sample of at least 10 ml of the pooled fluid shall be tested for the presence of cercopithecid herpesvirus 1 (B virus) and other viruses in rabbit kidney-cell cultures. Serum used in the nutrient medium of these cultures shall have been shown to be free from inhibitors.[1] The sample shall be inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm^2 per ml of pooled fluid. At least one bottle of the cell cultures shall remain uninoculated and serve as a control.

The cultures shall be incubated at a temperature of 35–37 °C and shall be observed for a period of at least two weeks.

It is suggested that, in addition to these tests, a further sample of 10 ml of the pooled fluid removed from the cell cultures on the day of inoculation with the seed lot virus should be tested for the presence of adventitious agents by inoculation into cell cultures sensitive to measles virus.

In some countries, a sample of 2% of the pooled fluid is tested in each of the culture systems specified.

For the tests to be valid, not more than 20% of the culture vessels shall have been discarded for nonspecific accidental reasons by the end of the respective test periods.

[1] Human herpesvirus has been used as an indicator for freedom from B virus inhibitors on account of the danger of handling cercopithecid herpesvirus 1 (B virus).

If, in these tests, evidence is found of the presence of an adventitious agent, the single harvest from the whole group of cell cultures concerned shall not be used for vaccine production.

If the presence of cercopithecid herpesvirus 1 (B virus) is demonstrated, the manufacture of oral poliomyelitis vaccine shall be discontinued and the national control authority shall be informed. Manufacturing shall not be resumed until a thorough investigation has been completed and precautions have been taken against any reappearance of the infection, and then only with the approval of the national control authority.

If these tests are not done immediately, the samples of pooled cell-culture fluid shall be kept at a temperature of −60 °C or below, with the exception of the sample for the test for B virus, which may be held at 4 C, provided that the test is done not more than seven days after it has been taken.

4.2.3 *Tests of control cell cultures*

Cultures prepared on the day of inoculation with the virus working seed lot from 25%, but not more than 2.5 litres, of the cell suspension obtained from the kidneys of each single monkey or from no more than 10 near-term monkeys shall remain uninoculated, and shall serve as controls. These control cell cultures shall be incubated under the same conditions as the inoculated cultures for at least two weeks, and shall be examined during this period for evidence of cytopathic changes. For the tests to be valid, not more than 20% of the control cell cultures shall have been discarded for nonspecific, accidental reasons. At the end of the observation period, the control cell cultures shall be examined for degeneration caused by an infectious agent. If this examination or any of the tests required in this section shows evidence of the presence in a control culture of any adventitious agent, the poliovirus grown in the corresponding inoculated cultures from the same group shall not be used for vaccine production.

4.2.3.1 *Tests for haemadsorbing viruses.* At the time of harvest or not more than four days after the day of inoculation of the production cultures with the virus working seed lot, a sample of 4% of the control cell cultures shall be taken and shall be tested for haemadsorbing viruses. At the end of the observation period, the remaining control cell cultures shall be similarly tested. The tests shall be made as described in Part A, section 4.1.1.

4.2.3.2 *Tests for other adventitious agents.* At the time of harvest, or not more than seven days after the day of inoculation of the production cultures with the working seed lot, a sample of at least 20 ml of the pooled fluid from each group of control cultures shall be taken and tested in two kinds of monkey kidney-cell culture, as described in Part C, section 4.2.2.

At the end of the observation period for the original control cell cultures, similar samples of the pooled fluid shall be taken and the tests referred to in this section in the two kinds of monkey kidney-cell culture and in the rabbit cell cultures shall be repeated, as described in Part C, section 4.2.2.

If the presence of cercopithecid herpesvirus 1 (B virus) is demonstrated, the production cell cultures shall not be used and the measures concerning vaccine production described in Part C, section 4.2.2, shall be taken.

> In some countries, fluids are collected from the control cell cultures at the time of virus harvest and at the end of the observation period. Such fluids may then be pooled before testing for adventitious agents.
>
> In several countries, a sample of 2% of the pooled fluid is tested in each of the cell culture systems specified.

4.3 Control of single harvests

4.3.1 *Single harvest*

4.3.1.1 *Tests for neutralized single harvests in monkey kidney-cell cultures.* A sample of at least 10 ml of each single harvest shall be neutralized by type-specific poliomyelitis antiserum prepared in animals other than monkeys. In preparing antisera for this purpose, the immunizing antigens used shall be prepared in nonsimian cells.

> Care should be taken to ensure that the antiserum used is monospecific. This may be demonstrated by titration of the antiserum against homotypic and heterotypic virus of known virus titre using the same dilution of the antiserum as that used for neutralization.

Half (corresponding to at least 5 ml of single harvest) of the neutralized suspension shall be tested in monkey kidney-cell cultures prepared from the same species, but not the same animal, as that

used for vaccine production. The other half of the neutralized suspension shall be tested in monkey kidney-cell cultures from another species, provided that the tests on the neutralized suspension shall be done in cell cultures from at least one species known to be sensitive to SV40 virus.

The neutralized suspensions shall be inoculated into bottles of these cell cultures in such a way that the dilution of the suspension in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm^2 per ml of neutralized suspension. At least one bottle of each kind of cell culture shall remain uninoculated, shall serve as a control and shall be maintained by nutrient medium containing the same concentration of the specific antiserum used for neutralization.

Animal serum may be used in the propagation of the cells, provided that it does not contain SV40 antibody or other inhibitors, but the maintenance medium after the inoculation of the test material should contain no added serum other than the poliovirus neutralizing antiserum, except as described below.

The cultures shall be incubated at a temperature of 35–37 C and shall be observed for a total period of at least four weeks. During this observation period and after no less than two weeks' incubation, at least one subculture of fluid shall be made from each of these cultures in the same tissue culture system. The subcultures shall also be observed for at least two weeks.

Serum may be added to the original cultures at the time of subculturing provided that the serum does not contain SV40 antibody or other inhibitors.

In several countries, additional tests must be made for adventitious agents on a further sample of the neutralized single harvests by inoculation of 10 ml into human cell cultures sensitive to measles virus.

Fluorescent antibody techniques may be useful for detecting SV40 virus and other viruses in the cells.

For the tests to be valid, not more than 20% of the culture vessels shall have been discarded for nonspecific accidental reasons by the end of the respective test periods.

If any cytopathic changes occur in any of the cultures, the causes of these changes shall be investigated. If the cytopathic changes are shown to be due to unneutralized poliovirus, the test shall be repeated. If there is evidence of the presence of SV40 virus or other adventitious agents attributable to the single harvest, that single harvest shall not be used for vaccine production.

4.4 Control of bulk suspension before filtration

4.4.1 *Bulk suspension*

4.4.1.1 *Tests in rabbits.* A sample of the bulk suspension shall be tested for the presence of cercopithecid herpesvirus 1 (B virus) and other viruses by injection into at least 10 healthy rabbits each weighing between 1.5 kg and 2.5 kg. The sample shall consist of at least 100 ml. Each rabbit shall receive not less than 10 ml nor more than 20 ml, of which 1 ml is given intradermally at multiple sites, and the remainder subcutaneously. The rabbits shall be observed for at least three weeks for death or signs of illness.

In some countries, the sample consists of at least 1% of the bulk suspension, provided that this is not less than 100 ml, up to a maximum of 500 ml, and the period of observation of the rabbits is four to five weeks.

All rabbits that die after the first 24 hours of the test shall be examined by autopsy, and the brain and organs removed for detailed examination to establish the cause of death; animals showing signs of illness shall be killed and subjected to a similar autopsy.

The bulk suspension passes the test if no more than 20% of the inoculated rabbits show signs of intercurrent infection during the observation period and if none of the rabbits shows evidence of infection with B virus or with other adventitious agents or lesions of any kind attributable to the bulk suspension.

If the presence of B virus is demonstrated, the measures concerning vaccine production described in Part C, section 4.2.2, shall be taken.

In some countries, a test for the presence of Marburg virus is carried out in guinea-pigs.

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ACKNOWLEDGEMENTS

Acknowledgements are due to the following experts for their comments and advice on the first draft of these Requirements: Dr A. Breschkin, Virology Unit, National Standards Laboratory, Commonwealth Department of Community Services and Health, Parkville, Victoria, Australia; Dr Dai Bin, Department of Viral Vaccine, National Institute for the Control of Pharmaceutical and Biological Products, Temple of Heaven, Beijing, China; Dr A. Fenyves, Head, Unit for the Control of Live Virus Vaccines, Paul Ehrlich Institute, Frankfurt, Federal Republic of Germany; Dr K. Frieling, Paul Ehrlich Institute, Frankfurt, Federal Republic of Germany; Dr R.K. Gupta, National Institute of Immunology, New Delhi, India; Dr C. Guthrie, Commonwealth Serum Laboratories, Parkville, Victoria, Australia; Dr R. Hassan Ali, Egyptian Organization for Biological Products and Vaccines, Agouza, Egypt; Dr D.E. Hazra, Consultant of the Nuclear Medicine Radioimmunoassay Unit, Post Graduate Department of Medicine, S.N. Medical College, Agra, India; Dr P. Lemoine, Institute of Hygiene and Epidemiology, Brussels, Belgium; Mr J. Lyng, State Serum Institute, Copenhagen, Denmark; Dr T. Matuhasi, Ministry of Health and Welfare, Tokyo, Japan; Dr B. Montagnon, Mérieux Institute, Marcy l'Etoile, France; Dr R. Netter, Director-General, National Health Laboratory, Ministry of Solidarity, Health, and Social Protection, Paris, France; Mr J. Peetermans, Technical Director, SmithKline Biologicals s.a., Rixensart, Belgium; Dr V. Takla-Rizk, Egyptian Organization for Biological Products and Vaccines, Agouza, Egypt; Dr T. Ubertini, Technical Director, Tuscan Institute for Serotherapy and Vaccine Production, (SCLAVO), Siena, Italy; Dr J.R. Vose, Connaught Laboratories Ltd., Willowdale, Ontario, Canada; Dr J.J. Walsh, Commonwealth Serum Laboratories, Parkville, Victoria, Australia.

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Appendix 1

TESTS FOR BOVINE VIRUSES IN SERUM

The serum to be tested is used in a growth medium to cultivate primary bovine testis cells or cells of a continuous bovine kidney-cell line known to be sensitive to bovine viruses.

The method, in which primary bovine testis cells are used as a suspension in Eagle's Minimum Essential Medium (MEM) containing 10^5 cells/ml, is as follows:

A 90-ml sample of the cell suspension is mixed with 10 ml of the test serum and dispensed into two 75-cm^2 plastic tissue-culture flasks. The process is repeated with a further 90 ml to which 10 ml of control serum (pretested for freedom from bovine viruses) is added. The cultures are incubated at 36.5 °C ± 1 °C for a period of 28 days with frequent microscopic examination for evidence of extraneous viruses. The growth medium may be replaced, as required, by fresh medium containing the relevant serum.

At least one subculture of the cells is carried out for both the test and control sera during the observation period in order to provide not less than 300 cm^2 (e.g., six bottles each of 25 cm^2) of cells of each group for further examination at 28 days after initiation.

At the end of the observation period, each of the following tests is applied to at least one 25-cm^2 culture each of cells grown on test serum and on control serum, appropriate controls being retained in each case:

(*a*) a test to show that the cells are sensitive to a suitable challenge virus,
(*b*) microscopic examination for abnormalities following staining by the May Grunwald–Giemsa technique,
(*c*) a test for haemadsorbing agents, using a mixed suspension of 0.2% chick, 0.2% guinea-pig, and 0.2% human group O erythrocytes,
(*d*) examination by electron microscopy of the negatively stained extract produced after disruption and clarification of the cell culture,
(*e*) examination for bovine viruses by direct or indirect immunofluorescence tests.

Appendix 2

ASSAY METHOD FOR THE DETERMINATION OF THE VIRUS CONTENT OF POLIOMYELITIS VACCINE (ORAL)

The preparation to be assayed and the reference preparation are diluted in an appropriate medium.

It is convenient to make tenfold dilution steps of the virus suspensions initially, but for dilutions that are to be inoculated into cell cultures the dilutions should be prepared in 0.5 $\log_{10}$ or smaller steps. A preliminary assay may be required to ensure that, in the test, the dilution range selected encompasses at least three dilutions that will infect between 10% and 90% of the cultures inoculated.

Groups of 8–12 flat-bottomed wells in a microtitre plate are inoculated with 0.1 ml of each of the selected dilutions of virus followed by 0.1 ml of a suitable cell suspension of the Hep-2 (Cincinnati) line. The plates are incubated at 35–36 °C for seven days.

The cultures are examined for the presence of a specific viral cytopathic effect on days 3–5 and again on day 7. The observations are recorded and the $CCID_{50}$ is calculated on the basis of the observation on day 7. The assay should be repeated on at least two occasions and a mean taken. Results should be presented for the test preparation and the reference. For the estimated titre to be accepted as valid, the observed mean for the reference should be within 0.5 $\log_{10}$ of the established mean for this preparation and the 95% confidence intervals of the assay should be within 0.5 $\log_{10}$ of the estimated number of infectious units in the vaccine.

Appendix 3

CRITERIA FOR THE ACCEPTANCE OF VACCINES AFTER NEUROVIRULENCE TESTING

It is recommended that each laboratory should perform a minimum of four neurovirulence tests (referred to here as "qualifying" tests) on each reference vaccine (Types 1, 2 and 3) to provide sufficient data on the activity of such vaccines for the development of criteria for the acceptability of test vaccines. On practical grounds, each of these tests should include a homotypic lot of production vaccine tested concurrently with the reference so that the results of the tests may be used in assessing vaccines in addition to providing information on the reference. The minimum number of animals in each of these tests is as specified on page 47 for each poliovirus type. The overall mean Lesion Score (M) for the replicate tests on each reference virus is calculated together with the pooled estimate (s^2) of the within-test variance and the within-test deviation (s).

Criteria for the validity of the results of a test of a reference preparation can be determined by each laboratory only on the basis of the data accumulated after the four qualifying tests. No generally applicable criteria can therefore be given. For laboratories with limited experience with neurovirulence testing, the following empirical method of establishing acceptable limits for the mean Lesion Score for the reference ($\bar{X}_{ref}$) may be helpful:

	Lower limit	*Upper limit*
For Types 1 and 2	$M - s$	$M + s$
For Type 3	$M - \frac{s}{2}$	$M + s$

If the mean Lesion Score for the test is $\bar{X}_{test}$, and C_1, C_2 and C_3 are constants, then:

The vaccine is not acceptable if:

$\bar{X}_{test} - \bar{X}_{ref} > C_1$.

The vaccine may be retested once at the discretion of the national control authority if:

$C_1 < \bar{X}_{test} - \bar{X}_{ref} < C_2$.

If the vaccine is retested, the means of the Lesion Scores for the test and reference vaccines are recalculated, and the vaccine is rejected if:

$$\frac{\bar{X}_{(test1 + test2)} - \bar{X}_{(ref1 + ref2)}}{2} > C_3.$$

The constants C_1, C_2, and C_3 are calculated as follows:

$$C_1 = 2.3\sqrt{\frac{2s^2}{N_1}}$$

$$C_2 = 2.6\sqrt{\frac{2s^2}{N_1}}$$

$$C_3 = 1.6\sqrt{\frac{2s^2}{N_2}}$$

where N_1 = number of positive monkeys per vaccine test,
N_2 = number of positive monkeys for the two tests,
2.3 = normal deviate at the 1% level,
2.6 = normal deviate at the 0.5% level,
1.6 = normal deviate at the 5% level.

In some countries, the national control authority may permit an experienced manufacturer to accumulate data on the qualifying tests of the Types 1 and 2 international preparations for neurovirulence as serial batches of vaccine are tested and released, rather than wait until the data are available from the four qualifying tests before the release of any future vaccine.

In some countries, however, the qualifying test may be required on only two occasions for a seed (Type 3) that has been extensively tested and used.

A neurovirulence test in which the mean Lesion Score for the reference ($\bar{X}_{ref}$) is not compatible with previous experience should not be used for assessing a test vaccine.

If the test is valid, the mean Lesion Score for the test vaccine ($\bar{X}_{test}$) is calculated and compared with that of the homotypic reference vaccine.

It is assumed that the values of the constants C_1, C_2, and C_3 will be calculated by each laboratory for each reference vaccine. As experience with the reference accumulates, it is recommended that laboratories should review the values of s^2 and M.

It is expected that, for a single test, the analysis recommended above will result in the rejection of approximately 1% of test vaccines that are identical to the homotypic reference, on the

assumption that, in each laboratory, the within-test variation is similar to that observed in the qualifying tests with that reference.

Estimates of the probability that test vaccines with a true Lesion Score double that of the reference vaccine will be rejected are given in Table 1 for different coefficients of variation.

Table 1. Estimated probability that a test vaccine for which the true Lesion Score is double that of the homotypic reference will be rejected

Total number of positive animals per test	Coefficient of variation[a]					
	0.4	0.6	0.8	1.0	1.2	1.4
24[b]	99%	96%[c]	77%	55%	39%	28%
40	99%	99%	95%	80%[d]	62%	47%

[a] The coefficient of variation is defined as the within-test standard deviation divided by the mean Lesion Score. Boxes show acceptable coefficients of variation.
[b] Divided equally between tests on Type 1 and 2 vaccines.
[c] Corresponds to tests on Type 1 and 2 vaccines.
[d] Corresponds to tests on Type 3 vaccines.

In tests on vaccines which satisfy the above criteria of acceptability, individual animals may occasionally develop extremely high Lesion Scores. Such findings should be taken into consideration in evaluating the acceptability of vaccines, but precise criteria for use in making a decision are difficult to define.

Sample calculations are shown in Tables 2 and 3.

Table 2. Sample calculations of results of qualifying tests of reference vaccines

Basic data	Type 1	Type 2	Type 3
Overall mean Lesion Score (M) (Initial four tests)	1.110	0.878	1.043
Within-test pooled variance (s^2) of M	0.444	0.236	0.686
Within-test pooled standard deviation (s) (square root of s^2)	0.666	0.486	0.828
Coefficient of variation (CV) (CV = s/M)	$\frac{0.666}{1.110} = 0.60$	$\frac{0.486}{0.878} = 0.55$	$\frac{0.828}{1.043} = 0.79$
Upper and lower limits for satisfactory test result. Mean Lesion Score of reference vaccine ($\bar{X}_{ref}$)	$M + s = 1.110 + 0.666 = 1.776$ $M - s = 1.110 - 0.666 = 0.444$	$M + s = 0.878 + 0.486 = 1.364$ $M - s = 0.878 - 0.486 = 0.392$	$M + s = 1.043 + 0.828 = 1.871$ $M - \frac{s}{2} = 1.043 - \frac{0.828}{2} = 0.629$
Constants for assessing acceptability of difference between mean Lesion Score of test vaccine ($\bar{X}_{test}$) and mean Lesion Score of reference vaccine ($\bar{X}_{ref}$)	$C_1 = 2.3\sqrt{\frac{2s^2}{N_1}} = 2.3\sqrt{\frac{2 \times 0.444}{12}} = 2.3\sqrt{0.074} = 0.626$ $C_2 = 2.6\sqrt{\frac{2s^2}{N_1}} = 2.6\sqrt{\frac{2 \times 0.444}{12}} = 2.6\sqrt{0.074} = 0.707$ $C_3 = 1.6\sqrt{\frac{2s^2}{N_2}} = 1.6\sqrt{\frac{2 \times 0.444}{24}} = 1.6\sqrt{0.037} = 0.308$	$2.3\sqrt{\frac{2 \times 0.236}{12}} = 2.3\sqrt{0.039} = 0.456$ $2.6\sqrt{\frac{2 \times 0.236}{12}} = 2.6\sqrt{0.039} = 0.516$ $1.6\sqrt{\frac{2 \times 0.236}{24}} = 1.6\sqrt{0.020} = 0.224$	$2.3\sqrt{\frac{2 \times 0.686}{20}} = 2.3\sqrt{0.069} = 0.602$ $2.6\sqrt{\frac{2 \times 0.686}{20}} = 2.6\sqrt{0.069} = 0.681$ $1.6\sqrt{\frac{2 \times 0.686}{40}} = 1.6\sqrt{0.034} = 0.296$

Table 3. Examples of tests with Type 1 reference and vaccine

Example	Mean Lesion Scores		Difference between vaccine and reference ($\bar{X}_{test} - \bar{X}_{ref}$)	Conclusion/Action*
	Reference ($\bar{X}_{ref}$)	Vaccine ($\bar{X}_{test}$)		
One test	0.826	1.188	0.362	Less than C_1: vaccine acceptable
Two tests 1st	0.826	1.493	0.667	More than C_1 but less than C_2: vaccine unacceptable: retest is permissible
2nd	1.285	1.209	−0.076	–
Combined tests	1.056	1.351	0.295	Less than C_3: vaccine acceptable
Two tests 1st	0.826	1.493	0.667	More than C_1 but less than C_2: vaccine unacceptable: retest is permissible
2nd	1.285	1.405	0.120	–
Combined tests	1.056	1.449	0.393	More than C_3: vaccine fails

*For values of C, see Table 2

Appendix 4

FORM ON WHICH TO REPORT THE SCORE OF VIRUS ACTIVITY FOR EACH HISTOLOGICAL SECTION FROM ALL MONKEYS INCLUDED IN THE NEUROVIRULENCE TEST

Although the test requires that at least 11 monkeys should be positive after inoculation with the vaccine and reference virus for Types 1 and Types 2, provision is made on the following form (pp. 74–75) for recording results for 12 monkeys that may be inoculated and survive the test. A separate form to record the lesions in 20 monkeys will be required for Type 3. Records for each vaccine and reference preparation must be on separate forms.

On the forms, the method of scoring the lesions used for all sections from all areas is that already indicated on p. 48, namely:

1. Cellular infiltration only.
2. Cellular infiltration with minimal neuronal damage.
3. Cellular infiltration with extensive neuronal damage.
4. Massive neuronal damage with or without cellular infiltration.

A model of the certificate of compliance with the international requirements for the neurovirulence testing of final OPV bulks in monkeys is given below.

Certificate of compliance with the requirements for the neurovirulence testing of final OPV bulks in monkeys

Final bulk No. _____

Date of certification _____

I certify that the above final bulk complies with the requirements for tests in monkeys for neurovirulence published in WHO Technical Report Series, No. 800, 1990.

Signature _____

Name (typed) _____

Date _____

74 All data from all monkeys must be recorded, which may require a larger form.
Clinical signs of paralysis must be recorded on a separate form.

Test No. _____ Type _____ Final Bulk No. _____ Species _____

Post-inoculation titre: Log_{10} $CCID_{50}$/ml _____ Dilution _____

Date of inoculation _____ Date of end of test _____

N	V		Histological lesions due to poliomyelitis																																				Lesion Score
			Lumbar enlargement																Cervical enlargement												Brain								
			1	2	3	4	5	6	7	8	9	10	11	12					Av	1	2	3	4	5	6	7	8	9	10		Av	Med 1	Med 2	Cb	P	M	T	Co	Av	
		L R																																							
		L R																																							
		L R																																							
		L R																																							
		L R																																							
		L R																																							

(continued on next page)

N	V		Histological lesions due to poliomyelitis																																				Lesion Score
			Lumbar enlargement																Cervical enlargement												Brain								
			1	2	3	4	5	6	7	8	9	10	11	12					Av	1	2	3	4	5	6	7	8	9	10		Av	Med 1	Med 2	Cb	P	M	T	Co	Av	
		L R																																							
		L R																																							
		L R																																							
		L R																																							
		L R																																							
		L R																																							

Mean Lesion Score

KEY

N = Monkey number
V = Virus titre
L = Left
R = Right
Av = Average
Med = Medulla
Cb = Cerebellum
P = Pons
M = Midbrain
T = Thalamus
Co = Cortex

75

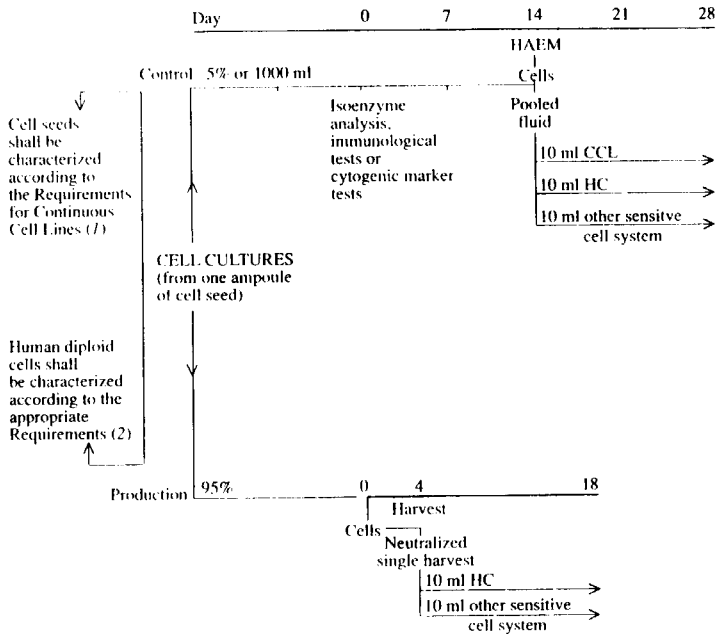
Signature _____ Date _____

Name (typed) _____

Appendix 5

PREPARATION OF POLIOMYELITIS VACCINE (ORAL) USING CELL BANKS

Example of a Flowsheet of Tests in Cell Cultures



HAEM = test for haemadsorbing viruses; CCL = continuous cell line; HC = human cells sensitive to measles.

Note. This example includes all tests, whether obligatory or not. Since the requirements applicable in a particular place are those authorized by the national control authority, this flowsheet should not be considered as an integral part of the requirements and has been included solely for guidance. Manufacturing establishments should prepare their own flowsheet in order to clarify the procedures used.

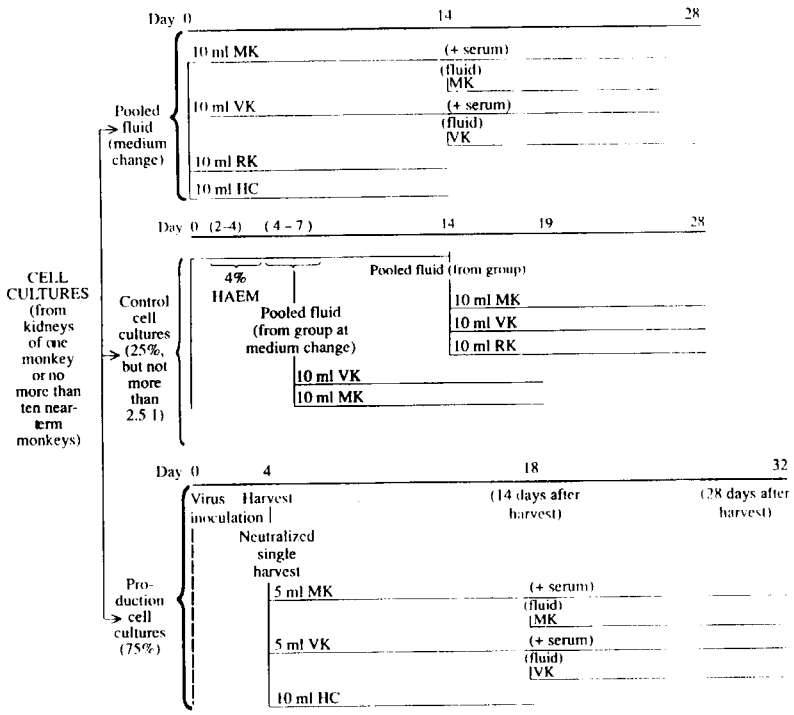
References

1. WHO Technical Report Series, No. 745, 1987.
2. WHO Technical Report Series, No. 687, 1983.

Appendix 6

PREPARATION OF POLIOMYELITIS VACCINE (ORAL) USING MONKEY KIDNEY-CELL CULTURES

Example of a Flowsheet of Tests in Cell Cultures



HAEM = test for haemadsorbing viruses; MK = monkey kidney cells from species used for production; VK = kidney cells from vervet monkey or one sensitive to SV40 virus; RK = rabbit kidney cells; HC = human cells sensitive to measles.

Note. This example includes all tests, whether obligatory or not. Since the requirements applicable in a particular place are those authorized by the national control authority, this flowsheet should not be considered as an integral part of the requirements and has been included solely for guidance. Manufacturing establishments should prepare their own flowsheet in order to clarify the procedures used.

Appendix 7

SUMMARY PROTOCOL FOR POLIOMYELITIS VACCINE (ORAL)

Based on Requirements for Biological Substances No. 7, revised 1989

Name and address of manufacturer .....
.....

Proprietary name .....

Lot No. of vaccine trivalent blend .....

Filling lot No. .....

No. of filled containers .....

Date on which last determination of virus concentration was started .....

Shelf-life .....

Expiry date .....

Nature and concentration of stabilizer .....

Volume of vaccine container .....

Volume of human dose (in drops and/or ml) .....

Prescribed virus concentration per human dose
Type 1 .....
Type 2 .....
Type 3 .....

Nature of any antibiotics present in vaccine and amount per human dose .....

Production cell tissue .....

	Type 1	*Type 2*	*Type 3*
Bulk Nos. of monovalent bulk suspensions blended in trivalent vaccine	.....	.....	.....
Date of approval of protocol indicating compliance with Requirements for Biological Substances No. 7	.....	.....	.....

The following sections are intended for the recording of the results of the tests performed during the production of the vaccine, so that the complete document will

provide evidence of consistency of production; thus if any test has h be repeated, this must be indicated. Any abnormal result must be recorded on a separate sheet.

If any cell lot or virus harvest intended for production was rejected during the control testing, this should also be recorded either in the following sections or on a separate sheet.

PRODUCTION IN CELL LINES

Control of source materials

Cell seed

Origin and short history of cell seed .....

Authority that approved cell seed .....

Total number of ampoules of cells stored .....

Method of preparation of cell seed in terms of number of freezes and efforts made to ensure that a homogeneous population is dispersed into the ampoules .....

Passage level (or No. of population doublings) of cell seed .....

Storage conditions .....

Percentage of total cell-seed ampoules tested .....

Growth characteristics .....

Morphological characteristics .....

Immunological markers .....

Cytogenetic data .....

Biochemical data .....

Results of other identity tests .....

Results of tests for adventitious agents .....

Results of tests for tumorigenicity .....

Capacity for interferon production (if determined) .....

Viral susceptibility .....

Manufacturer's working cell bank (MWCB)

Date MWCB was established .....

Quantity of cells stored .....

Passage level of MWCB .....

Storage conditions .....

Percentage of total MWCB ampoules tested .....

Results of identity tests .....

Results of tests for adventitious agents .....

Results of tests for tumorigenicity .....

Virus strains

Reference No. of seed lot .....

Seed virus strain .....

Substrate used for preparing seed lot .....

Date(s) of satisfactory test(s) for freedom from adventitious agents .....

Sterility test .....

Identity test .....

Virus concentration .....

Test for consistency of virus characteristics

Neurovirulence test in monkeys .....

Result of blood serum test in monkeys prior to inoculation .....

Date of inoculation of seed lot .....

Number and species of monkeys inoculated } control ..... test .....

Quantity ($CCID_{50}$) inoculated in each test monkey .....

Number of monkeys surviving without specific symptoms } control ..... test .....

Result of histopathological examination (specify any abnormal findings) } control ..... test .....

Test *in vitro* .....

Control of vaccine production

Control of cell cultures

Ratio of control to production cell cultures or control cultures as proportion of production cell cultures .....

Period of observation of cultures .....

Ratio or proportion of cultures discarded for nonspecific reasons .....

Results of observation .....

Tests for haemadsorbing viruses:
- Methods .....
- Results .....

Tests for adventitious agents:
- Methods .....
- Results .....

Identity test .....

Cell cultures for vaccine production

Tests for adventitious agents:
- Methods .....
- Results .....

Tests for bacteri, fungi and mycoplasmas:
- Methods .....
- Results .....

Control of single harvests

Volume harvested .....

Date of sampling .....

Tests of neutralized single harvests for adventitious agents:
- Methods .....
- Results .....

Sterility tests:
- Methods .....
- Results .....

Control of bulk suspension

Date of filtration of bulk .....

Porosity of filters used .....

Date of sampling .....

Identity test:
- Method .....
- Result .....

Virus concentration:
- Method .....
- Result .....

Tests for consistency of virus characteristics

Neurovirulence tests in monkeys:
- Species of monkey inoculated .....
- Dose of vaccine virus injected .....
- No. of "valid" monkeys inoculated with test sample .....
- No. of positive monkeys observed .....
- Reference preparation .....
- Dose of reference virus injected .....
- No. of "valid" monkeys inoculated with reference .....
- No. of positive monkeys observed .....
- Mean Lesion Score of test sample .....
- Mean Lesion Score of reference (see also attached forms giving details of the histological observations and assessment)[1] .....

In vitro rct/40 marker test:
- Reduction of titre of bulk sample .....
- Reduction of titre of negative reference .....
- Reduction of titre of positive reference .....
- Result .....

Additional *in vitro* marker tests:
- Test method .....
- Details .....
- Date of test .....
- Result .....

Final bulk

Preparation of trivalent bulk:

	Type 1	*Type 2*	*Type 3*
Lot No. of trivalent blend	.....	.....	.....
Monovalent bulks in blend	.....	.....	.....
Volume in blend	.....	.....	.....
Nature and volume of stabilizer	.....	.....	.....
Nature and volume of diluent	.....	.....	.....
Total volume of blend	.....	.....	.....

[1] Completed forms in the format given in Appendix 4, pages 74–75, should be attached.

Sterility test:
- Date .....
- Media used .....
- Result .....

Filling and containers:
- Total volume of final filling .....
- Date of final filling .....
- No. of vials filled .....
- Test on final filling .....

Control tests on final product

Identity test
- Methods .....
- Result .....

Tests for bacteria and fungi
- Number of containers examined .....
- Method .....
- Result .....

Virus titration
- Identity of reference preparations .....
- Titre of individual virus types .....
- Batch Nos. of antiserum used in test .....
- Date of test .....

	Vaccine	*Reference*
Results:		
Type 1	.....	.....
Type 2	.....	.....
Type 3	.....	.....

Stability
- Method .....
- Result .....

PRODUCTION IN MONKEY KIDNEY-CELL CULTURES

Control of vaccine production

- Monkey species used for production .....
- Quarantine batch No. .....

Percentage of monkeys surviving quarantine period .....

Nature and concentration of antibiotics used in production cell culture maintenance medium .....

Tests for antibodies to simian immunodeficiency virus and SV40:
- Methods .....
- Results .....

Production details:
- Production monkey No. .....
- Date of trypsinizing .....
- No. of cultures prepared .....

Production cell cultures:
- Virus seed lot No. .....
- Virus infectivity/cell ratio .....
- No. of cultures inoculated .....
- Date of inoculation .....
- Date of harvest .....
- Temperature of incubation .....
- Period of incubation .....
- No. of cultures harvested .....

Tests on pooled supernatant fluids:
- Date of sampling from production cell cultures .....
- Tests for adventitious agents .....
- Volume tested/cell culture type .....
- Observation period .....
- Date of completion of tests .....
- Result .....
- Date of sampling from cell cultures inoculated with the pooled fluid .....

Tests for adventitious agents:
- Volume tested/cell culture type .....
- Date of completion of tests .....
- Results .....

Tests in rabbit kidney-cell cultures:
- Volume tested .....
- Date of completion of test .....
- Result .....

Tests of control cell cultures:
- Ratio of control to production cell cultures or control cell cultures as proportion of production cell cultures .....
- Period of observation of cultures .....
- Ratio or proportion of cultures discarded for nonspecific reasons .....
- Result .....

Tests for haemadsorbing viruses:
- Methods .....
- Results .....

Test for other adventitious agents:
- Methods .....
- Results .....

Control of single harvests

Volume harvested .....

Date of sampling .....

Tests for bacteria, fungi, and mycoplasmas:
- Result .....

Tests on neutralized single harvests in monkey kidney-cell and human cell cultures:
- Batch No. of antiserum used .....
- Volume tested .....
- Date of starting primary cell culture tests .....
- Period of observation .....
- Date of sampling cell culture fluids .....
- Period of observation .....
- Date of completion of test .....
- Result .....

Control of bulk suspension

Date of filtration of bulk .....

Porosity of filters used .....

Date of sampling .....

Tests in rabbits:

No. and weight of animals .....

Date of inoculation .....

Results of injection .....

Quantity injected .....

Results (survival Nos., etc.) .....

Tests in guinea-pigs:

No. and weight of animals .....

Date of inoculation .....

Results of injection .....

Quantity injected .....

Results (survival Nos., etc.) .....

Certification by the manufacturer

Name of head of production (typed) .....

Certification by person from the control laboratory of the manufacturing company taking overall responsibility for the production and control of the vaccine

I certify that lot No. . . . of poliomyelitis vaccine (oral), whose number appears on the label of the final container, meets all national requirements[1] and satisfies Part A of the Requirements for Biological Substances No. 7, revised 1989, and (if applicable) addenda 19. . . for poliomyelitis vaccine (oral).

Signature .....

Name (typed) .....

Date .....

Certification by the national control authority

If the vaccine is to be exported, attach a certificate from the national control authority as described in Part B, section 2, a label from a final container, and an instruction leaflet for users.

[1] If any national requirement(s) is (are) not met, specify which one(s) and indicate why release of the lot has nevertheless been authorized.

F. HEPATITIS B VACCINE

Documents provided in this section include:

1. Types of Hepatitis B Vaccines 458
2. Sample Procurement Requirements Hepatitis B Vaccine 459
3. HB Vaccine Model Package Insert (UNICEF) 467
4. Hepatitis B Virus Vaccine Inactivated, *USP 23 - NF 18*, 1994 468
5. Hepatitis Requirements, US Food and Drug Administration HHS 21 CFR, Part 610, Subpart E 470
6. Requirements for Hepatitis B Vaccine Prepared from Plasma (Requirements for Biological Substances No. 31, revised 1994), WHO *Technical Report Series* No. 858, Annex 3, 1995 473
7. WHO Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques (Requirements for Biological Substances No. 45), WHO *Technical Report Series* No. 786, 1989 490

Types of Hepatitis B Vaccines

There are two basic types of Hepatitis B vaccine:

Plasma-derived vaccine is prepared from particles of HBsAg obtained from human plasma. Purification yields products that are at least 95% pure. The major impurities result from trace materials found in human plasma such as albumin, alpha-2 macroglobulins and a small amount of immunoglobulin G. These vaccines are subjected to inactivation with formalin and/or heat and are adjuvanted with a compound of aluminium. Plasma-derived vaccine has been recently available for large-scale, public-sector purchase at between US $0.55 and US $1 per paediatric dose.

Recombinant DNA vaccine is prepared from particles produced in host cells (usually yeast) transformed through insertion by recombinant DNA genetic material from the surface proteins of HBV. These vaccines are of extremely high purity, having been purified by complex biophysical and biochemical techniques. They do not require inactivation and are adjuvanted with aluminium compounds. Recombinant DNA HB vaccines have been recently available for large-scale, public-sector purchase at between US $1.00 and US $2.50 per paediatric dose. The World Health Organization has stated that plasma-derived and recombinant DNA vaccines are equally safe and effective.

In 1989 the Global Advisory Group of EPI officially recommended that HB vaccination be administered to all infants as part of the EPI in countries where the prevalence of chronic carriage of HBV exceeded 2%. In 1991 the Global Advisory Group committee established targets for full incorporation of HB vaccination into the EPI globally, with countries where the HBV carrier rate exceeded 8% to establish such programs by 1995, and all countries to undertake universal infant immunization with HB vaccine by 1997. The 1992 World Health Assembly endorsed these targets, and HB vaccine should now be considered the seventh EPI vaccine (*Perspectives on Hepatitis* 4(1), 1993).

SAMPLE
PROCUREMENT REQUIREMENTS

HEPATITIS B VACCINE

For Paediatric Use

A. General Description of Intended Use

This vaccine is intended for use in a public-sector health programme for immunization of newborns in _____ [*place*] beginning on _____ [*date*].

The total requirement for this solicitation is _____ [*quantity*] doses. _____ [*name*] expects to procure additional quantities of vaccine annually for use in this project beginning in _____ [*month, year*] and subsequent years as follows: _____ [*quantity*], _____ [*quantity*] and _____ [*quantity*].

B. Specific Requirements

Item: Hepatitis B Vaccine for intramuscular injection.

Vaccine manufactured from purified, inactivated hepatitis B surface antigen (HBsAg) particles obtained from human plasma or vaccine manufactured using recombinant DNA technology. Intended for intramuscular injection.

Each dose shall contain that amount of HBsAg protein with micrograms/ml specified by the manufacturer for either paediatric or adult dosage that, when given as part of a primary immunization series (3 doses), is capable of producing specific humeral antibody (anti HBs) at a level of at least 10 milli international units in $\geq$ 90% of recipients.

The vaccine must be free from all demonstrable viable microbial agents and found suitable for human immunization. It may contain a suitable stabilising agent with antimicrobial properties.

The vaccine shall meet _____ [*specify*] requirements for Hepatitis B Vaccines, either for plasma-derived or recombinant DNA vaccine (WHO Requirements for Biological Substances No. 31, revised 1994; Requirements for Biological Substances No. 45, revised 1989 or equivalent requirements of the USFDA as detailed in 21 CFR, or another fully equivalent set of biological requirements). *Editors' note: It is recognized that equivalence does not mean identical, only that both provide the same level of potency, safety and efficacy.*

The vaccine shall be currently registered in the country of manufacture and shall meet all requirements of the licensing authority of the country of manufacture. The vaccine also shall be currently registered in the country of use and shall meet all requirements of the licensing authority of the country of use.

Quantity:	_____ doses from one production lot.
Dosage Size:	0.5 ml, adjuvant and vehicle.
Dose Package:	Single-dose vials; multi-dose vials.
Filling Volume:	Final product should contain 15% overfill.
Storage Temperature:	2 - 8 degrees C. Do not freeze!
Shelf Life:	The shelf life of the product provided under this solicitation will be at least 24 months from date of manufacture when stored between +2 to +8 degrees C. The supplier will provide

manufacturer's stability test data substantiating this 24-month shelf life in the proposed vial.

At the time of inspection or acceptance for delivery to the country of destination, no more than 6 months shall have expired since the date of manufacture (or date of the beginning of the last satisfactory test for potency) shown on the Certificate of Analysis.

Stability: Stable for a minimum of 2 years after production at +2 to +8 degrees C. Manufacturer to specify additional thermal stability characteristics.

Labelling: The label on each vial shall conform to the requirements of the country of use and shall appear in the language of _____.

All labelling shall withstand immersion in water and remain intact.

All labels shall state the name of the vaccine, name of the manufacturer, place of manufacture, lot number, composition, concentration, dose and mode for administration, expiry date, storage temperature and any other marking that is appropriate.

Optional Labelling: If vaccine is destined for use in public-sector immunization programs, the statement "Not for Sale; For Use Only in Government-Approved Immunization Programmes" shall appear on each label in the language of the purchasing country.

Closures: Vaccine vials shall be fitted with closures that conform to ISO standard 8362-2.

Printed Materials:	Two (2) information sheets, printed in _____ [*language*], shall be included in each box of ___ vials (see Model Package Insert, attached)[29].

C. Quality Assurance

Compliance:	The Supplier shall guarantee that the products as packed for shipment (a) comply with all provisions of the specification and related documents; (b) meet internationally recognized standards for safety, efficacy and quality; (c) are fit for the purposes made known to the Seller and (d) are free from defects in workmanship and in materials.
Evidence:	The Supplier shall provide objective evidence, acceptable to the Purchaser, of the satisfaction of the requirements of this document for which no specific inspection has been mentioned. The Supplier shall provide a copy of the manufacturing record and procedures to the Purchaser for each lot intended for shipment. The Supplier shall provide a copy of the batch record and all quality assurance documentation to the Purchaser for each lot being supplied. The Supplier shall provide a copy of the Certificate of Analysis to the Purchaser for each lot intended for shipment. The Supplier shall provide to the Purchaser a copy of the approval of each source material, constituent material and component for each lot intended for shipment.

[29] See example, page 467.

The Supplier shall retain a sample of twenty (20) vials from each lot shipped for two years beyond the printed expiration date.

Chemical, physical and microbiological test data for in-process and finished product testing must be on record for each lot shipped and must be available to Purchaser's representatives when requested.

Inspection: The Purchaser may inspect and sample, or cause to be sampled, the product at the Supplier's factory and/or warehouse at a mutually agreeable time prior to shipment of the product.

Testing: The Purchaser may cause independent laboratory testing to be performed as deemed necessary to assure that the goods conform to prescribed requirements. Said testing laboratory shall be of the Purchaser's choice and suitably equipped and qualified to conduct quality assurance tests on biological products.

D. Packing

Storage: Supplier shall state storage volume occupied per dose of vaccine (storage volume includes the vaccine vial, packet containing the vaccine vial and any intermediate packaging).

Inner Boxes: _____ [*number*] individual vials shall be supplied in sturdy white cardboard boxes outfitted with individual segments for protecting and separating each vial.

Cold Chain Monitor Cards: One cold chain monitor card (description attached)[30] in _____ language shall be packed with each ___ doses of vaccine. Freeze

[30] See example in Supplement VI.G.1.a., page 783.

watch indicators shall be included with each carton.

Overpacking: Boxes shall be overpacked so that the vaccine remains refrigerated at between +2 and +8 degrees C. The overpacking must be suitable for export handling in accordance with WHO/EPI Guidelines on the International Packaging and Shipping of Vaccine. It must have adequate insulation and sufficient refrigerant to ensure that the warmest storage temperature of the vaccine does not rise above +8 degrees C in continuous outside ambient temperature of +43 degrees C, or fall below +2 degrees C in continuous outside ambient temperature of -20 degrees C during transit and for a period of at least 48 hours after arrival at the airport of destination.

Additional cushioning shall be provided, sufficient to protect the vials from breakage during transit and handling.

Exterior Shipping Cartons: Product and printed materials, packaged as specified above, shall be packed in weather-resistant, triple-wall corrugated fibreboard cartons with a bursting test strength of not less than 1,900 kPa.

The overall dimensions of the exterior shipping cartons should be such that the product does not become damaged during transportation and storage.

E. Markings

All containers and invoices must bear the name of the vaccine, expiry dates of the vaccine and appropriate storage temperature.

Inner Boxes: The inner boxes containing vaccine vials shall be marked with the following information in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Manufacturer's name and registered address
- Manufacturer's national registration number
- Lot or batch number
- Composition and concentration
- Number of vials contained in box
- Expiration date (month and year)
- Instructions for storage and handling†
- Place of manufacture (Made in _____)

Exterior Shipping Cartons: The following information shall be stencilled or labelled on the exterior shipping cartons on two opposing sides in bold letters at least __ mm high with waterproof ink in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Lot or batch number
- Expiration date (month and year)
- Manufacturer's name and registered address
- Manufacturer's national registration number
- Destination country license or registration number
- Consignee's address and emergency phone number
- Destination airport
- Contract number
- Number of vials contained in the carton
- Gross weight of each carton (in kg)
- Carton #___ of ___

† To be provided by Purchaser.

- Instructions for storage and handling†
- Place of manufacture (Made in _____)

F. Documentation

Supplier shall provide to Purchaser a copy of the batch record, including all quality assurance documentation for the vaccine being supplied.

† To be provided by Purchaser.

HEPATITIS B VACCINE

DESCRIPTION
EITHER*: The vaccine is a subunit viral vaccine containing highly purified, non-infectious particles of hepatitis B surface antigen (HbsAg) adsorbed onto aluminium salts as an adjuvant and preserved with thimerosal. It is a plasma-derived vaccine purified from the plasma of HbsAg-positive donors and is inactivated with formalin/heat (state which).

OR*: The vaccine is a subunit vial vaccine containing highly purified, non-infectious particles of hepatitis B surface antigen (HbsAg) adsorbed onto aluminium salts as an adjuvant and preserved with thimerosal. It is a DNA recombinant vaccine derived from HbsAg produced by DNA recombinant technology in yeast/mammalian (state which) cells.

ADMINISTRATION
The vaccine should be shaken before use. It should be injected intramuscularly into the anterolateral aspect of the thigh in infants, or into the deltoid muscles of older children or adults. One dose is 0.5 ml and this contains _____ HbsAg. Once opened, multi-dose vials should be kept at between +2°C and 8°C. A sterile syringe and needle should be used for each injection.

IMMUNIZATION SCHEDULES
In countries where perinatal transmission of hepatitis B is common, the first dose should be given as soon as possible after birth. If perinatal transmission is uncommon, or if delivery at birth is not feasible, the first dose can be given with the first dose of DTP. The second dose should be administered one month after the first dose, during a visit for a dose of DTP. The third dose should be administered one to twelve months after the second dose, during a visit for DTP-3 or measles vaccine.

HB vaccine can be given safely and effectively at the same time as BCG, DTP, measles, polio vaccines (OPV or IPV) or yellow fever vaccines. If HB vaccine is given at the same time as other vaccines, it should be administered at a separate site. It should not be mixed in the vial or syringe with any other vaccine.

SIDE EFFECTS
The vaccine is very well tolerated. Some transitional swelling, tenderness and redness at the site of the injection occur in some individuals (about 12%). Other minor reactions such as malaise or fever occur in less than 2% of individuals. More serious reactions are rare: a causal relationship between more serious reactions and the vaccine has not been established.

CONTRAINDICATIONS
Known hypersensitivity to any component of the vaccine, or a severe reaction to a previous dose. The vaccine will not harm individuals currently or previously infected with the HB virus. HB vaccine may be administered to individuals known or suspected to be HIV-infected infants.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED PERSONS: HIV-infected infants, both asymptomatic and symptomatic, should be immunized with Hepatitis B, according to standard schedules.

STORAGE
HB vaccine should be stored and transported between +2°C and +8°C. IT MUST NOT BE FROZEN.

PRESENTATION
The vaccine comes in single dose vials of _____ doses.

*Manufacturers should include the appropriate paragraph, depending on whether the vaccine is plasma-derived or recombinant.

Hepatitis B Virus Vaccine Inactivated

» Hepatitis B Virus Vaccine Inactivated is a ster preparation consisting of a suspension of particles Hepatitis B surface antigen (HBsAg) isolated fro the plasma of HBsAg carriers; treated with pepsin pH 2, 8 *M* urea, and 1:4,000 formalin so as to i activate any hepatitis B virus and any representati viruses from all known virus groups that may be pre ent; purified by ultracentrifugation and biochemic procedures and standardized to a concentration of : μg to 55 μg of Lowry (HBsAg) protein per mL. Tl preparation is adsorbed on aluminum hydroxide ar diluted to a concentration of 20 μg Lowry protein p mL or other appropriate concentration, depending (the intended use. The Vaccine meets the requir ments for potency in animal tests using mice and t a quantitative parallél line radioimmunoassay. contains not more than 0.62 mg of aluminum per m and not more than 0.02 percent of residual free forn aldehyde. It contains thimerosal as a preservativ It meets the requirements of the tests for pyrogen an for general safety (see *Safety Tests—General* und *Biological Reactivity Tests, In Vivo* ⟨88⟩).

Packaging and storage—Preserve at a temperature between 2 and 8°.

Expiration date—The expiration date is 3 years from the date (manufacture, the date of manufacture being the date on whic the last valid potency test was initiated.

Labeling—Label it to state the content of HBsAg protein recommended dose. Label it also to state that it is to be sh before use and that it is not to be frozen.

Subpart E—Hepatitis Requirements

§ 610.40 Test for hepatitis B surface antigen.

(a) *Test sensitivity.* Each donation of blood, plasma, or serum to be used in preparing a biological product shall be tested for the presence of hepatitis B surface antigen by a method of sufficient sensitivity to detect all sera labeled A, (A), B, (B), and C in the Reference Hepatitis B Surface Antigen Panel distributed by the Center for Biologics Evaluation and Research; except that, in emergency situations, a test method of sufficient sensitivity to detect all sera labeled A, (A), and B in the Reference Hepatitis B Surface Antigen Panel may be used and, in dire emergency situations, blood and blood products may be issued without any HB_8 Ag testing, provided that a test otherwise required by this paragraph is performed as soon as possible after issuance of the blood and blood product.

(b) *Procedures.* Only Antibody to Hepatitis B Surface Antigen licensed under this subchapter shall be used in performing the test and the test method(s) used shall be that for which the antibody product is specifically designed to be effective as recommended by the manufacturer in the package insert. The sample of blood, plasma, or serum to be tested shall have been taken from the donor at the time of donation of that unit. The test need not be performed on the day of the withdrawal of the sample. If the radioimmunoassay method is used, it must be performed in one of the following ways:

(1) The complete test is performed at the collection facility.

(2) The test is performed at the collection facility up to the point of counting the radioactivity of the samples, which counting, thereafter, is performed at another facility by personnel from the collection facility or by personnel from the counting facility.

(3) The complete test is performed by the personnel at an establishment licensed to manufacture blood or blood derivatives under section 351(a) of the Public Health Service Act (42 U.S.C. 262(a)), or by a clinical laboratory which meets the standards of the Clinical Laboratories Improvement Act of 1967 (CLIA) (42 U.S.C. 263a), provided the establishment or the clinical laboratory is qualified to perform radioimmunoassay testing for the presence of hepatitis B surface antigen.

(4) Except as provided in this paragraph (b)(4), a collection facility shall not ship any blood product as a biological product or ship such a blood product where it is intended for use in manufacturing a biological product until the test for hepatitis B surface antigen is completed and the written test results are received by the collection facility. Notwithstanding the provisions of § 610.1 of this chapter, in the case of an emergency, or as otherwise approved in writing by the Director, Center for Biologics Evaluation and Research, a collection facility may ship a blood product before the test for hepatitis B surface antigen is completed. To obtain approval for such shipments, the collection facility shall submit a description of the control procedures to be used by both the collection facility and the manufacturing facility to the Director, Center for Biologics Evaluation and Research (HFB-1), Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892. The control procedures to be used by the collection facility and the manufacturing facility shall include, but may not be limited to, a system of communicating the test results to the manufacturing facility, use of specific labeling warnings for the product to ensure that persons handling the shipment know that it may be infectious, procedures for quarantine of the untested or incompletely tested product both at the collection facility and at the manufacturing facility, and a procedure at the manufacturing facility to identify, preclude use of, and dispose of any blood product that is received and later found to be reactive for hepatitis B surface antigen.

(c) *Materials in storage.* All blood, plasma, or serum in storage which has not been tested for the presence of the hepatitis B surface antigen shall be tested as required in paragraphs (a) and

(b) of this section before use as a biological product, or before use in the manufacture of a biological product. All blood, plasma, or serum in storage which has been tested for the presence of the hepatitis B surface antigen by a method of second generation sensitivity may be used as a biological product or in manufacture of a biological product, provided it is used on or before March 15, 1976.

(d) *Restrictions on use.* Blood, plasma, or serum that is reactive when tested for hepatitis B surface antigen or that was collected from a donor known to be reactive for hepatitis B surface antigen shall not be used in manufacturing biological products except as provided in paragraphs (d) (1) and (2) of this section.

(1) *Injectable biological products and licensed in vitro diagnostic biological products.* Blood, plasma, or serum that is reactive when tested for hepatitis B surface antigen or that was collected from a donor known to be reactive for hepatitis B surface antigen may be used in manufacturing hepatitis B vaccine and licensed in vitro diagnostic biological products if all of the following conditions are met:

(i) The final product cannot be prepared from blood, plasma, or serum that is nonreactive when tested for hepatitis B surface antigen, due either to the nature or to the scarcity of the final product.

(ii) The label of the source blood, plasma, or serum conspicuously states either that it is reactive when tested for hepatitis B surface antigen and it may transmit viral hepatitis; or that the source blood, plasma, or serum was collected from a donor known to be reactive for hepatitis B surface antigen and it may transmit viral hepatitis, although confirmatory hepatitis testing has not been done.

(iii) The package label of the licensed in vitro diagnostic biological product prepared from such blood, plasma, or serum states conspicuously that either the product was prepared from source material that was reactive when tested for hepatitis B surface antigen and it may transmit viral hepatitis; or that the source material was collected from a donor known to be reactive for hepatitis B surface antigen and it may transmit viral hepatitis, although confirmatory hepatitis testing has not been done.

(iv) The package label of the licensed injectable biological product prepared from such blood, plasma, or serum states that the product has been inactivated.

(v) The Director, Center for Biologics Evaluation and Research (HFB-1), Food and Drug Administration, 8800 Rockville Pike, Bethesda MD 20892, is notified in writing at the time of the shipment, or in the case of repetitive shipments, or April 1 and October 1 of each year, of each shipment of source blood, plasma, or serum for manufacture into hepatitis B vaccine or into a licensed in vitro diagnostic biological product. Such shipments shall not be subject to the requirements of paragraph (b)(3) of this section. Each notification shall identify the kind and amount of source material shipped, the name and address of the consignee, the date of shipment, and the manner in which the source material is labeled.

(2) *Unlicensed in vitro diagnostic biological products.* Blood, plasma, or serum that is reactive when tested for hepatitis B surface antigen or that was collected from a donor known to be reactive for hepatitis B surface antigen may be used in manufacturing unlicensed in vitro diagnostic biological products including clinical chemistry control reagents if all of the following conditions are met:

(i) The final product cannot be prepared from blood, plasma, or serum that is nonreactive when tested for hepatitis B surface antigen, due either to the nature or to the scarcity of the final product.

(ii) The label of the source blood, plasma, or serum states conspicuously that either it is reactive when tested for hepatitis B surface antigen and it may transmit viral hepatitis; or that the source blood, plasma, or serum was collected from a donor known to be reactive for hepatitis B surface antigen and it may transmit viral hepatitis, although confirmatory hepatitis testing has not been done.

(iii) The manufacturer of the source blood, plasma, or serum obtains written assurance from the manufacturer(s) of the final unlicensed product

that package labels of all unlicensed products will conspicuously state, as required by §809.10(a)(4) of this chapter, that the product was prepared from blood, plasma, or serum that was reactive when tested for hepatitis B surface antigen and it may transmit viral hepatitis; or that the source material was collected from a donor known to be reactive for hepatitis B surface antigen and it may transmit viral hepatitis, although confirmatory hepatitis testing has not been done.

(iv) At the time of shipment, the Director, Center for Biologics Evaluation and Research (HFB-1), Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892, is notified in writing of each shipment of source blood, plasma, or serum signifying the kind and the amount of source material shipped, the name and address of the consignee, the date of shipment, and the manner in which such source material was labeled. Such shipments shall not be subject to the requirements of paragraph (b)(3) of this section.

(e) *Manufacturing responsibility.* When the radioimmunoassay method for hepatitis B surface antigen testing is performed by personnel other than those of the facility collecting the blood, plasma, or serum, as provided in paragraph (b) of this section, it shall not be considered as divided manufacturing as described in §610.63, provided the following conditions are met:

(1) The collecting facility has obtained a written agreement that the testing laboratory will permit authorized representatives of the Food and Drug Administration to inspect its testing procedures and facilities during reasonable business hours.

(2) The testing laboratory will participate in any proficiency testing programs undertaken by the Center for Biologics Evaluation and Research, Food and Drug Administration.

(f) The information collection requirements in paragraph (d) of this section were approved by the Office of Management and Budget and assigned OMB control number 0910-0136.

(Information collection requirements contained in paragraph (b)(4) were approved by the Office of Management and Budget under control number 0910-0168)

[40 FR 29710, July 15, 1975, as amended at 48 FR 23181, May 24, 1983; 49 FR 23834, June 8, 1984; 49 FR 26718, June 29, 1984; 51 FR 15607, Apr. 25, 1986; 55 FR 11013 and 11014, Mar. 26, 1990]

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WHO Technical Report Series, No. 858, 1995

Annex 3
Requirements for hepatitis B vaccine prep from plasma

(Requirements for Biological Substances No. 31, revised 1994)

Introduction

General considerations

Part A. Manufacturing requirements
A.1 Definitions
A.2 General manufacturing requirements
A.3 Production control
A.4 Filling and containers
A.5 Control tests on final product
A.6 Records
A.7 Samples
A.8 Labelling
A.9 Distribution and shipping
A.10 Storage and expiry date

Part B. National control requirements
B.1 General
B.2 Release and certification

Authors

Acknowledgements

References

Appendix 1
Example, for guidance, of a summary protocol for the production and testing of hepatitis B vaccine prepared from plasma

Appendix 2
Model certificate for the release of hepatitis B vaccine prepared from plasma by national control authorities

ntroduction

Viral hepatitis type B is a major public health problem occurring endemically in all parts of the world, more than 2000 million people having been infected with the hepatitis B virus (HBV). The disease is characterized by a relatively long incubation period, high carrier rate, and diverse manifestations, including the serious consequences associated with cirrhosis and hepatocellular carcinoma. More than 80% of all cases of the latter are attributable to HBV, making it second only to tobacco among the known causes of human cancer. In some regions of the world, coinfection and superinfection with hepatitis delta virus have been associated with high morbidity and mortality in HBV-positive individuals. Although rare in infants and children, severe fulminant hepatitis B with hepatic failure and coma may develop in adults. Approximately 300 million people, most of whom live in developing countries, are lifelong chronic carriers of HBV. Such carriers in adulthood are at high risk of serious illness and death from cirrhosis of the liver and primary liver cancer, diseases that kill 1-2 million carriers per year. Primary liver cancer caused by HBV infection is one of the top three causes of cancer-related deaths in much of Africa, Asia and the Pacific Basin. In addition, carriers constitute a permanent reservoir of infected individuals who perpetuate the infection from generation to generation. HBV infection occurring at, or shortly after, birth or in early childhood is especially likely to lead to the chronic carrier state.

In the absence of any specific antiviral therapy for HBV infection, immunization, both passive and active, is the only available means of controlling the disease. Hyperimmune gamma globulin in combination with hepatitis B vaccine has been shown to be extremely effective in preventing infection in children born to HBV-positive mothers. The use of the vaccine alone, although slightly less effective, has also been shown to be capable of reducing this mode of infection in many countries throughout the world. In addition, the widespread use of hepatitis B vaccine in newborn children has been shown to reduce the rate of transmission among older children in the household.

Hepatitis B vaccine, if given before exposure, can prevent infection and disease and the development of a carrier state in almost all individuals. The vaccine is highly effective when included in the infant immunization schedule, although it can be used at any age. Two types of hepatitis B vaccine exist, namely plasma-derived vaccine and recombinant DNA-derived vaccine. Immunity in individuals given the recombinant DNA vaccine can be boosted with plasma-derived vaccine and vice versa. Seroconversion rates with the two vaccines are comparable. Plasma-derived vaccines have been shown to be safe, and transmission of HBV and other viruses, including the human immunodeficiency virus (HIV) and hepatitis C virus (HCV), has not been documented following extensive surveillance. The present document concerns only the plasma-derived vaccine.

HBV is a 42-nm enveloped particle containing a partially do stranded DNA genome. The envelope consists of host lipids and v specific proteins encoded by a large open reading frame that has thre phase translation start codons. In addition to the infectious virus, non-infectious morphological forms are produced during infection three morphological forms share common antigenic epitopes referr as the hepatitis B surface antigen (HBsAg), which is antigeni complex and is composed of proteins (75% protein by wei carbohydrates (in the form of glycoproteins) and host-derived l (25%) (*1*). During infection, hepatocytes synthesize and secrete HE in excess, mainly as lipoprotein in the form of 22-nm diameter part (*2, 3*). All HBsAg-containing particles have a group-specific determi "a", which confers protection. Additional complexities have led t recognition of 10 different serotypes of HBsAg (*4*). However, there known improvement in vaccine efficacy when all of the various subt are included in the final vaccine formulation. The small 22-nm sphe morphological form can circulate in chronic carriers in very concentrations sometimes exceeding 100 µg/ml. This form is the so antigen from which plasma-derived HBV vaccine is prepared.

General considerations

Because of the worldwide prevalence of hepatitis B infection Requirements for Hepatitis B Vaccine Prepared from Plasma formulated in 1980 (*6*), and revised in 1984 (*7*), and again in 1987 Since immunization with purified 22-nm spherical particles contai HBsAg leads to the production of protective antibody against the g "a" determinant, these particles represent a convenient source of sta material for the production of hepatitis B vaccines.

HBsAg-positive source plasma may contain infectious agents posses a wide range of physicochemical and biological characteristics, an various degrees of resistance to different modes of inactiva Consequently, it is essential that, in addition to the careful selectio donors and methods of separating and purifying the 22-nm HBs containing particles, procedures that are known to inactivate infectious agents potentially present in human plasma should be app during the vaccine manufacturing process. Inactivation steps such as and formalin treatment have been successfully used alone o combination to render the purified material free from infectious ag including HIV. Whatever the procedures used to purify HBsAg parti and to inactivate the final product, the resultant vaccines mus demonstrated to be safe (i.e. free from demonstrable virus and c microbial agents), immunogenic (i.e. capable of eliciting antib against the virus in both animals and humans by the administration standardized dose of antigen), and efficacious (i.e. protective agains disease).

Because HBsAg particles are relatively stable, harsh and rigorous treatment can be employed during the purification and inactivation steps. This permits the production of an essentially pure vaccine antigen of uniform composition. Most manufacturers employ an initial fractionation step followed by either chromatographic or ultracentrifugal separation before inactivation. All these procedures are known to be capable of purifying the particles by removing any contaminating blood products and other infectious agents that may be present in the starting material.

For vaccines prepared by urea, pepsin and formalin treatment, it has been shown that each of the three steps can inactivate at least 10^5 (5 $\log_{10}$) infectious units of HBV infectivity (*9*). Moreover, these processes also inactivate members of all known groups of animal viruses (*10*). Examination of vaccines prepared by two-step heat inactivation has demonstrated that each step can inactivate at least 10^6 (6 $\log_{10}$) infectious units of HBV (*11*), and that these procedures are capable of inactivating 12 different animal viruses (*12*). No transmission of HIV, HBV, HCV or any other virus has been documented following the use of the vaccines currently available.

The safety of most plasma-derived hepatitis B vaccines was initially ensured by safety tests in two to four hepatitis B antibody-free chimpanzees per lot. Because of the safety record of these vaccines in humans, the shortage of animals, and the costs associated with chimpanzee safety trials, any requirement to continue to use chimpanzees to demonstrate safety must be fully justified. Model viruses such as duck hepatitis B virus may be used in validation studies. Nevertheless, the national control authority must be satisfied that any production process used will yield a safe and effective vaccine. This may mean that a safety test using chimpanzees has to form part of the validation of a process proposed by a new manufacturer, or a revised process proposed by an existing one.

The immunogenicity of plasma-derived hepatitis B vaccines has been routinely assessed in mice by a quantitative assay performed with each lot by injecting serial dilutions of alum-adjuvanted vaccine intra-peritoneally (*13, 14*). This procedure provides an effective dose 50% (ED_{50}), defined as the dose at which 50% of the animals seroconvert, which can be compared with that of a simultaneously titrated standard preparation. When carried out properly this test can provide a useful measure of immunogenicity, but there is considerable variation from laboratory to laboratory (*15, 16*). Thus, if this test is used, it should be highly standardized, and the immunogenicity results for each new lot of vaccine should be compared with those for an internal standard, measured at the same time, rather than with a historical ED_{50} value. An International Reference Reagent has been established for this purpose. It is acknowledged that vaccines that are immunogenic in mice may not be highly immunogenic in humans. However, correlations between the responses in humans and mice should be assessed during clinical trials.

Immunogenicity in humans is expressed as the geometric mean level (anti-HBs antibodies in terms of milli-International Units per ml using a international reference material for comparison.

The efficacy of HBV vaccines can be assessed in terms of their ability protect against infection, disease or the development of a chronic carri state. These three end-points are different and may require differe levels of immunity. This distinction is important since dosage ar schedule regimens designed on the basis of clinical trials and using on the prevention of HBV infection as the end-point may overestimate tl dosage and schedule required to prevent the chronic carrier state. Th may be the primary goal of immunization in countries of high HB endemicity, where infections in childhood seldom cause disease b rather lead to the development of the chronic carrier state which accoun for most of the morbidity and mortality in later life.

In the first large-scale studies of the efficacy of a hepatitis B vaccine, classical randomized double-blind placebo-controlled trial was carri out in a high-risk population of homosexual men (*16*). This showed 90% reduction in the incidence of HBsAg antigenaemia and hepatitis th correlated with a 95% seroconversion rate.

New data have suggested that some of the initial requirements for vacci production and testing, although appropriate at the time, may no long be relevant. In this revision, the Requirements for Hepatitis B Vacci Prepared from Plasma (*8*) have been modified in the light of experien with the use of seven different vaccines over several years.

Each of the following sections constitutes a recommendation. The par of each section printed in normal type have been written in the form requirements, so that, if a national control authority so desires, they m be adopted as they stand as definitive national requirements. The parts each section printed in small type are comments and recommendatio for guidance. To facilitate the international distribution of vaccine ma in accordance with these Requirements, a summary protocol f recording the results of tests is provided as Appendix 1.

Should individual countries wish to adopt these Requirements as tl basis of their national regulations concerning hepatitis B vaccine, it recommended that modifications be made only on condition that tl modified requirements ensure at least an equal degree of safety ar potency of the vaccine. It is desirable that the World Health Organizati be informed of any such changes.

Part A. Manufacturing requirements

A.1 Definitions

A.1.1 *International name and proper name*

The international name shall be "Vaccinum hepatitidis B explasma humanum". The proper name shall be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

A.1.2 *Descriptive definition*

"Vaccinum hepatitidis B explasma humanum" is a preparation of purified hepatitis B surface antigen (HBsAg) that has been treated to inactivate HBV and other pathogens known to be present in human blood. The preparation shall satisfy all the requirements formulated below.

A.1.3 *International reference materials*

An International Reference Reagent for Plasma-derived Hepatitis B Vaccine for Immunogenicity Studies is available from the National Institute for Biological Standards and Control, Potters Bar, Herts., England.

An International Reference Preparation of Anti-Hepatitis B Immunoglobulin is available from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands.

These materials are distributed free of charge, on request, to national control authorities.

A.1.4 *Terminology*

The following definitions are given for the purposes of these Requirements only.

Whole blood (sometimes referred to as blood): blood collected in an anticoagulant solution with or without the addition of nutrients such as glucose or adenine.

Plasma: the liquid part of blood collected in a receptable containing an anticoagulant.

HBV: hepatitis B virus, a 42-nm enveloped virus.

HBsAg: hepatitis B surface antigen, the complex of antigens associated with the virus envelope and subviral forms (22-nm spherical and tubular particles). Native HBsAg is coded for by envelope sequences encoded by the *S* gene plus *pre-*S_2 and *pre-*S_1.

Anti-HBs: antibody to hepatitis B surface antigen.

Single-donor plasma: plasma obtained from a single donation of whole blood or obtained from a single donor by plasmapheresis.

Plasma pools: pools of single-donor plasma that have been shown t satisfactory before pooling.

Purified HBsAg batch: purified HBsAg prepared from one or r plasma pools by suitable procedures.

Purified, inactivated HBsAg batch: purified HBsAg prepared from or more plasma pools by suitable procedures and further treate inactivate HBV and other pathogens that may be present in human b or plasma.

Final aqueous bulk: the final bulk before the addition of adjuvar consists of one or more purified, concentrated HBsAg batches that been treated to inactivate infectious agents that may be present in hu blood.

Final bulk: The homogeneous adjuvanted finished material prep from one or more batches of final aqueous bulk from which the containers are filled.

Final lot: a collection of sealed final containers that are homogen with respect to the risk of contamination during filling or preparatic the finished vaccine. A final lot must therefore consist of fini material dispensed into containers in one working session from a si final bulk under standardized conditions in a common chamber.

A.2 General manufacturing requirements

The general manufacturing requirements contained in Good Manu turing Practices for Pharmaceutical (*17*) and Biological (*18*) Prod shall apply to establishments manufacturing hepatitis B vaccine, wit addition of the following directives:

Completely separate areas shall be used for the separation inactivation steps. All separation and inactivation steps shall be ca out in closed systems and closely monitored.

Steps should be taken to ensure that no persons in the production a are chronic carriers of HBsAg and that all such persons are immun HBV as shown by the presence of anti-HBs, either as a result of nat infection or by immunization.

A.3 Production control

The general production precautions formulated in Good Manufactu Practices for Biological Products (*18,* section 6) shall apply to manufacture of hepatitis B vaccine.

A.3.1 *Collection of blood and plasma and selection of donors*

Appropriate information is given in Requirements for the Collect Processing and Quality Control of Blood, Blood Components and Pla Derivatives (revised 1992) (*19*).

The criteria for the selection of donors of whole blood or plasma by plasmapheresis, their physical fitness, including a medical history and physical examination, are described in the above-mentioned Requirements, except that the donors must be HBsAg-positive and are not subject to the exclusions relating to HBV. Plasmapheresis of donors of HBsAg-positive plasma shall be permitted, even if the liver function test values are above normal limits in individual donors, provided that the values obtained are stable.

In some countries plasma from donors who are seropositive for malaria, sexually transmitted diseases, human (gamma) herpes virus 4 (Epstein-Barr virus) and cytomegalovirus is excluded from use for hepatitis B vaccine production.

In some countries separate areas or special times are set aside for the collection of plasma known to be HBsAg-positive.

Records should be kept of the identity of the donors for the identification of each batch of vaccine.

When plasma is collected in regions or from populations with a high prevalence of hepatitis delta virus infection, screening of individual donors for evidence of chronic delta virus infection should be considered, although the presence of delta infection need not disqualify a donor.

The acceptable upper limits of liver function tests for donor exclusion should be specified by the national control authority.

Appropriate guidelines to define donor changes of such significance as to justify discontinuation of plasmapheresis should be established by the responsible physician.

No guidelines have been established for the maximum volume of plasma that can be taken in any one year from a chronic HBV carrier, but this volume should not exceed the limits imposed in certain countries for healthy HBsAg-negative donors (*19*).

Where plasmapheresis is regularly performed on HBsAg-positive donors, it is advisable for equipment to be dedicated specifically for this purpose.

.3.2 *Tests on single-donor plasma*

Each single-donor plasma shall be shown by sensitive tests to be seronegative for HIV-1 and HIV-2 (*20*) and for HCV. Each single-donor plasma, whether obtained from whole blood or by plasmapheresis, shall be tested for HBsAg content by a method approved by the national control authority.

The subtype of HBsAg is irrelevant since protective immunity is not related to it. Thus, mixing of whole blood or plasma containing different subtypes of HBV is of no concern and may even be desirable.

Many countries have minimum standards of HBsAg content for the acceptance of whole blood or single-donor plasma as the starting material. A convenient and suitable method is to make a 1:100 000 dilution of test plasma in human serum seronegative for HBsAg and anti-HBs and retain only those plasma units that test positive by radioimmunoassay (RIA) or enzyme immunoassay (EIA) at this dilution. In countries where RIA and EIA are readily available, a titre of greater than or equal to 1:16 by countercu immunoelectrophoresis is acceptable.

A.3.3 *Pooling of single-donor plasma*

Only plasma meeting the requirements of sections A.3.1 and A.3.2 having a defined minimum amount of HBsAg shall be included i plasma pool.

A.3.4 *Tests on plasma pools*

Some countries may require the tests of section A.3.2 to be repeated on plasma pools.

A.3.4.1 Sterility tests

Each plasma pool shall be tested for microbial contamination as specif in Part A, section 5, of the revised Requirements for Biologi Substances No. 6 (General Requirements for the Sterility of Biologi Substances) (*21*). If testing demonstrates microbial contamination, pool shall be destroyed. The final decision on sample size shall be tal by the national control authority.

A.3.4.2 Tests for adventitious viruses

Tests in cell cultures. A sample of at least 5 ml of each plasma pool sh be tested for adventitious viruses by inoculation into simian cell cultur Similar volumes of the plasma pool shall likewise be tested in hum diploid cell cultures. The inoculated cell cultures and uninocula control cultures shall be observed for at least 14 days. At the end of t observation period a subculture in the same cell system shall be made a cultures observed for at least 14 days.

In some countries larger volumes of the plasma pool are required to be test

Simian cell cultures are prepared from the kidneys of *Cercopithecus* monkeys from Vero cells. For the human cell cultures, either WI-38 or MRC-5 may be us

The plasma pool passes the test if none of the cell cultures show eviden of the presence of any adventitious virus attributable to the plasma po For a test to be valid, no more than 20% of the culture vessels should ha been discarded for non-specific reasons by the end of the test period.

Tests in adult mice. Each plasma pool shall be tested in adult mice f adventitious viruses pathogenic to mice. Each of at least 10 adult mice, 15-20 g weight, shall be inoculated intracerebrally with 0.03 ml, and least 10 mice intraperitoneally with at least 0.5 ml of the plasma po The mice shall be observed for at least 21 days. All mice that die after t first 24 hours of the test or that show signs of illness shall be autopsi and examined for evidence of viral infection, both macroscopically l direct observation and by subinoculation of appropriate tissue su pensions by the intracerebral and intraperitoneal route into at least fi additional mice, which shall be observed for 21 days.

The plasma pool shall pass the test if at least 80% of the original inoculated mice survive the observation period and if no mice show evidence of infection with adventitious transmissible viruses attributable to the plasma pool.

Tests in suckling mice. Each plasma pool shall be tested in suckling mice for adventitious viruses pathogenic to mice. Each of at least 20 mice less than 24 hours old shall be inoculated intracerebrally with 0.01 ml and intraperitoneally with at least 0.1 ml of the plasma pool. The mice shall be observed daily for at least 14 days. All mice that die after the first 24 hours of the test or that show signs of illness shall be autopsied and examined to determine the cause of death or illness.

In some countries a subinoculation (second passage) is suggested and in others an additional blind passage is made of a suspension of the pooled emulsified tissue (minus skin and viscera) of all mice surviving the original 14-day test.

The plasma pool passes the test if at least 80% of the original inoculated mice survive the observation period and if no mice show evidence of infection with adventitious transmissible viruses attributable to the plasma pool.

Tests in embryonated eggs. A sample of at least 5 ml of each plasma pool shall be tested in a group of embryonated hens' eggs by the allantoic route of inoculation and a similar sample in a separate group of eggs by the yolk-sac route of inoculation, using at least 0.25 ml of the plasma pool per egg for each route of inoculation. The incubation of the eggs and the observation time shall be approved by the national control authority. The embryo shall remain normal throughout the observation period.

The plasma pool passes the test if there is no evidence of the presence of any adventitious agents attributable to it.

Other tests

In some countries the national control authority may require tests on the plasma pools for HBV DNA to be carried out by sensitive DNA hybridization assays to monitor the elimination of HBV DNA during the subsequent purification steps.

Concentration, purification and inactivation

Each plasma pool shall be subjected to procedures that concentrate and purify HBsAg reproducibly and result in the inactivation of residual HBV. The methods used shall remove the bulk of extraneous substances and inactivate infectious agents so that the resultant purified product is safe when administered to humans.

A.3.5.1 *Concentration and purification*

Since the purity of the final aqueous bulk is highly dependent on the method of purification, the national control authority shall approve the degree of purity.

The many licensed vaccines that exist worldwide are all freed from contaminating substances by means of similar biochemical and biophysical separation methods. Various combinations of biophysical, biochemical and chemical treatments may be used to concentrate and purify HBsAg from the plasma pool (*22-33*). The national control authority shall approve the exact combination of methods and shall ensure that the production process is reproducible and will give rise to consecutive lots that do not substantially differ with respect to degree of purity.

In some countries the required HBsAg content of vaccines is not less than 95% of the total protein content of the finished vaccine.

When a new process for the concentration and purification of HBsAg from the plasma pool is introduced, the efficiency of HBV DNA removal at each step during the purification process shall be validated. The national control authority shall approve the acceptable level of residual HBV DNA, the permitted level of contaminating plasma proteins, and the method for determining the identity of non-HBsAg proteins.

Removal of HBV DNA (3.2 kilobases) can be monitored by Southern blot hybridization assay. This assay should be sensitive to 1 pg of HBV DNA equivalent to approximately 300 000 copies of HBV DNA.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions followed by staining with a sensitive silver stain will permit the detection of all polypeptides. The electropherogram may be scanned by a densitometer to determine what proportion of the total polypeptides is accounted for by each individual polypeptide.

Native PAGE under non-denaturing conditions followed by staining with a sensitive silver stain will permit the detection of all polypeptides not associated with the purified HBsAg; this will remain at the top of the gel while the free polypeptides will enter the gel.

A convenient and suitable way of measuring purity is to determine the specific activity of the purified HBsAg (*34*). This can be done by dividing the reciprocal of the end-point dilution per ml, as determined by a sensitive RIA or EIA, by the protein concentration quantified by the Lowry method. This method can be used to determine the degree of purity as compared with that of the starting material and of other purified, inactivated HBsAg batches. The higher the specific activity, the greater the degree of purity.

A.3.5.2 *Inactivation*

The national control authority shall approve the method for the inactivation of HBV and other potential contaminating agents in the purified HBsAg batch. It shall also approve the number and types of inactivation steps that shall be used and the procedures to be used in validating the method. In assessing the efficiency of a procedure or procedures for inactivation the national control authority shall take into consideration data demonstrating the ability of each method to inactivate infectious agents that may be found in human blood. Each of the procedures shall be validated in order to demonstrate lot-to-lot consistency.

A procedure involving two heating cycles, namely 90 seconds at 103 °C followed by 10 hours at 65 °C, has been shown to inactivate members of 12 different virus families (*29*). A period of warming up to 65 °C has been shown to inactivate representatives of the following virus families: *Poxviridae* (vaccinia), *Picornaviridae* (encephalomyocarditis virus), *Togaviridae* (sindbis virus), *Coronaviridae* (murine hepatitis virus), *Orthomyxoviridae* (influenza virus), *Rhabdoviridae* (vesicular stomatitis virus), *Herpesviridae* (cytomegalovirus), and *Retroviridae* (HIV-1, murine leukaemia virus). After prolonged heating at 65 °C or heating for 90 seconds at 103 °C, parvoviruses and the phage ΦX174 were also completely inactivated (*29*). Papovaviruses, represented by simian virus 40, were the most heat-resistant, with a reduction of only 10^4 tissue culture infectious doses ($TCID_{50}$) per ml after 90 seconds at 103 °C.

In a study of the safety of plasma-derived hepatitis B vaccine prepared following two heating cycles, 10^6 chimpanzee infectious doses (CID_{50}) of HBV heated at 103 °C for 90 seconds and another 10^6 doses heated at 65 °C for 10 hours were not infectious in two hepatitis B antibody-free chimpanzees. Thus, the two-step heat inactivation process may be expected to inactivate 10^{12} CID_{50} of HBV DNA (*11*), which is approximately equivalent to 3 µg of HBV DNA on the assumption that 1 CID_{50} contains one copy of HBV DNA.

Dilutions of human sera containing between 10^3 and 10^5 CID_{50} of HBV per ml were treated with 1 µg/ml pepsin at pH 2.0 for 18 hours, urea (8 mol/litre) for 4 hours, or 1:4000 formalin for 72 hours, after which 1 ml of each treated serum was inoculated intravenously into one or two chimpanzees. No evidence of HBV infection was seen during a 6-month observation period. Thus, each of these procedures inactivated a minimum of 10^3 CID_{50} of HBV DNA in the presence of unfractionated human serum (*9*), which is approximately equivalent to 3 fg of HBV DNA on the assumption that 1 CID_{50} contains one copy of HBV DNA.

Inactivation procedures successfully employed in the production of plasma-derived hepatitis B vaccine include: (a) 1:4000 formalin at 37 °C for 48 hours (*9*); (b) 1:4000 formalin at 30 °C for 48 hours (*31*); (c) 1:4000 formalin at 36 °C for 72 hours (*20*); (d) heat treatment at 101-104 °C for 1.5 minutes followed by pasteurization for 10 hours at 65 °C (*22*), (e) 1:500 formalin at 37 °C for 4 days (*35*); (f) continuous-flow flash heating at 102 °C for 160 seconds followed by pasteurization for 10 hours at 65 °C (*36*); and (g) heat inactivation at 101 °C for 90 seconds and, after adsorption to aluminium phosphate, an additional heat inactivation at 65 °C for 10 hours (*37*).

Heat-inactivation procedures used in some vaccine manufacturing processes induce particle aggregation, which prevents subsequent accurate determination of the HBsAg content. Such aggregation may result in enhanced immunogenicity *in vivo*, but decreased antigenicity *in vitro*, as measured by immunological assays, presumably because of steric hindrance of antibody-binding activity. Therefore, if *in vitro* methods are being used, the HBsAg content of the purified HBsAg batch should be determined before the inactivation step.

A decrease in the concentration of free formaldehyde occurs in the course of the inactivation process. Tests for residual free formaldehyde should therefore be performed at intervals, and the concentration maintained at the desired level by intermittent readjustments.

Some authorities have suggested the use of model viruses, such as duck hepatitis B virus, in validation studies.

The production of hepatitis B vaccine shall be based on a method of inactivation shown to give a consistently safe product. A record of consistency shall have been established by the testing of five consecutive lots (see section A.3.7.1).

The method of inactivation is independent of the method of purification. The national control authority shall approve the combination of procedures used for purification and for inactivation.

A.3.6 ***Tests on purified, inactivated HBsAg batches***

A.3.6.1 Sterility tests

A volume at least 10 ml of the purified, inactivated HBsAg batch shall be tested for sterility as specified in Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*21*).

A.3.6.2 Tests for HBsAg and total protein

After purification, the protein content shall be measured for both total protein and HBsAg-specific protein. The latter shall be compared with a suitable reference preparation. If the procedure used for inactivation can interfere with the tests described in sections A.3.6.3 to A.3.6.5, these tests shall be performed before the inactivation step.

> It has been found suitable to measure the total concentration of protein by means of extinction coefficient or by the micro-Kjeldahl technique, the Lowry test or another appropriate reproducible method.

The content of HBsAg shall be determined by a sensitive serological test such as EIA or RIA for which the level of sensitivity is known. The concentration of HBsAg shall be related to the total protein. The purity and minimum content of HBsAg shall be approved by the national control authority.

> By making limiting two-fold dilutions of the purified, inactivated batch in human sera seronegative for HBsAg and anti-HBs, it is possible to determine the concentration of the starting material by multiplying the limit of sensitivity of the test by the dilution factor.
>
> The degree of purity of the purified, inactivated HBsAg batch can be determined by the methods described in section A.3.5.1.
>
> For the assay of antigenic content by standard immunological tests, a purified International Reference Reagent without adjuvant is recommended. Although such a reference reagent is not currently available, an estimate of the antigenic content may be obtained by preparing serial two-fold dilutions of the purified antigen in normal human serum and determining the end-point by an immunological test for which the sensitivity is known.

A.3.6.3 Tests for extraneous substances

Tests shall be made for the presence of blood group substances and other blood proteins, including liver-specific membrane proteins, by method approved by the national control authority.

enzyme-linked immunosorbent assay (ELISA) and PAGE have been used to test for other extraneous proteins.

The preparations shall be free from detectable blood group substances.

The permitted concentration of non-HBsAg proteins present in the vaccine shall be determined by the national control authority.

A.3.6.4 Test for HBV DNA

The preparation shall be tested for residual HBV DNA using a sensitive test approved by the national control authority and the level shown to be less than 1 pg HBV DNA per 50 doses.

A.3.6.5 Test for antigen purity

The degree of purity of the purified, inactivated HBsAg batch shall be determined.

The methods described in section A.3.5.1 (*25*) may be used.

The national control authority shall determine the electrophoretic pattern permitted as a demonstration of purity.

A.3.6.6 Test for reagents used during manufacture

A test shall be made for the presence of any potentially hazardous reagent, including inactivating reagents such as free formaldehyde, that may have been used during the manufacture of the purified, inactivated HBsAg batch.

The method used and the permitted concentration shall be approved by the national control authority.

A suitable procedure for the detection of free formaldehyde is described by Frerichs & Chandler (*38*).

' Final aqueous bulk

Only batches that have satisfied the requirements in sections A.3.5 and A.3.6 shall be included in the final aqueous bulk.

A.3.7.1 Safety test

In view of the concern about the possibility of infectious agents being present in the plasma used for the production of hepatitis B vaccine, the national control authority shall ensure that the procedures for the collection of plasma and the production process, including purification and inactivation, are well regulated, validated and reproducible, and will give rise to consecutive lots that will not differ with respect to safety.

In the light of the known safety record of currently licensed hepatitis B vaccine when validated inactivation procedures are used, and the high cost and shortage of chimpanzees, the testing of initial lots for the presence of infectious HBV in chimpanzees is no longer considered essential in the control of the vaccine produced by these methods, provided that an alternative procedure is approved by the national control authority.

All final aqueous bulks shall be tested for sterility as described in secti A.3.4.1 and for adventitious viruses as described in section A.3.4.2.

A.3.7.3 Test for HBsAg

The quantity of HBsAg compared with the total protein in the fin aqueous bulk shall be determined by a quantitative serological procedu in comparison with a suitable reference reagent (see section A.1.3). T purity and minimum content of HBsAg shall be approved by the nation control authority.

The tests referred to in section A.3.6.2 have been found to be suitable. T samples for this test should be taken before the addition of preservative.

A.3.7.4 Pyrogenicity test

In order to detect non-endotoxin pyrogens, each final aqueous bulk sha be tested for pyrogenicity by a suitable test. The test shall be approved b the national control authority.

A.3.8 Final bulk

A.3.8.1 Addition of adjuvant

Where the final bulk contains an adjuvant, each final bulk or final l shall be assayed for the content of adjuvant. The adjuvant and t concentration used shall be approved by the national control authorit Where aluminium salts are used, the concentration of aluminium sha not exceed 1.25 mg per single human dose. The national control authori shall determine the method used to quantify aluminium (*39-45*).

A.3.8.2 Tests for completeness of adsorption to adjuvant

Tests shall be carried out to confirm that HBsAg is adsorbed to t adjuvant. The tests and limits shall be approved by the national contr authority.

A convenient and suitable method is to centrifuge the preparation ar determine the end-point titre of HBsAg in the supernatant by EIA or RIA. T amount of HBsAg not bound to the adjuvant can be determined by multiplyi the end-point dilution by the known sensitivity of the EIA or RIA. This value c be compared with the value obtained before adding adjuvant.

A.3.8.3 Tests for sterility

A volume of at least 10 ml of the final aqueous bulk shall be tested f bacterial and mycotic sterility as specified in Part A, section 5.2, of t revised Requirements for Biological Substances No. 6 (Gener Requirements for the Sterility of Biological Substances) (*21*).

A.3.8.4 Tests for preservative

Each final bulk or final lot shall be tested for the presence of preservativ The method used and the permitted concentration shall be approved b the national control authority.

analytical methods for organic mercurials involved the decomposition of th metallo-organic compounds (*46*) and detection with a complexing agent suc as diphenylthiocarbazone (*47, 48*). Greater specificity was achieved by the us of better extraction procedures (*49*) or column chromatography (*50*). Mor sophisticated analytical methods, such as atomic absorption (*51*), neutro activation (*52*), gas-liquid chromatography (*53*) and high-pressure liqui chromatography (*54*), detect degradation products as well as intact thiomersa A suitable and simple procedure described by Christensen & Weis Bentzon (*5* relies on microbiological methods using agar diffusion from paper discs.

A.3.8.5 Tests for inactivating agent

The concentration of any inactivating agent remaining in the final vaccin shall be determined by the methods approved by the national contro authority. This concentration shall not exceed a specified upper limit.

If the manufacturer has validated a process for the total clearance c inactivating agent and if this has been approved by the national contrc authority, a test may not be required on each batch.

A.3.8.6 Test for potency

An appropriate quantitative potency assay shall be performed on eacl final bulk or final lot. The vaccine potency shall be compared with that o a reference preparation calibrated against the International Referenc Reagent (see section A.1.3). The required potency and the assay metho shall be approved by the national control authority. The national contro authority may approve the use of a validated *in vitro* assay.

The following is an example of a method that has been found suitable. Group of at least 20 suitable mice, 5 weeks of age, are vaccinated intraperitoneall with graded doses of adjuvanted hepatitis B vaccine diluted in the adjuvan used in the vaccine. Similar groups of mice are inoculated with the adjuvantec reference preparation. The mice are bled 28 days later and the sera are kep separate. Antibody determinations are performed by a sensitive quantitative method such as RIA or EIA. The data are analysed according to sero conversion and the geometric mean titre of anti-HBs for each antigen dose The strain of mice used for this test must give a steep dose-response curve with the reference antigen.

A collaborative study was carried out to examine the suitability of plasma-derived hepatitis B vaccine as an immunogenicity reference (*14, 16*). The dilution of vaccine required to induce antibodies in 50% of test animals anc potency estimates varied widely between laboratories.

In some countries a quantitative extinction test in guinea-pigs has been showr to be suitable.

The potency in an *in vivo* assay should be measured in terms of the quantity o vaccine giving an antibody response in 50% of the animals (ED_{50}). Since it has been shown that seroconversion in mice is influenced by the amount of pre-S_2 polypeptide in the vaccine, the potency in mice may not reflect immunogenicity in humans. The purpose of the potency test is thus to establish an ED_{50} value to quantify lot-to-lot variation and ensure conformity with the material used ir the clinical trial that showed the product to be suitable.

A.4 Filling and containers

The requirements concerning filling and containers in Good Manu facturing Practices for Biological Products (*18*, section 4) shall apply.

Care should be taken to ensure that the material of which the containe is made does not adversely affect the HBsAg under the recommende conditions of storage.

A.5 Control tests on final product

A.5.1 *Identity test*

An identity test using an appropriate method shall be performed on th vaccine in the final container.

ELISA, immunoblot or single-dose immunogenicity tests have been four suitable, depending on whether the antigen can be removed readily from th adjuvant.

A.5.2 *Potency test*

An appropriate quantitative potency assay shall be performed on eac final lot or final bulk by the methods described in section A.3.8.6.

A.5.3 *Sterility tests*

The final lot shall be tested for sterility as specified in Part A, section of the revised Requirements for Biological Substances No. 6 (Gener Requirements for the Sterility of Biological Substances) (*21*).

A.5.4 *General safety tests*

Each final lot shall be tested for innocuity by appropriate tests in mic and guinea-pigs, using parenteral injections. The tests shall be thos approved by the national control authority.

A.5.5 *Pyrogenicity test*

Each final lot shall be tested for pyrogenicity by a suitable test. The te shall be approved by the national control authority.

The national control authority may approve replacement of th pyrogenicity test on the final lot by a validated test for endotoxin.

A.5.6 *Test for preservative*

Each final lot or final bulk shall be tested for the presence of preservativ The test used and the permitted concentration shall be approved by th national control authority.

Suitable procedures for quantifying thiomersal, the most common preservativ are described in section A.3.8.4.

Each final lot or final bulk shall be assayed for the content of adjuvant. The method used and permitted concentration shall be approved by the national control authority. Where aluminium compounds are used, the concentration of aluminium shall not be greater than 1.25 mg per single human dose.

A.6 Records

The requirements in Good Manufacturing Practices for Biological Products (*18*, section 8) shall apply.

A.7 Samples

The requirements in Good Manufacturing Practices for Biological Products (*18*, section 9) shall apply.

A.8 Labelling

The requirements in Good Manufacturing Practices for Biological Products (*18*, section 7) shall apply, with the addition of the following directive:

The leaflet accompanying the package shall include the following information:

- the method used to inactivate HBV;
- the nature and amount of any preservative, adjuvant or stabilizer present in the vaccine;
- the volume of one recommended human dose, immunization schedules and the recommended routes of administration; these shall be given for newborn babies, children, adults and immunosuppressed individuals and shall be the same for a given vaccine for all regions of the world;
- the amount of HBsAg contained in one recommended human dose.

A.9 Distribution and shipping

The requirements in Good Manufacturing Practices for Biological Products (*18*, section 8) shall apply. In addition, the conditions of shipping shall be such that the vaccine does not freeze.

Temperature indicators should be packaged with each vaccine shipment to show that freezing did not occur.

If freezing has occurred, the vaccine should not be used.

A.10 Storage and expiry date

The requirements in Good Manufacturing Practices for Biological Products (*18*) shall apply.

A.10.1 *Storage conditions and stability*

Before being distributed by the manufacturing establishment, or before being issued from a depot for the maintenance of reserves of vaccines, all vaccines in bulk form or in final containers shall be kept at 2–8 °C. After distribution or issue, the vaccine shall be stored at a temperature not exceeding 8 °C. The vaccine shall have been shown to maintain potency for a period equal to that between the date of issue and the expiry date. During storage the vaccine shall not be frozen.

A.10.2 *Expiry date*

The expiry date shall be fixed with the approval of the national control authority and shall relate to the date of the last satisfactory potency test, the date of this test being that on which the test system was inoculated.

Part B. National control requirements

B.1 General

The general requirements for control laboratories in the Guidelines for National Authorities on Quality Assurance for Biological Products (*56*) and the revised Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives (*19*) shall apply.

The national control authority shall:

- approve the degree of purity;
- approve the method for inactivation and the permitted level of extraneous protein;
- approve the method for determining potency;
- ensure that a reference preparation is available for the expression of activity of HBsAg contained in a given quantity of protein.

B.2 Release and certification

A vaccine lot shall be released only if it fulfils the national requirements and/or part A of the present Requirements. A protocol based on the model given in Appendix 1, signed by the responsible official of the manufacturing establishment, shall be prepared and submitted to the national control authority in support of a request for release of vaccine for use.

At the request of the manufacturing establishment, the national control authority shall provide a certificate that states whether the vaccine meets all national requirements and/or Part A of the present Requirements. The certificate shall be based on the model given in Appendix 2.

The purpose of the certificate is to facilitate the exchange of hepatitis B vaccines among countries.

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cknowledgements

Acknowledgements are due to the following experts for their comments and advice: Professor J. P. Cano, Director, National Health Laboratory, Montpellier, France; Ms M. Cone, Vice-President for Scientific Affairs, International Federation of Pharmaceutical Manufacturers Associations, Geneva, Switzerland; Dr Darodjatum, President Director, Perum Bio Farma (Pasteur Institute), Bandung, Indonesia; Dr M. Ferguson, Division of Virology, National Institute for Biological Standards and Control, Potters Bar, Herts., England; Professor D. K. Hazra, Chief Consultant, Nuclear Medicine and Radioimmunoassay Unit, Post Graduate Department of Medicine, S. N. Medical College, Agra, India; Dr K. J. Healy, Head, Quality Assurance, CSL Ltd, Victoria, Australia; Professor M. Kantoch, Head, Virology Department, National Institute of Hygiene, Warsaw, Poland; Mr P. E. Lemoine, formerly Head, Biological Standardization, Institute of Hygiene and Epidemiology, Brussels, Belgium; Dr M. Mirchamsy, Associate Director, Razi State Institute of Sera and Vaccines, Teheran, Islamic Republic of Iran; Dr R. Netter, formerly General Inspectorate of Social Affairs, Ministry of Solidarity, Health and Social Protection, Paris, France; Dr J. C. Petricciani, Vice President, Regulatory Affairs, Genetic Institute, Cambridge, MA, USA; Professor G. R. E. Swaniker, Division of Biochemistry, Department of Basic Medical Sciences, University of Papua New Guinea, Boroko, Papua New Guinea; Dr M. Weeke-Lüttmann, Paul Ehrlich Institute, Langen, Germany.

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Appendix 1

Example, for guidance, of a summary protocol for the production and testing of hepatitis B vaccine prepared from plasma[1]

Identification of final lot

Name and address of manufacturer _____

_____

_____

International name and proprietary name of vaccine _____

Lot no. of final product _____

Date of manufacture of final lot _____

Date containers were filled _____

Number of containers and nature (ampoules or vials) _____

Date of last potency test _____

Number of doses in each container _____

Volume of single dose _____

Expiry date _____

Production control (A.3)

Collection of blood and plasma and selection of donors (A.3.1)

Results _____

Tests on single-donor plasma (A.3.2)

Methods _____

Results _____

HBsAg content _____

Pooling of single-donor plasma (A.3.3)

Volume of plasma pools _____

Number of single-donor plasmas pooled _____

[1] Based on Requirements for Hepatitis B Vaccine Prepared from Plasma (Requirements for Biological Substances No. 31, revised 1994). In: *WHO Expert Committee on Biological Standardization. Forty-fifth Report:* Geneva, World Health Organization, 1995, Annex 3 (WHO Technical Report Series, No. 858).

Tests on plasma pools (A.3.4)

Sterility tests (A.3.4.1)

	bacteria	*fungi*	*mycoplasma*
Date of inoculation	_____	_____	_____
Media used	_____	_____	_____
Observation period	_____	_____	_____
Results	_____	_____	_____

Tests for adventitious viruses (A.3.4.2)

Tests in cell cultures

Method _____

Results _____

Date _____

Tests in adult mice

Method _____

Results _____

Date _____

Tests in suckling mice

Method _____

Results _____

Date _____

Tests in embryonated eggs

Method _____

Results _____

Date _____

Other tests (if applicable)

Method _____

Results _____

Date _____

Concentration, purification and inactivation (A.3.5)

Concentration and purification (A.3.5.1)

Method _____

Inactivation (A.3.5.2)

Method _____

Concentration of inactivator _____

Date of starting inactivation _____

Date of finishing inactivation _____

Results _____

Tests on purified, inactivated HBsAg (A.3.6)

Tests for sterility (A.3.6.1)

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Media used	_____	_____	_____
Observation period	_____	_____	_____
Results	_____	_____	_____

Tests for HBsAg and total protein (A.3.6.2)

Method _____

Results _____

Date _____

Tests for extraneous substances (A.3.6.3)

Method _____

Results _____

Date _____

Tests for HBV DNA (A.3.6.4)

Method _____

Results _____

Date _____

Tests for antigen purity (A.3.6.5)

Method _____

Results _____

Date _____

Method _____

Results _____

Date _____

Final aqueous bulk (A.3.7)

Safety test (A.3.7.1)

Method _____

Results _____

Date _____

Sterility tests and tests for adventitious viruses (A.3.7.2)

	bacteria	*fungi*	*mycoplasma*
Sterility tests			
Date of inoculation	_____	_____	_____
Media used	_____	_____	_____
Observation period	_____	_____	_____
Results	_____	_____	_____

Tests for adventitious viruses

Tests in cell cultures:

Method _____

Results _____

Date _____

Tests in adult mice:

Method _____

Results _____

Date _____

Tests in suckling mice:

Method _____

Results _____

Date _____

Tests in embryonated eggs:

Method _____

Results _____

Other tests (if applicable):

Method _____

Results _____

Date _____

Test for HBsAg (A.3.7.3)

Method _____

Results _____

Date _____

Pyrogenicity test (A.3.7.4)

Method _____

Results _____

Date _____

Final bulk (A.3.8)

Composition of final bulk (after mixing of all ingredients) and identification number _____

Addition of adjuvant (A.3.8.1)

Volume of bulk _____

Nature and volume of adjuvant added and final concentration _____

Tests for completeness of adsorption to adjuvant (A.3.8.2)

Method _____

Results _____

Date _____

Tests for sterility (A.3.8.3)

	bacteria	*fungi*
Date of inoculation	_____	_____
Media used	_____	_____
Observation period	_____	_____
Results	_____	_____

Method _____

Results _____

Date _____

Tests for inactivating agent (A.3.8.5)

Method _____

Results _____

Date _____

Tests for potency (A.3.8.6)

Method _____

Results _____

Date _____

Filling and containers (A.4)

Date of filling _____

Quantity of containers _____

Volume of vaccine per container _____

Control for defective containers _____

Control tests on final product (A.5)

Identity test (A.5.1)

Method _____

Results _____

Date _____

Potency test (A.5.2)[1]

Method _____

Results _____

Date _____

Sterility tests (A.5.3)

	bacteria	*fungi*	*mycoplasm*
Date of inoculation	_____	_____	_____
Media used	_____	_____	_____
Observation period	_____	_____	_____
Results	_____	_____	_____

[1] If not done on final bulk.

Date of inoculation _____

No. of mice tested _____

Volume and route of injection _____

Observation period _____

Results (give details of deaths) _____

Test in guinea-pigs

Date of inoculation _____

No. of guinea-pigs tested _____

Volume and route of injection _____

Observation period _____

Results (give details of deaths) _____

Pyrogenicity test (A.5.5)

Method _____

Results _____

Date _____

Test for preservative (A.5.6)[1]

Method _____

Results _____

Date _____

Assay of adjuvant (A.5.7)[1]

Method _____

Results _____

Date _____

[1] If not done on final bulk.

of head of production laboratory _____

_____

Date _____

Certification by person taking overall responsibility for production and control of the vaccine:

I certify that lot no. _____ of hepatitis B vaccine satisfies national requirements and/or Part A of the Requirements for Biological Substances No. 31 (Requirements for Hepatitis B Vaccine Prepared from Plasma), revised 1994.

Signature _____

Name (typed) _____

Date _____

Appendix 2

Model certificate for the release of hepatitis B vaccine prepared from plasma by national control authorities[1]

The following lots of hepatitis B vaccine produced by _____[2] in _____,[3] whose numbers appear on the labels of the final containers, meet all national requirements[4] and Part A[5] of Requirements for Biological Substances No. 31 (Requirements for Hepatitis B Vaccine Prepared from Plasma), revised 1994[6] and comply with Good Manufacturing Practices for Pharmaceutical[7] and Biological[8] Products.

Lot no.	Date of last potency test by manufacturer	Expiry date	Lot no.	Date of last potency test by manufacturer	Expiry date

As a minimum, this certificate is based on examination of the manufacturing protocol.

The Director of the National Control Laboratory (or Authority as appropriate):[9]

Name (typed) _____

Signature _____

Date _____

[1] To be completed by the national control authority of the country where the vaccine has been manufactured, and to be provided by the vaccine manufacturer to importers.
[2] Name of manufacturer.
[3] Country.
[4] If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the national control authority.
[5] With the exception of the provisions on distribution and shipping, which the national control authority may not be in a position to assess.
[6] WHO Technical Report Series, No. 858, 1995, Annex 3.
[7] WHO Technical Report Series, No. 823, 1992, Annex 1.
[8] WHO Technical Report Series, No. 822, 1992, Annex 1.
[9] Or his or her representative.

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World Health Organization, Technical Report Series, No. 786, 1989

Annex 2

REQUIREMENTS FOR HEPATITIS B VACCINES MADE BY RECOMBINANT DNA TECHNIQUES

(Requirements for Biological Substances No. 45)

	Page
General considerations	38
Part A. Manufacturing requirements	42
1. Definitions	42
2. General manufacturing requirements	44
3. Validation and control of manufacturing procedures	45
4. Manufacturer's working cell bank (MWCB)	48
5. Production precautions	49
6. Single harvests	50
7. Purification	50
8. Final bulk before addition of adjuvant (aqueous bulk)	53
9. Final bulk	54
10. Filling and containers	55
11. Control of final lot	55
12. Records	57
13. Samples	57
14. Labelling	57
15. Distribution and shipping	57
16. Storage and expiry date	58
Part B. National control requirements	58
1. General	58
2. Release and certification	59
Authors and acknowledgements	60
References	60
Appendix. Summary protocol	61

GENERAL CONSIDERATIONS

Advances in molecular genetics and nucleic acid chemistry have made it possible to identify genes coding for biologically active substances, to analyse them in detail, to transfer them within and

between organisms, and to obtain gene expression under controlled conditions with efficient synthesis of the encoded product. A gene that codes for a specific product can be isolated and propagated by insertion into a suitable vector with the aid of highly specific restriction endonuclease enzymes (which cleave the vector DNA at predetermined sites) and ligases (which join the gene insert to the vector). The vector can then be introduced into host organisms, and individual clones that carry the desired gene can be selected and propagated in mass culture.

A gene is characterized by a specific nucleotide sequence in one strand of the double-stranded DNA molecule. When the strands are separated, each forms a template for the synthesis of a complementary copy, thus providing a mechanism for the faithful reproduction of genes with conservation of the linear sequence of the four types of mononucleotides. The process of decoding the genetic information and of synthesizing the gene product occurs in two phases, of which the first is transcription of the DNA coding strand in the form of messenger RNA, and the second translation of the information carried by the messenger RNA molecule into an amino acid sequence. The factors affecting the expression of foreign genes introduced into prokaryotic and eukaryotic cells are complex; the efficient and controlled expression of stable, cloned DNA sequences is an important field of current research.

Hepatitis B virus has several characteristics that distinguish it from the other families of DNA viruses. It has an outer coat (more substantial than a membrane or envelope) consisting of protein, lipid, and carbohydrate and bearing a unique antigen complex, hepatitis B surface antigen (HBsAg). Its nucleic acid consists of a circular DNA genome of relative molecular mass about 2 million, part of which is double-stranded and part single-stranded — an unusual feature among viruses. Virus recovered from the plasma of a hepatitis B carrier has been used to prepare viral DNA; that DNA has been cloned in *Escherichia coli* and the gene coding for HBsAg has been isolated.

This gene has been inserted into yeast and mammalian cells by means of appropriate expression vectors. Purified antigens obtained from transfected cultures containing it have been shown to induce antibodies in mice and guinea-pigs and have been formulated into vaccines. Electron microscopy has revealed that the purified HBsAg used for these vaccines exists as particles 15–30 nm in diameter, with the morphological characteristics of free surface antigen in plasma

and of the purified antigen now used in plasma-derived hepatitis B vaccines. Some of the vaccine formulations containing these materials have already been shown to be immunogenic in mice, chimpanzees and other monkeys, and human beings, with antigenic potencies similar to those of vaccine made from plasma-derived antigen.[1]

The requirements that follow apply to the control and testing of hepatitis B vaccine made by recombinant DNA methods, and have been formulated to take account of the scale-up required for commercial production. Particular emphasis is placed on introducing "in-process" control, which has been highly effective for other bacterial and viral vaccines, rather than relying entirely on tests on the end-products. General requirements, such as tests for potency, purity, toxicity, pyrogenicity, and sterility, will apply as much to hepatitis B vaccines made by recombinant DNA methods as to those derived from human plasma. Certain tests will be required on every production batch of vaccine, whereas others will be required only to establish the validity, acceptability, and consistency of a given manufacturing process.

A detailed description of how the product is made should be given. Evidence should be presented to show that any HBsAg made by recombinant DNA techniques possesses the characteristics of an immunogen that protects against hepatitis B virus.

Rigorous identification and characterization of recombinant DNA-derived vaccines will be required. The ways in which these products differ chemically, structurally, biologically, or immunologically from the naturally occurring antigen must be fully documented. Such differences could arise during processing at the genetic or post-translational level, or during purification. Differences between batches of the product may result from genetic instability during serial cultivation. Microbial contamination during fermentation may occur, and tests for contaminants must be thorough.

Special attention should be given to purity because:

- unwanted gene products may be co-expressed unexpectedly with the HBsAg, for example if transcription is initiated at several sites, or if changes occur during culture that affect transcription, initiation, or termination processes or favour the expression of other genes in the vector or the host cell;
- biologically active extraneous components, such as DNA, proteins, and endogenous retroviruses derived from the host-cell system may be found in the final product;
- agents used in the purification process (column matrices, antibodies) may give rise to specific contaminants in the final product.

[1] For further details and a discussion of hepatitis B vaccines produced by recombinant DNA techniques, see WHO Technical Report Series, No. 760, 1987, Annex 5.

The product arising from the recombinant system must be shown to elicit specific antibody responses to HBsAg in laboratory animals, including, where possible, a non-human primate species. HBsAg of diverse subtypes should be used to characterize the specificity of the response fully.

One approach to evaluating the protective potential of recombinant DNA-derived vaccines in human beings is through immunization and challenge studies in chimpanzees. The requirement for a test of vaccine efficacy in chimpanzees should be at the discretion of the national control authority but, if possible, on at least one occasion, chimpanzees given the recommended human immunization schedule should be shown to be protected against challenge with hepatitis B virus. In some studies, more than 1000 chimpanzee infectious doses of hepatitis B virus of homotypic and heterotypic subtypes, given intravenously, have been used as a challenge.

The vaccine should reliably induce antibody responses to HBsAg in human recipients. The frequency and titre of the antibody responses should be at least equivalent to those induced by plasma-derived vaccines that fulfil WHO requirements. The aims of immunogenicity studies in human subjects should be to define the quantity of antigen and number of doses required to elicit reliably antibody responses to HBsAg. The titre, duration, and quality of the responses should be clearly defined. *In vitro* tests of antigenicity may be difficult to standardize, and it is therefore proposed that the content of pure HBsAg in the product should be used as the basis for comparing immunogenicity in mice and responses in human subjects. The relationship between the antigenicity of the product in *in vitro* tests, in mouse immunogenicity tests, and in human beings should be established. Studies in human subjects should be designed to provide information on the frequency and severity of any local and systemic adverse reactions to vaccination. Va[illegible] ecipients in

such studies should be representative of the intended target group for immunization in terms of age and risk.

Each of the following sections constitutes a recommendation. The parts of each section that are printed in large type have been written in the form of requirements, so that, if a health administration so desires, these parts as they appear may be included in definitive national requirements. The parts of each section that are printed in small type are comments and recommendations for guidance.

Should individual countries wish to adopt these requirements as the basis of their national regulations concerning hepatitis B vaccine, it is recommended that a clause should be included permitting modifications of the manufacturing requirements on the condition that it can be demonstrated, to the satisfaction of the national control authority, that such modifications ensure a degree of safety and potency of the vaccine at least equal to those provided by the requirements formulated below. The World Health Organization should then be informed of the action taken.

The terms "national control authority" and "national control laboratory", as used in these requirements, always refer to the country in which the vaccine is manufactured and/or used.

PART A. MANUFACTURING REQUIREMENTS

1. Definitions

1.1 International name and proper name

The international name shall be *Vaccinum hepatitidis B recombinatum*. The proper name shall be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

1.2 Descriptive definition

Vaccinum hepatitidis B recombinatum is a preparation of purified hepatitis B surface antigen (HBsAg) that has been produced by recombinant DNA techniques. The preparation shall satisfy all the requirements formulated below.

1.3 International reference materials

International standards and reference reagents are needed for the control of HBsAg or to determine antigenic content and potency.

The reference materials mentioned below are available but are not specified for use in tests in these requirements.

For the assay of antigenic content by techniques such as radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), single radial immunodiffusion, and rocket electrophoresis, an international reference material consisting of an aqueous preparation without an adjuvant is required (see Part A, section 7.3). Such a preparation was established in 1985 as the first International Standard for Hepatitis B Surface Antigen *ad* Subtype (100 IU per ampoule).

The International Reference Reagent of Plasma-Derived Hepatitis B Vaccine for Immunogenicity Studies was established in 1986. It is a liquid preparation containing approximately 20 µg/ml of plasma-derived hepatitis B surface antigen and contains aluminium hydroxide as an adjuvant.

Both the above-mentioned reference materials are available on request from the National Institute for Biological Standards and Control, Potters Bar, England.

For the calibration of techniques used to measure antibody responses to hepatitis B vaccines, an international reference preparation is available. This International Reference Preparation of Hepatitis B Immunoglobulin (established in 1977) is dispensed in ampoules containing 50 IU of hepatitis B immunoglobulin (from fractionated human plasma, and freeze-dried). This preparation is in the custody of the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands.

1.4 Terminology

Hepatitis B virus: a 42-nm double-shelled virus particle, originally known as the Dane particle, which contains the DNA genome of the virus.

HBsAg: hepatitis B surface antigen, comprising a complex of antigens associated with the virus envelope and subviral forms (22-nm spherical and tubular particles). Native HBsAg is encoded by envelope gene sequences (S plus pre-S) in the viral DNA. Recombinant DNA-derived hepatitis B vaccines may contain the S gene product or products of the S/pre-S combination.

Cell seed lot: a quantity of cells stored frozen at −70 °C or below in aliquots of uniform composition, one or more of which would be used for the production of a manufacturer's working cell bank.

Manufacturer's working cell bank (MWCB): a quantity of cells, derived from one or more ampoules of the cell seed, stored frozen at −70 °C or below in aliquots of uniform composition.

In normal practice, a cell seed is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer, at which point the cells are combined to give a single pool and preserved cryogenically to form the MWCB.

Production cell culture: a collection of cell cultures being used for biological production that have been derived from one or more ampoules of the MWCB.

Single harvest: the biological material prepared from a single production run.

Final aqueous bulk: the final bulk before the addition of an adjuvant.

Final bulk: the finished biological material prepared from one or more batches of purified HBsAg, and present in the container from which the final containers are filled.

Final lot: a collection of sealed final containers that are homogeneous with respect to the risk of contamination during filling and during the preparation of the finished vaccine. A final lot must therefore consist of finished material distributed into containers in one working session from a single final bulk.

2. General manufacturing requirements

The general manufacturing requirements contained in the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 11) shall apply to establishments manufacturing hepatitis B vaccine, with the addition of the following directives:

(*a*) Production areas shall be decontaminated before they are used for the manufacture of hepatitis B vaccine.

(*b*) Hepatitis B vaccine shall be produced by staff who have not handled animals or infectious microorganisms in the same working day. The staff shall consist of persons who have been examined medically and have been found to be healthy and not to be carriers of hepatitis B.

(*c*) No cult[illegible] of microorganisms or eukaryotic cells other than those a[illegible]ed by the national control authority shall be introduced into or handled in the production area at any time during manufacture of the vaccine.

(*d*) Persons not directly concerned with the production processes, other than official representatives of the national control authority, shall not be permitted to enter the production area.

Particular attention is drawn to the recommendations contained in Part A, section 1 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 13) regarding the training and experience of persons in charge of production and testing and of those assigned to various positions of responsibility in the manufacturing establishment, and to the registration of such personnel with the national control authority.

The preparation of hepatitis B vaccine shall be based strictly on the cell seed lot system. A description of the system used should be provided, including the number of vials of seed available and details of their storage. Particular attention should be paid to the stability of the expression vector and to the plasmid copy number in the seed stock under conditions of storage and recovery.

Full details of the cell culture process used in manufacture should be provided to the national control authority with particular reference to tests to monitor microbial contamination in the cell culture vessels. Information on the sensitivity of methods of detecting such contamination and the frequency of testing should be provided, together with criteria for the rejection of contaminated materials.

The yield of HBsAg shall be monitored during the course of individual production runs. Criteria, based on yield, for the acceptance of culture harvests for further processing into vaccine shall be defined, and consistency of production shall be established by testing at least five consecutive lots prepared by the same procedures.

3. Validation and control of manufacturing procedures

The general production precautions formulated in Part A, section 3 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p 15) shall apply to the manufacture of hepatitis B vaccine.

3.1 Strategy for cloning and expressing the gene

A full description of the biological characteristics of the host cell and expression vectors used in production should be given. This should include details of: (*a*) potential retrovirus-like particles in and genetic markers of the host cell; (*b*) the construction, genetics, and structure of the expression vector; and (*c*) the origin and identification of the gene that is being cloned.

The physiological measures used to promote and control the expression of the cloned gene in the host cell should be described in detail.

Data that demonstrate the stability of the expression system during storage of the MWCB and beyond the passage level used for production should be provided. Any instability of the expression system occurring in the seed culture or after a production-scale run, for example involving rearrangements, deletions, or insertions of nucleotides, must be documented. Unstable preparations must *not* be used until approval to continue use has been obtained from the national control authority.

3.2 Biochemical characterization of recombinant vector

The nucleotide sequence of the gene insert and of adjacent segments of the vector and restriction-enzyme mapping of the vector containing the gene insert shall be provided as required by the national control authority.

3.3 Purification procedures

The methods used to purify the HBsAg from culture harvests should be fully described. The capacity of each step of the purification procedure to remove and/or inactivate substances other than HBsAg derived from the host cell or culture medium, including, in particular, virus particles, proteins, and nucleic acids, shall be evaluated. If individual contaminants are difficult to monitor, the results of pilot-scale studies to follow the removal of individual, deliberately added contaminants at appropriate stages of purification will provide valuable information.

If antibodies are used in the purification procedures, their origins and characteristics should be fully described. The degree of purity of monoclonal antibodies produced from hybridoma cell lines and the criteria for freedom from cell-derived DNA and murine viruses should conform to the regulations laid down by the national control authority.

3.4 Characterization of gene products (HBsAg)

3.4.1 *Particle characterization*

The morphological characteristics of the HBsAg particles and degree of aggregation should be established by electron microscopy and by physicochemical methods, for example by gradient centrifugation. In addition, the protein, lipid, nucleic acid, and carbohydrate content should be measured.

3.4.2 *Determination of protein content*

The protein content (composition) in comparison with a reference reagent should be established by quantitative amino acid analysis or by another accurate method.

3.4.3 *Protein characterization*

An ultraviolet absorption spectrum should be recorded. The protein composition should be established by techniques such as sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) under reducing conditions. The bands should be identified by sensitive staining techniques and, where possible, by specific antibodies to confirm the presence of the expected products of the hepatitis B virus envelope gene. The identity of the protein should be established by partial N-terminal and C-terminal sequence analysis.

3.4.4 *Antibody responses*

The recombinant DNA-derived vaccine should be shown to induce antibody responses in humans comparable to those elicited by plasma-derived vaccines that have proved effective in the field. The antibodies induced by the vaccine in human beings should be titrated and characterized with respect to their activities against relevant determinants of the hepatitis B virus envelope, for example group and subtype determinants.

3.4.5 *Consistency of yield*

Data on the consistency of yield between runs and during individual production runs shall be provided, and the national control authority shall approve the criteria for an acceptable production run.

4. Manufacturer's working cell bank (MWCB)

4.1 Origin of cell banks

Only cells approved by and registered with the national control authority shall be used to produce HBsAg. The national control authority shall have responsibility for approving the cell seed. A short history of the cell banks shall be provided.

4.2 Characteristics of cell seed lot

The characteristics of the cell seed (host cell in combination with the expression vector system) shall be fully described, and information given on the absence of extraneous agents and on genetic homogeneity. The nucleotide sequence of the HBsAg gene insert and its flanking regions shall be specified where relevant. A peptide map and/or terminal amino acid sequence of the gene products shall be obtained.

4.3 Phenotypic indicators of purity and genetic consistency of recombinant cultures

Cells must be maintained in a frozen state that allows recovery of viable cells without alteration of genotype. The cells should be recovered from the frozen state, if necessary, in selective media such that the genotype and phenotype consistent with the unmodified host and unmodified recombinant DNA vector are maintained and are clearly identifiable. The cell seed must be identified by means of appropriate tests.

4.4 Sterility tests

Tests for bacteria, fungi, and mycoplasmas shall be performed in accordance with the requirements of Part A, section 5 of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*2*, p. 49).

4.5 Additional tests for MWCB of mammalian cells

The cells shall have been characterized as specified under the Requirements for Biological Substances No. 37 (Requirements for Continuous Cell Lines Used for Biologicals Production) (*3*).

The national control authority shall have the responsibility for approving the cell seed.

5. Production precautions

5.1 Production cell cultures

Only cell cultures derived from the MWCB shall be used for production. All processing of cells shall be done in an area in which no cells or organisms are handled other than those directly required for the process.

5.2 Culture conditions for production cell cultures

Production cell cultures shall be grown under conditions agreed with the national control authority. These conditions shall include details of the culture system used, the cell doubling time, the number of subcultures or the duration of the period of subcultivation permitted, and the incubation temperature.

Cell cultures shall be monitored for freedom from microbial contamination as required by the national control authority.

5.3 Cell culture medium

If serum is used for the propagation of cells for vaccine production, it shall be tested by methods approved by the national control authority, to demonstrate freedom from bacteria, fungi, viruses, and mycoplasmas, according to the requirements given in Part A, sections 5.2 and 5.3 of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*2*, p. 49) as well as freedom from pathogens of the species of origin of the serum.

In some countries, sera are examined for freedom from phages.

Antibiotics of the β-lactam type shall not be used at any stage in the production.

Minimal concentrations of other suitable antibiotics may be used if approved by the national control authority. Nontoxic pH indicators may be added, e.g., phenol red in a concentration of 20 mg/l.

6. Single harvests

6.1 Sterility

Microbial contamination in the cell culture vessels shall be monitored during and at the end of the production runs by methods approved by the national control authority.

6.2 Consistency of yield

The yield of HBsAg from each single harvest shall be shown to be within the limits approved by the national control authority (see Part A, section 3.4.5).

6.3 Plasmid retention

A sample of cells that are representative of each harvest must be tested to confirm that the recombinant phenotype has been retained. The method used shall be approved by the national control authority.

7. Purification

The purification procedure can be applied to a single harvest, a part of a single harvest, or a pool of single harvests. The maximum number of single harvests that may be pooled shall be fixed by the national control authority. The antigen must be purified before adsorption on to an adjuvant. Adequate purification may require several purification steps based on different principles. This will minimize the possibility of co-purification of extraneous cellular materials. The methods used for the purification of the HBsAg

should be appropriately validated (see Part A, section 3.3) and approved by the national control authority.

The experience of several manufacturers has shown that it is possible to produce batches in which HBsAg accounts for at least 95% of the total protein. One suitable method of analysing the proportion of potential contaminant proteins in the total protein of the product is separation of the proteins by polyacrylamide gel electrophoresis under both non-reducing and reducing denaturing conditions. Individual gels should be stained by Coomassie Blue and by silver stain, or a single gel may be successively stained by both methods. The silver stain is considerably more sensitive for the detection of very small quantities of proteins and is also useful for identifying any non-protein materials, such as nucleic acids, carbohydrates, and lipids, which may be present. The protein in each band can be quantified by densitometric analysis. The staining procedure should be sensitive enough to reveal a potential contaminant protein present at a level of 1% of total protein.

High-performance liquid chromatography is another very useful method for determining the purity of a protein or peptide and for investigating its molecular configuration. A widely used method is reverse-phase high-performance liquid chromatography. A protein or peptide that elutes as a single symmetrical peak in two markedly different systems, including an ion-pair system, is generally of high purity.

7.1 Protein and other components of the vaccine

The total protein content of the vaccine should be determined (see also Part A, section 3.4.2).

The total concentration of protein may be determined by measuring absorbance at 280 nm by the micro-Kjeldahl method, the Lowry technique, or another suitable method.

Lipid and carbohydrate contents may also provide useful information.

If serum is used in the medium for the production cell cultures or at any stage in the purification process, for example as a reagent in immunoabsorption chromatography, tests shall be made for residual serum in the purified vaccine.

The concentration of animal serum in the vaccine shall be not more than 1 µl per litre of vaccine.

A concentration of 1 µl of animal serum per litre of vaccine is approximately equivalent to 50 ng of albumin per ml. Methods such as enzyme-linked immunosorbent assay (ELISA) and

immunoprecipitation can detect as little as 1 ng of albumin per ml.

7.2 Tests for agents used during purification or other phases of manufacture

A test shall be made for the presence of any potentially hazardous agent used in manufacture. The method used and the permitted concentration shall be approved by the national control authority.

7.2.1 *Monoclonal antibodies*

Where a monoclonal antibody is used in vaccine preparation, for example for immunological affinity chromatography to purify HBsAg, the product should be tested for residual antibody. The methods used and the permitted concentrations of antibody shall be approved by the national control authority.

Several national control authorities have drafted guidelines for the control of monoclonal antibody preparations used for the manufacture of biological products for human use.

7.3 Determination of HBsAg content

The HBsAg content of the purified preparation shall be determined by an appropriate method.

Tests that have been found suitable include radioimmunoassay, enzme-linked immunosorbent assay (ELISA), single radial immunodiffusion, and rocket electrophoresis in which the purified preparation is compared with a known standard. Analysis of the results by the parallel-line method has been found suitable for most of these techniques.

7.4 Test for antigenic identity (molecular and immunochemical identity)

A test shall be made for the molecular characteristics of the HBsAg gene product by polyacrylamide gel electrophoresis under reducing denaturing conditions.

In one country, the detection of pre-S antigen by SDS PAGE is required.

The g[illegible] products shall be shown to possess antigenic determinan[illegible] characteristic of HBsAg by means of tests with monoclonal antibodies or other antibodies of defined specificity directed against epitopes of HBsAg known to be relevant to the protective efficacy of the vaccine. Such tests shall be approved by the national control authority.

7.5 Test for sterility of purified surface antigen

Each batch shall be tested for sterility according to the requirements given in Part A, section 5 of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (2, p. 48).

7.6 Test for inactivating agents

If the HBsAg has been treated with formaldehyde and or other inactivating agents, then the material shall be tested for the presence of free formaldehyde and/or the other agents. The method used and the permitted concentration shall be approved by the national control authority.

8. Final bulk before addition of adjuvant (aqueous bulk)

The final aqueous bulk consists of one or more purified HBsAg batches. Only batches that have satisfied the requirements of sections 2–7 above shall be included in the final bulk. The national control authority shall determine the maximum number of batches that may be pooled.

8.1 Test for sterility

The final aqueous bulk shall be tested for bacterial and mycotic sterility according to the requirements given in Part A, section 5.2 of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (2, p. 49).

8.2 Test for HBsAg

The quantity of HBsAg as a proportion of the total protein in the final bulk shall be determined by an appropri[illegible] quantitative procedure. The protein load should be specified s[illegible] ensure that

the assay is of reasonable sensitivity. The lower limit of the ratio of HBsAg to total protein shall be approved by the national control authority. The final aqueous bulk should contain at least 90% of the total protein as HBsAg.

In one country, the amount of pre-S antigen is determined by means of a suitable quantitative assay.

8.3 Test for DNA

The amount of residual cell or plasmid DNA in each batch of vaccine should be determined by sensitive methods which must be validated and approved by the national control authority. Suitable tests for the homologous vector used in the production of the HBsAg should be included. Maximum acceptable levels of DNA per vaccine dose shall be approved by the national control authority.

The WHO Study Group on Biologicals concluded that the probability of risk associated with heterogeneous contaminating DNA in a product derived from a continuous cell line is negligible when the amount of such DNA is 100 pg or less in a single dose given parenterally (*4*).

9. Final bulk

9.1 Addition of adjuvant

The adjuvant and the concentration used shall be approved by the national control authority.

A preservative may need to be added at this stage.

9.2 Tests on final bulk

9.2.1 *Tests for sterility*

The final bulk shall be tested for bacterial and mycotic sterility according to the requirements given in Part A, section 5.2 of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*2*, p. 49).

9.2.2 *Tests for preservative*

The final bulk may be tested for the presence of preservative. The method used and the permitted concentration should be approved by the national control authority.

9.2.3 *Assay for adjuvant*

Each final lot may be assayed for the content of adjuvant. The method used and the permitted concentration should be approved by the national control authority. Where aluminium compounds are used, the concentration of aluminium should not be greater than 1.25 mg per single human dose.

10. Filling and containers

The requirements concerning filling and containers given in Part A, section 4 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 16) shall apply.

Care should be taken to ensure that the material of which the container is made does not adversely affect the HBsAg under the recommended storage conditions. Adjuvanted HBsAg vaccine must be stored at 5–8 °C.

11. Control of final lot

Samples shall be taken from each final lot for the tests described in the following sections.

11.1 Sterility test

Each final lot shall be tested for sterility according to the requirements given in Part A, section 5 of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*6*).

11.2 Innocuity tests

Each final lot shall be tested for innocuity by appropriate tests in mice and guinea-pigs, using parenteral injections. The tests shall be approved by the national control authority.

11.3 Test for pyrogenic substances

Each final lot shall be tested for pyrogenic substances. The test shall be approved by the national control authority.

11.4 Test for preservative

Each final lot shall be tested for the presence of preservative. The test used and the permitted concentration shall be approved by the national control authority.

11.5 Assay for adjuvant

Each final lot shall be assayed for the content of adjuvant. The method used and the permitted concentration shall be approved by the national control authority. Where aluminium compounds are used, the concentration of aluminium shall not be greater than 1.25 mg per single human dose.

11.6 Potency and identity tests

The vaccine shall be identified as envelope antigens of hepatitis B virus by appropriate methods. An appropriate quantitative test for antigen content and an immunogenicity assay shall be performed on samples representative of the final filling lots. The vaccine potency shall be compared with that of a reference preparation, and the national control authority shall determine the lower limit of potency.

A suitable quantitative extinction test in mice is as follows. Several groups of at least 20 suitable mice, five weeks of age, are tested. Each mouse is vaccinated intraperitoneally with the adjuvanted hepatitis B vaccine diluted in the adjuvant used in the vaccine; a different graded dose is used for each group of mice. Similar groups of mice are inoculated with the adjuvanted reference preparation. The reactivity of positive sera towards important epitopes on HBsAg should be verified by means of appropriate tests. Blood samples are taken from the mice after sufficient time has elapsed to permit the development of an adequate antibody response, and individual sera are assayed for antibodies to products of the envelope gene of hepatitis B virus by means of sensitive quantitative tests such as radioimmunoassay. The concentrations of vaccine tested should be selected to permit the calculation of 50% seroconversion to antibodies against HBsAg. The strain of mice used for this test must give a suitable dose response curve with the reference and test antigens. In any test, a calibrated reference preparation should be included and the potency of the vaccine expressed in terms of it.

12. Records

The requirements given in Part A, section 6 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 17) shall apply.

13. Samples

The requirements given in Part A, section 7 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 18) shall apply.

14. Labelling

The requirements given in Part A, section 8 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 18) shall apply, with the addition of the following directive.

The leaflet accompanying the package shall include the following information:

- the nature of the cells used to produce the antigen;
- the nature and amount of any preservative, adjuvant, or stabilizer present in the vaccine;
- the volume of one recommended human dose, the immunization schedules, and the recommended routes of administration (this information shall be given for newborn babies, children, adults, and immunosuppressed individuals, and shall be the same for a given vaccine for all regions of the world);
- the amount of total protein contained in one recommended human dose; and
- the amount of HBsAg protein contained in one recommended human dose.

15. Distribution and shipping

The requirements given in Part A, section [illegible] the revised Requirements for Biological Substances No. 1 ([illegible]al Require-

ments for Manufacturing Establishments and Control Laboratories (*1*, p. 18) shall apply.

In addition, the conditions of shipping shall be such as to ensure that the adjuvanted vaccine does not freeze.

Temperature indicators should be packaged with each vaccine shipment to show whether freezing occurs. If freezing has occurred, the vaccine should not be used.

16. Storage and expiry date

The requirements given in Part A, section 10 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 19) shall apply.

16.1 Storage conditions and stability

Before being distributed by the manufacturing establishment, or before being issued from a depot for the maintenance of reserves of vaccines, all vaccine in bulk form or in final containers shall be kept at 5–8 °C. The vaccine shall also be stored at this temperature after distribution or issue. The vaccine shall have been shown to maintain its potency for a period equal to that between the date of issue and the expiry date. During storage the vaccine shall not be frozen.

16.2 Expiry date

The expiry date shall be fixed with the approval of the national control authority and shall relate to the date of the last satisfactory potency test, i.e., the date on which the animals were inoculated with the vaccine.

PART B. NATIONAL CONTROL REQUIREMENTS

1. General

The general requirements for control laboratories contained in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 19) shall apply.

The national control authority shall:

approve the methods for producing HBsAg by recombinant DNA techniques;
approve the tests for HBsAg concentration and define its minimum value;
approve the methods used for purification;
approve the purity of the final product;
approve the tests for extraneous substances and total protein;
approve the tests for preservative and the agents used for purification and in other aspects of manufacture;
approve the tests used to determine whether the final product is free from abnormal toxicity;
approve the adjuvant assay and define the permitted concentration of adjuvant in the final product;
approve the types of animals used in the assay of potency; and
approve the data showing that the vaccine produces an adequate antibody response (titre, duration, and quality) in human beings.

The national control authority shall also provide national reference preparations for defining the activity of HBsAg in a given quantity of protein.

Before releasing a vaccine, the national control authority shall be satisfied that the results of all tests, including those on individual batches during the process of manufacture, are satisfactory and that consistency has been established.

2. Release and certification

A hepatitis B vaccine shall be released only if it satisfies Part A of these requirements.

A statement signed by the appropriate official of the national control laboratory shall be provided at the request of the manufacturing establishment and shall certify whether or not the final lot of vaccine in question meets all national requirements as well as Part A of these requirements. The certificate shall state the date of the last satisfactory HBsAg potency test, the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, a copy of the official national release document shall be attached.

The purpose of the certificate is to facilitate the exchange of hepatitis B vaccine between countries.

AUTHORS AND ACKNOWLEDGEMENTS

These Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques are based on four previous drafts; for the authors of the first three of these drafts and corresponding acknowledgements, please see WHO Technical Report Series, No. 760, 1987, Annex 6. For the fourth draft, acknowledgements are also due to Dr G.R.E. Swaniker, University of Papua New Guinea, Boroko, Papua New Guinea. The fourth draft was prepared in January 1988 by Dr V. Grachev, Scientist, and Dr D. Magrath, Chief, Biologicals, WHO, Geneva, Switzerland.

REFERENCES

1. WHO Technical Report Series, No. 323, 1966.
2. WHO Technical Report Series, No. 530, 1973.
3. WHO Technical Report Series, No. 745, 1987, Annex 3.
4. WHO Technical Report Series, No. 747, 1987 (*Acceptability of cell substrates for production of biologicals*: report of a WHO Study Group).

Appendix

SUMMARY PROTOCOL FOR PRODUCTION AND TESTING OF HEPATITIS B VACCINES MADE BY RECOMBINANT DNA TECHNIQUES

Based on Requirements for Biological Substances No. 45 (Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques) (WHO Technical Report Series, No. 786, 1989, Annex 2)

Identification of final lot

Name and address of manufacturer _____

International name and proprietary name of vaccine _____

Lot number of final product _____

Date of manufacture of final lot _____

Date of filling containers _____

Number and nature of containers (ampoules or vials) _____

Date of last potency test _____

Number of doses in each container _____

Volume of single dose _____

Expiry date _____

3.[1] Validation and control of manufacturing procedures

3.1 Strategy for cloning and expressing the gene[2]

Details of:

(*a*) potential retrovirus-like particles in and genetic markers of the host cell _____

(*b*) construction, genetics, and structure of the expression vector _____

(*c*) origin and identity of the gene that was cloned _____

[1] Numbers refer to the corresponding numbered sections in the text of the Requirements.

[2] Section 3.1 does not necessarily need to accompany each [illegible] of vaccine. If necessary, it should be typed on a separate sheet.

Description, in detail, of the measures taken to promote and control the expression of the cloned gene in the host cells

Information on the stability of the expression system during storage and cell propagation to the production level

3.2 Biochemical characterization of recombinant vector

Nucleotide sequence of the surface-antigen gene insert

Restriction-endonuclease mapping of the recombinant vector, where relevant

3.3 Purification procedures

Methods used to purify the HBsAg

Results

Origins and characteristics of antibodies, if used

3.4 Characterization of gene products (HBsAg)

3.4.1 *Particle characterization*

Morphological characteristics of the particles
- Results of examination by electron microscopy

Degree of aggregation
- Method
- Results

Quantity of protein
- Method
- Results

Quantity of lipid
- Method
- Results

Quantity of nucleic acid
- Method
- Results

Quantity of carbohydrate
- Method
- Results

3.4.2 *Determination of protein content*

Method

Results

3.4.3 *Protein characterization*

Ultraviolet absorption spectrum

Protein composition
- Method
- Results

Identity of the protein by partial N-terminal and C-terminal analysis
- Method
- Results

3.4.4 *Antibodies induced by the vaccine in human beings*

Titres

Characteristics

3.4.5 *Consistency of yield*

Between runs

During individual runs

4. Manufacturer's working cell bank (MWCB)

4.1 Origin of cell banks

Date of establishment of cell banks

Quantity of cells stored

Passage level of the MWCB

Storage conditions

4.2 Characteristics of cell seed lot

Purity and homogeneity of the cell seed lot
- Method
- Results

Genetic characteristics of the cell seed lot
- Method
- Results

Purity of recombinant DNA vector
- Method
- Results

Genetic characteristics of recombinant DNA vector
- Methods
- Results

Nucleotide sequence of HBsAg gene insert

Peptide map of gene product (HBsAg)

Terminal amino acid sequence of gene product

4.3 Phenotypic indicators of purity and genetic consistency of recombinant cultures

Tests on cells after recovery from preserved state
- Methods
- Results

Identity of cell seed
- Method
- Results

4.4 Sterility tests

Results

4.5 Additional tests for MWCB of mammalian cells

Tests for adventitious agents

Tests in suckling mice
- No. and weight of animals
- Quantity injected (intramuscular)
- Observation period
- Results of injection (pathological changes, no. survived, etc.)

Tests in adult mice
- No. and weight of animals
- Quantity injected (intramuscular)
- Observation period
- Results of injection (pathological changes, no. survived, etc.)

Tests in guinea-pigs
- No. and weight of animals
- Quantity injected (intramuscular)
- Observation period
- Results of injection (pathological changes, no. survived, etc.)

Tests in rabbits
- No. and weight of animals
- Quantity injected (intramuscular)
- Observation period
- Results of injection (pathological changes, no. survived, etc.)

Tests in embryonated chicken eggs
- No. and age of eggs
- Quantity of cells injected
- Observation period
- Results of injection (pathological changes, no. survived, etc.)

Tests in adult mice
- No. and weight of animals
- Quantity injected (intracerebral)
- Observation period
- Results of injection (pathological changes, no. survived, etc.)

Tests for bacteria, fungi, and mycoplasmas
- Media used
- Results

Morphological tests
- Examination by light microscopy
- Examination by electron microscopy (if this test has not been done on the cell seed)

Tests on cell cultures
- Percentage of MWCB tested
- Tests performed on cell system
- Period of observation of cell cultures
- Results

Tests for haemagglutinating viruses
- Percentage of MWCB tested
- Method
- Results

Tests for retroviruses

Percentage of MWCB tested

Method

Results

Tests for tumorigenicity

Method

Results

Identity tests

Method

Results

5. Production precautions

5.1 Production cell cultures

5.2 Culture conditions for production cell cultures

Cell doubling time

Number of subcultures

Duration of permitted subculture

Incubation temperature

Sterility test

Media

Results

5.3 Cell culture medium

6. Single harvests

6.1 Sterility test performed at the end of the culture

Media

Results

6.2 Consistency of yield of HBsAg

Method

Results

6.3 Plasmid retention

Method

Results (proportion of cells still possessing the plasmid at the end of the culture)

7. Purification

Methods

Results

7.1 Protein and other components of the vaccine

Protein content

Methods

Results

Lipid content

Methods

Results

Carbohydrate content (as compared with total proteins)

Methods

Results (%)

Residual amounts of animal serum

Methods

Results

7.2 Tests for agents used during purification or other phases of manufacture

Methods

Results

7.2.1 *Monoclonal antibodies*[1]

Methods

Results

7.3 Determination of HBsAg content

Methods

Results

7.4 Test for antigenic identity

Methods

Results

[1] Only if immunoadsorption was used in the purification process.

7.5 Test for sterility of purified surface antigen

Methods

Results

7.6 Test for inactivating agents

Methods

Results

8. Final bulk before addition of adjuvant (aqueous bulk)

Volume of purified bulk

Number of batches pooled

Nature and volume of diluent added

8.1 Test for sterility

Method

Results

8.2 Test for HBsAg

Quantity of HBsAg
Method

Results

Ratio of HBsAg to total protein
Method

Results

8.3 Test for DNA

Method

Results

9. Final bulk

9.1 Addition of adjuvant

Volume of bulk

Nature and volume of adjuvant added and final concentration

Nature and volume of preservative added and final concentration

Comp[illegible] of final bulk (mixing of all inpu[illegible]s) and identification number

9.2 Tests on final bulk

9.2.1 *Tests for sterility*

Method

Results

9.2.2 *Tests for preservatives*

Method

Results

9.2.3 *Assay for adjuvant*

Method

Results

10. Filling and containers

Date of filling

Quantity of containers

Volume of vaccine per container

Control for defective containers

11. Control of final lot

11.1 Sterility test

Method

Results

11.2 Innocuity tests

In mice

No. and weight of animals

Quantity injected

Observation period

Results

In guinea-pigs

No. and weight of animals

Quantity injected

Observation period

Results

11.3 Test for pyrogenic substances

Method _____

Results _____

11.4 Test for preservative

Method _____

Results _____

11.5 Assay for adjuvant

Method _____

Results _____

11.6 Potency and identity tests

Identification of vaccine as HBsAg
- Method _____
- Results _____

Antigen content
- Method _____
- Results _____

Immunogenicity assay
- Method _____
- Results _____

Vaccine potency
- Method _____
- Results _____

Internal certification

Certification by person taking overall responsibility for production of the vaccine

I certify that lot no. ..... of the vaccine satisfies Part A of the Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques published by WHO.

Signature _____

Name (typed) _____

Date _____

The protocol must be accompanied by a sample of the label and a copy of the leaflet.

Release certification by the national control authority

Whenever hepatitis B vaccines produced by recombinant DNA techniques are to be exported, they should be accompanied by a release certificate from the national control authority.

Sample release certificate

I hereby certify that batch no. ..... of hepatitis B vaccine produced by (*name of producer*) by recombinant DNA techniques meets all national requirements as well as Part A of the Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques published by WHO.

The date of the last satisfactory potency test carried out by the national control laboratory is .....

The final lot has been released by us under no. .....

The number appearing on the label of the containers is .....

Signature _____

Name (typed) _____

Date _____

G. YELLOW FEVER VACCINE

Documents provided in this section include:

1. Sample Procurement Requirements Yellow Fever Vaccine 508
2. Yellow Fever Vaccine, *USP 23 - NF 18*, 1994 515
3. Laboratories Approved by WHO for the Production of Yellow Fever Vaccine, WHO *Technical Report Series* No. 872, 1998 516
4. Requirements for Yellow Fever Vaccine (Requirements for Biological Substances No. 3, revised 1995), WHO *Technical Report Series* No. 872, 1998 517

SAMPLE
PROCUREMENT REQUIREMENTS

YELLOW FEVER VACCINE

For Paediatric Use

A. General Description of Intended Use

This vaccine is intended for use in a public-sector health programme for immunization of children over six months of age in _____ [*place*] beginning on _____ [*date*].

The total requirement for this solicitation is _____ [*quantity*] doses. _____ [*name*] expects to procure additional quantities of vaccine annually for use in this project beginning in _____ [*month, year*] and subsequent years as follows: _____ [*quantity*], _____ [*quantity*] and _____ [*quantity*].

B. Specific Requirements

Item: Yellow Fever Vaccine for intramuscular injection, live, attenuated, freeze-dried with sterile diluent packed separately.

Bacterially sterile preparation of live, attenuated yellow fever virus grown in a suitable cell culture; tested for neurovirulence in monkeys, safety and immunogenicity; free from all demonstrable viable microbial agents except unavoidable bacteriophage; and found suitable for human immunization.

Each dose shall contain not less than ____ [*specify*] infective units of viral vaccine strain 17D and not more than ___gm _____residual antibiotic [*specify the antibiotic*].

	The vaccine shall meet _____ [*specify*] requirements for Yellow Fever Vaccine (Live) (WHO Requirements for Biological Substances No. 3, revised 1995 or equivalent requirements of the USFDA as detailed in USP 23 or another fully equivalent set of biological requirements for Yellow Fever Vaccine). *Editors' note: It is recognized that equivalence does not mean identical, only that both provide the same minimum level of potency, safety and efficacy.* The vaccine shall be currently registered in the country of manufacture and shall meet all requirements of the licensing authority of the country of manufacture. The vaccine also shall be currently registered in the country of use and shall meet all requirements of the licensing authority of the country of use.
Quantity:	_____ doses from one production lot.
Dosage Size:	0.5 ml.
Dose Package:	Single-dose vials, multi-dose vials with diluent in corresponding quantity; container must protect vaccine from exposure to light.
Filling Volume:	15 % overfill applicable only to the diluent.
Storage Temperature:	+2 to +8 degrees C; may be frozen.
Shelf Life:	The shelf life of the product provided under this solicitation will be at least 12 months from date of manufacture when stored between +2 and +8 degrees C. The Supplier will provide manufacturer's stability test data substantiating this 12-month shelf life in the proposed vial.

	At the time of inspection or acceptance for delivery to the country of destination, no more than six months shall have expired since the date of manufacture (or date of the beginning of the last satisfactory test for potency) shown on the Certificate of Analysis.
Stability:	Stable for a minimum of two years after production at +2 to +8 degrees C. Manufacturer to specify additional thermal stability characteristics.
Labelling:	The label on each vial shall conform to the requirements of the country of use and shall appear in the language of _____. All labelling shall withstand immersion in water and remain intact. All labels shall state the name of the vaccine, name of the manufacturer, place of manufacture, lot number, composition, concentration, dose and mode for administration, expiry date, storage temperature and any other marking that is appropriate.
Optional Labelling:	If vaccine is destined for use in public-sector immunization programmes, the statement "Not for Sale; For Use Only in Government-Approved Immunization Programmes" shall appear on each label in the language of the purchasing country.
Closures:	Vaccine vials shall be fitted with closures that conform to ISO standard 8362-2.
Printed Materials:	Two (2) information sheets, printed in _____ [language], shall be included in each box of ___ vials.

C. Quality Assurance

Compliance: The Supplier shall guarantee that the products as packed for shipment (a) comply with all provisions of the specification and related documents; (b) meet internationally recognized standards for safety, efficacy and quality; (c) are fit for the purposes made known to the Seller and (d) are free from defects in workmanship and in materials.

Evidence: The Supplier shall provide objective evidence, acceptable to the Purchaser, of the satisfaction of the requirements of this document for which no specific inspection has been mentioned.

The Supplier shall provide a copy of the manufacturing record and procedures to the Purchaser for each lot intended for shipment.

The Supplier shall provide a copy of the batch record and all quality assurance documentation to the Purchaser for each lot being supplied.

The Supplier shall provide a copy of the Certificate of Analysis to the Purchaser for each lot intended for shipment.

The Supplier shall provide to the Purchaser a copy of the approval of each source material, constituent material and component for each lot intended for shipment.

The Supplier shall retain a sample of twenty (20) vials from each lot shipped for two years beyond the printed expiration date.

Chemical, physical and microbiological test data for in-process and finished product testing must be on record for each lot shipped and must be

available to Purchaser's representatives when requested.

Inspection: The Purchaser may inspect and sample, or cause to be sampled, the product at the Supplier's factory and/or warehouse at a mutually agreeable time prior to shipment of the product.

Testing: The Purchaser may cause independent laboratory testing to be performed as deemed necessary to assure that the goods conform to prescribed requirements. Said testing laboratory shall be of the Purchaser's choice and suitably equipped and qualified to conduct quality assurance tests on biological products.

D. Packing

Storage: Supplier shall state storage volume occupied per dose of vaccine (storage volume includes the vaccine vial, packet containing the vaccine vial and any intermediate packaging).

Inner Boxes: _____ [*number*] individual vials shall be supplied in sturdy white cardboard boxes outfitted with individual segments for protecting and separating each vial. Vaccine and diluent will be packaged to correspond; i.e., one box of diluent will constitute one box of vaccine with no deficit or excess.

Cold Chain Monitor Cards: One cold chain monitor card (description attached)[31] in _____ language shall be packed with each ___ doses of vaccine.

Overpacking: Boxes shall be overpacked so that the vaccine remains refrigerated below +8 degrees C. The overpacking must be suitable for export

[31] See example in Supplement VI.G.1.a., page 783.

handling in accordance with WHO/EPI Guidelines on the International Packaging and Shipping of Vaccines. It must have adequate insulation and sufficient refrigerant to ensure that the warmest storage temperature of the vaccine does not rise above +8 degrees C in continuous outside ambient temperature of +43 degrees C, or fall below +2 degrees C in continuous outside ambient temperature of -20 degrees C during transit and for a period of at least 48 hours after arrival at the airport of destination.

Additional cushioning shall be provided, sufficient to protect the vials from breakage during transit and handling.

Exterior Shipping Cartons: Product and printed materials, packaged as specified above, shall be packed in weather-resistant, triple-wall corrugated fibreboard cartons with a bursting test strength of not less than 1,900 kPa.

The overall dimensions of the exterior shipping cartons should be such that the product does not become damaged during transportation and storage.

E. Markings

All containers and invoices must bear the name of the vaccine, expiry dates of the vaccine and appropriate storage temperature.

Inner Boxes: The inner boxes containing vaccine vials shall be marked with the following information in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Manufacturer's name and registered address

- Manufacturer's national registration number
- Lot or batch number
- Composition and concentration
- Number of vials contained in box
- Expiration date (month and year)
- Instructions for storage and handling†
- Place of manufacture (Made in _____)

Exterior Shipping Cartons: The following information shall be stencilled or labelled on the exterior shipping cartons on two opposing sides in bold letters at least __ mm high with waterproof ink in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Lot or batch number
- Expiration date (month and year)
- Manufacturer's name and registered address
- Manufacturer's national registration number
- Destination country license or registration number
- Consignee's address and emergency phone number
- Destination airport
- Contract number
- Number of vials contained in the carton
- Gross weight of each carton (in kg)
- Carton #___ of ___
- Instructions for storage and handling†
- Place of manufacture (Made in _____)

F. Documentation

Supplier shall provide to purchaser a copy of the batch record, including all quality assurance documentation for the vaccine being supplied.

† To be provided by Purchaser.

Yellow Fever Vaccine

» Yellow Fever Vaccine conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is the attenuated strain that has been tested in monkeys for viscerotropism, immunogenicity, and neurotropism, of living yellow fever virus selected for high antigenic activity and safety. It is prepared by the culturing of the virus in the living embryos of chicken eggs, from which a suspension is prepared, processed with aseptic precautions, and finally dried from the frozen state. It meets the requirements of the specific mouse potency test in titer of mouse LD_{50} (quantity of virus estimated to produce fatal specific encephalitis in 50% of the mice) or the requirements for plaque-forming units in a suitable cell culture system, such as a Vero cell system for which the relationship between mouse LD_{50} and plaque-forming units has been established, in which cell monolayers in 35 mm petri dishes are inoculated for a specified time with dilutions of Vaccine, after which the dilutions are replaced with 0.5% agarose-containing medium. Following adsorption and incubation for five days an overlay is added of the 0.5% agarose medium containing 1:50,000 neutral red and the plaques are counted on the sixth day following inoculation. It is sterile, and contains no human serum and no antimicrobial agent.

Yellow Fever Vaccine is constituted, with Sodium Chloride Injection containing no antimicrobial agent, just prior to use.

Packaging and storage—Preserve in nitrogen-filled, flame-sealed ampuls or suitable stoppered vials at a temperature preferably below 0° but never above 5°, throughout the dating period. Preserve it during shipment in a suitable container adequately packed in solid carbon dioxide, or provided with other means of refrigeration, so as to insure a temperature constantly below 0°.

Expiration date—The expiration date is not later than 1 year after date of issue from manufacturer's cold storage (−20°, 1 year).

Labeling—Label it to state that it is to be well shaken before use and that the constituted vaccine is to be used entirely or discarded within 1 hour of opening the container. Label it also to state that it is the living yellow fever vaccine virus prepared from chicken embryos and that the dose is the same for persons of all ages, but that it is not recommended for infants under six months of age.

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Annex 1
Laboratories approved by WHO for the production of yellow fever vaccine

This list supersedes Annex 5 in WHO Technical Report Series, No. 822, 1992.

Bio-Manguinhos
Oswaldo Cruz Foundation
Rio de Janeiro
Brazil

Connaught Laboratories Inc.
Swiftwater, PA
USA

Evans Medical
Speke
Liverpool
England

Institute of Poliomyelitis and Viral Encephalitides
Moscow
Russian Federation

Pasteur Institute of Dakar
Dakar
Senegal

Pasteur Mérieux Sera and Vaccines
Marcy l'Etoile
France

Robert Koch Institute
Berlin
Germany

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Annex 2
Requirements for yellow fever vaccine
(Requirements for Biological Substances No. 3, revised 1995)

Introduction 31

General considerations 32

Part A. Manufacturing requirements 34
A.1 Definitions 34
A.2 Certification of the substrain of 17D virus for use in vaccine production 35
A.3 General manufacturing requirements 35
A.4 Production control 36
A.5 Filling and containers 42
A.6 Control tests on final lot 43
A.7 Records 45
A.8 Samples 45
A.9 Labelling 45
A.10 Distribution and shipping 46
A.11 Storage and expiry date 46

Part B. National control requirements 47
B.1 General 47
B.2 Release and certification 47

Authors 47

Acknowledgements 48

References 48

Appendix 1
Example, for guidance, of a summary protocol for the production and testing of yellow fever vaccine 51

Appendix 2
Model certificate for the release of yellow fever vaccine by national control authorities 66

Appendix 3
Techniques for the potency evaluation of yellow fever vaccine 67

Introduction

Yellow fever is a viral haemorrhagic fever that, each year, strikes an estimated 200000 persons worldwide and causes an estimated 30000 deaths. The case-fatality rate can reach as high as 20% to 80% in severely ill patients who are hospitalized (*1*). However, the overall case-fatality rate is lower.

The yellow fever virus is small (35–45 nm) and consists of a core containing single-stranded RNA surrounded by a lipoprotein envelope. The yellow fever virus genome has been completely sequenced and has been found to contain 10862 nucleotides (*2*). The lipoprotein envelope contains a single glycoprotein with type- and group-specific antigenic determinants.

Yellow fever was first distinguished from malaria, dengue and other tropical diseases during a series of epidemics between 1647 and 1649 in Barbados, Cuba, Guadeloupe and Mexico. Recognized yellow fever epidemics have periodically raged since then in parts of the Americas and Africa. In 1900, a commission headed by the American physician Walter Reed confirmed that the disease was transmitted from human to human by the mosquito *Aedes aegypti*, a hypothesis proposed earlier by the Cuban physician Carlos Finlay in 1881 (*3*).

There are two epidemiological patterns of yellow fever transmission: the urban cycle and the forest cycle (also known as the jungle or sylvan cycle). The two patterns of transmission lead to a clinically identical disease, since the same virus is responsible. In the Americas, the yellow fever virus circulates today by means of an endemic, forest cycle that results in up to several hundred reports of infection in non-immune forest workers per year. In Africa, the virus circulates by means of both urban and forest cycles and periodically breaks out of its endemic pattern to infect large numbers of non-immune persons in the course of major epidemics.

Two live attenuated yellow fever vaccines were developed in the 1930s; the French neurotropic vaccine (FNV), prepared from human virus passaged in mouse brain, and the "17D" vaccine, prepared from the 17D strain of human virus passaged in embryonated chicken eggs.

Today, 17D vaccine is the only type of yellow fever vaccine produced, as the use of FNV was found to be associated with a high incidence of encephalitic reactions in children (*4*). The 17D vaccine was developed by Theiler and Smith in 1937.

More than 200 million doses of 17D vaccine have been administered, and it has proved to be one of the safest vaccines ever developed. This

vaccine has also been shown to be very effective for the control of yellow fever during outbreaks and between epidemics. In 1990, the Global Advisory Group of the Expanded Programme on Immunization recommended that all countries at risk of yellow fever should incorporate the vaccine in their routine immunization programmes. As of 1992, 16 of 33 such countries in Africa included yellow fever vaccine in their national immunization programmes.

Requirements for yellow fever vaccine (Requirements for Biological Substances No. 3) were first formulated by a WHO Study Group in 1958 (*5*). The Requirements embodied recommendations made by the first WHO Expert Committee on Yellow Fever Vaccine (*6*), and they applied to vaccine prepared from a suitable strain of yellow fever virus. The vaccine was intended to be given by subcutaneous injection. Conformity with these Requirements has been the basis for WHO approval of yellow fever vaccine used for vaccination and revaccination against yellow fever in connection with certification for the purposes of international travel (*7*), and such approval has been given only to vaccine prepared using seed derived from the 17D strain of yellow fever virus. The Requirements have been used also by national authorities for the control and approval of yellow fever vaccine used in national immunization programmes. In 1969 the twenty-second meeting of the WHO Expert Committee on Biological Standardization agreed that developments in virology in general and in the manufacture and control of yellow fever vaccine in particular warranted a revision of the existing Requirements, with due consideration of both their national and international application (*8*). In 1975 the twenty-seventh meeting of the WHO Expert Committee on Biological Standardization formulated revised Requirements for Yellow Fever Vaccine (*9*). Much experience has been gained with the preparation of yellow fever vaccine since 1975, and a further revision of the Requirements has therefore been prepared.

General considerations

Yellow fever vaccine has been proved to be safe. Only 19 cases of encephalitis temporally associated with yellow fever vaccine prepared from the 17D virus strain have been reported, mostly in children, despite over 200 million doses having been delivered since 1945. Since all but six of these occurred in children immunized at 4 months of age or younger, a review by a panel of experts recommended that yellow fever vaccine should not be routinely given before 6 months of age (*10*, *11*).

Formerly, there were some problems associated with under- or over-attenuation of the 17D strain on passage. These problems were resolved by the establishment of a virus seed-lot system. There are a number of substrains in use today for the manufacture of 17D vaccine (*12*). A detailed study using oligonucleotide mapping and monoclonal antibody analysis of the genetic and antigenic variation among these substrains showed an RNA-sequence homology of 98–100%, which demonstrates a high degree of genetic and antigenic similarity among substrains (*13*).

Although all countries now use seed virus free from avian leukosis viruses (ALV), some permit the production of vaccine in embryonated chicken eggs not proved to be free from ALV. This practice may be justified where the cost and difficulty of obtaining ALV-free embryonated eggs would restrict the availability of yellow fever vaccine, particularly in view of epidemiological evidence that shows no relationship between yellow fever vaccination and leukaemia, lymphoma or other cancers (*14*). For this reason, the revised Requirements do not specify ALV-free eggs.

It is important to ensure that new pools of virus seed, whether master or working seed, have levels of neurotropism and viscerotropism within safe limits. The relevant safety test, performed on monkeys, has therefore been retained in the revised Requirements.

One important amendment to the Requirements concerns the inclusion of a test of the thermal stability of the vaccine as a requirement instead of as a recommendation (*15*). Each batch of yellow fever vaccine should accordingly be evaluated by testing for potency before and after storage at 37 °C for 2 weeks.

The National Institute for Biological Standards and Control, Potters Bar, distributes the International Reference Preparation of Anti-Yellow-Fever Serum. Such a preparation is needed as a basis for comparison of antibody responses in the monkey neurovirulence test. WHO can provide primary seed virus for the production of yellow fever vaccine (*16*).

Each of the following sections constitutes a recommendation. The parts of each section that are printed in normal type have been written in the form of requirements so that, if a national control authority so desires, they may be adopted as they stand as definitive national requirements. The parts of each section printed in small type are comments or recommendations for guidance. To facilitate the licensing and international distribution of vaccines made in accordance with these Requirements, a summary protocol for recording the results of tests is provided as Appendix 1. A model summary protocol

for the routine lot release of virus vaccines is provided as Annex 4 of this volume of the WHO Technical Report Series.

Should individual countries wish to adopt these Requirements as the basis for their national regulations concerning yellow fever vaccine, it is recommended that modifications be made only on condition that the modified requirements ensure at least an equal degree of safety and potency of the vaccine. It is desirable that the World Health Organization should be informed of any such changes.

Part A. Manufacturing requirements

A.1 Definitions

A.1.1 *International name and proper name*

The international name shall be "Vaccinum febris flavae". The proper name shall be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

A.1.2 *Descriptive definition*

Yellow fever vaccine shall consist of a freeze-dried preparation of viable, attenuated yellow fever virus (*Flavivirus hominis*). The preparation shall satisfy all the requirements formulated below.

A.1.3 *International reference materials*

An International Reference Preparation of Anti-Yellow-Fever Serum is available from the National Institute for Biological Standards and Control, Potters Bar, England. A nonimmune control serum is also available. Samples are distributed free of charge, on request, to national control laboratories.

A.1.4 *Terminology*

The following definitions are given for the purpose of these Requirements only.

Primary or *master seed lot.* A quantity of virus suspension that has been processed in a single production run and has a uniform composition. It is used for the preparation of secondary (working) seed lots.

Secondary or *working seed lot.* A quantity of virus suspension that has been processed in a single production run, and is uniform with respect to composition, fully characterized and only one passage from a master seed lot. Material drawn from secondary seed lots is used for inoculating embryonated eggs in the preparation of vaccine.

Single harvest. A quantity of virus suspension, derived from tissues of the same origin that were inoculated with the same working seed lot, that has been collected and processed in a single production run.

Final bulk. The material prepared from one or more single harvests in the vessel from which the final containers are filled.

Filling or *final lot.* A collection of sealed final containers of finished vaccine that are homogeneous with respect to the risk of contamination during filling and freeze-drying. All the final containers must, therefore, have been filled from a single vessel of final bulk in one working session and lyophilized under standardized conditions in a common chamber.

Plaque-forming unit (PFU). The smallest quantity of a virus suspension that will produce a plaque in monolayer cell cultures.

Median mouse lethal dose (mouse LD_{50}). The quantity of a virus suspension that will kill 50% of the mice injected with it.

A.2 Certification of the substrain of 17D virus for use in vaccine production

The seed lot of the substrain of 17D yellow fever virus used in the production of yellow fever vaccine shall be identified by historical records that include information on the origin of the substrain, its method of attenuation and the passage level at which attenuation and immunogenicity were demonstrated by clinical evaluation.

The master and working seed lots of yellow fever virus used in the production of vaccine shall have been shown to be safe and immunogenic by appropriate laboratory tests (see section A.4 of these Requirements), and the master seed lot shall be shown to be immunogenic by clinical trials in susceptible humans. Only seed lots that are approved by the national control authority shall be used.

Seed lots that have been certified previously can be used.

A suitable primary seed lot (213–77) is available from WHO (*16*).

A.3 General manufacturing requirements

The general manufacturing requirements contained in Good Manufacturing Practices for Pharmaceutical (*17*) and Biological (*18*) Products shall apply to establishments manufacturing yellow fever vaccine, with the following additions: those staff directly involved with the production and testing of yellow fever vaccine shall be shown to be immune to yellow fever.

A.4 Production control

A.4.1 *Control of source materials*

A.4.1.1 Tissues for virus production

Virus for the preparation of master and working seed lots shall be grown in the tissue of chicken embryos obtained from a closed, specific-pathogen-free, healthy flock. This flock shall be monitored at regular intervals for agents pathogenic to birds. However, the national control authority may vary these requirements where the cost and difficulty of obtaining such tissue may restrict the availability of the vaccine and where the vaccine produced has been demonstrated to be both safe and effective.

Examples of agents that it may be necessary to monitor include *Mycobacterium avium*, fowl pox virus, ALV and other avian retroviruses, Newcastle disease virus and other avian parainfluenza viruses, avian encephalomyelitis virus, infectious laryngotracheitis virus, avian reticuloendotheliosis virus, Marek's disease virus, infectious bursal disease virus, *Haemophilus paragallinarum*, *Salmonella gallinarum*, *Salmonella pullorum*, *Mycoplasma gallisepticum* and *Mycoplasma synoviae*.

In some countries, all birds are bled when a colony is established, and thereafter 5% of the birds are bled each month. The resulting serum samples are screened for antibodies to the relevant pathogens. Any bird that dies is investigated to determine the cause of death.

Virus for the preparation of all vaccine production lots shall be grown in the tissue of chicken embryos obtained from a healthy flock. Monitoring of the flock or embryos shall include at least tests for exclusion of infection by *Salmonella* spp., *Mycobacterium avium* and fowl pox virus. The flock must not have been vaccinated with live Newcastle disease virus vaccine.

In some countries, the national control authority requires that the tissue used for production of vaccine should be shown by suitable tests to be free from ALV.

It is recommended that eggs should be obtained from young birds.

A.4.1.2 Seed-lot system

The production of vaccine shall be based on the master- and working-seed-lot system. All seed lots shall be stored at a temperature of –60 °C or below. Primary and secondary seed lots shall not contain any human protein or added serum or antibiotics.

In some laboratories, the master and working seed lots are stored in more than one location.

The master and working seed lots shall be free from ALV, mycoplasmas or other adventitious agents as shown by suitable tests (A.4.1.1 and A.4.1.5).

The inoculum for infecting tissues used in the production of vaccine shall be a working seed lot without intervening passage, in order to ensure that no vaccine shall be manufactured that is more than one passage removed from a seed lot that has passed all safety tests.

A.4.1.3 Monkey safety test

New master and working seed lots shall be tested for viscerotropism, immunogenicity and neurotropism in a group of 10 test monkeys. For the neurotropism test, the test monkeys inoculated with the virus seed lot shall be compared with a similar group of 10 monkeys injected with a reference virus. The reference virus shall be approved by the national control authority.

The monkeys shall be *Macaca mulatta* (i.e. rhesus monkeys) or *Macaca fascicularis* (i.e. cynomolgus monkeys) and shall have been demonstrated to be non-immune to yellow fever immediately prior to injection of the seed virus. They shall be healthy and shall not have been previously subjected to intracerebral or intraspinal inoculation. Furthermore, they shall not have been inoculated by any route with neurotropic viruses or antigens related to yellow fever.

The test dose shall consist of 0.25 ml containing the equivalent of not less than 5000 and not more than 50 000 mouse LD_{50}, as shown by a titration conducted by the method described in Appendix 3. The test dose shall be injected into one frontal lobe of each monkey under anaesthetic, and the monkeys shall be observed for a minimum of 30 days.

Viscerotropism test. The criterion of viscerotropism (indicated by the amount of circulating virus) shall be fulfilled as follows. Sera obtained from each of the test monkeys on the second, fourth and sixth days after injection of the test dose shall be inoculated at dilutions of 1:10, 1:100 and 1:1000 into at least 4 cell-culture vessels (or intracerebrally in 0.03 ml aliquots into at least 6 mice) per dilution, as specified in Appendix 3. In no case shall 0.03 ml of serum contain more than 500 mouse LD_{50} or the equivalent in PFU (see section A.6.2) and in no more than one case shall 0.03 ml of serum contain more than the equivalent of 100 mouse LD_{50} (appropriate techniques for potency testing are given in Appendix 3).

Immunogenicity test. The criterion of sufficient virus-neutralizing antibody in the sera (immunogenicity) shall be fulfilled as follows. At

least 90% of the test monkeys shall be shown to have become immune within 30 days following injection of the test dose, as determined by examining their sera in the test for neutralization of yellow fever virus described below.

In some countries, it has been shown that, at low dilutions, some sera contain non-specific inhibitors that interfere with this test. The national control authority may require sera to be treated to remove such substances.

Dilutions of 1:10, 1:40 and 1:160 of serum from each test monkey shall be mixed with an equal volume of strain 17D vaccine virus at a dilution that has been shown to yield an optimum number of plaques when assayed according to one of the cell-culture methods given in Appendix 3. These serum–virus mixtures shall be incubated in a water bath at 37 °C for 1 hour and then chilled in an ice–water bath before inoculation of 0.2 ml aliquots of each mixture into each of 4 separate cell-culture vessels. The vessels shall be handled according to one of the cell-culture techniques described in Appendix 3. In addition, 10 vessels shall be similarly inoculated with virus as above and an equal volume of a 1:10 dilution of monkey serum known to contain no neutralizing antibodies to yellow fever virus. At the end of the observation period, the mean number of plaques in the vessels receiving virus and non-immune serum shall be compared with the mean number of plaques in the vessels receiving virus and serum from test monkeys. For the immunogenicity test to be satisfied, serum at the 1:10 dilution from no more than 10% of the test monkeys shall fail to reduce the mean number of plaques by 50% as compared with the vessels containing non-immune serum.

Neurotropism test. Monkeys in the test group shall be compared with 10 monkeys injected with the reference virus with respect to both the clinical evidence of encephalitis and the severity of histological lesions of the nervous system (*19*, *20*).

The onset and duration of the febrile reaction should not differ between monkeys injected with the test or reference virus.

The monkeys shall be examined daily for 30 days by personnel familiar with the clinical signs of encephalitis in primates.

If necessary, the monkeys may be removed from their cages and examined for signs of motor weakness or spasticity as described elsewhere (*20*).

Signs of encephalitis, such as paresis, incoordination, lethargy, tremors or spasticity, shall be assigned numerical values for severity by the following grading method. Each day each monkey shall be given a numerical score based on the scale:

1: rough coat, not eating;
2: high-pitched voice, inactive, slow moving;

3: shaky movements, tremors, incoordination, limb weakness;
4: inability to stand, limb paralysis or death.

A monkey that dies receives the score "4" from the day of death until day 30.

The clinical score for a monkey is the average of its daily scores; the clinical score for a group is the arithmetic mean of the individual scores. For the clinical criterion of the neurotropism test to be satisfied, the clinical score of the monkeys injected with the virus being tested shall not exceed the clinical score of the monkeys injected with the reference virus.

The cervical and lumbar enlargements of the spinal cord and specific structures at five levels of the brain shall be examined (*20*) (see Appendix 1). The cervical and lumbar enlargements shall each be divided equally into six blocks. The blocks shall be dehydrated and embedded in paraffin wax; 15-µm sections shall be cut and stained with gallocyanin. One section, consisting of two hemisections, shall be cut from each block.

Tissue blocks 3–4 mm thick shall be taken from the brain by making the following frontal cuts:

Block I: the corpus striatum at the level of the optic chiasma;
Block II: the thalamus at the level of the mamillary bodies;
Block III: the mesencephalon at the level of the superior colliculi;
Block IV: the pons and cerebellum at the level of the superior olives;
Block V: the medulla oblongata at the mid-level of the inferior olives.

These blocks shall be dehydrated and embedded in paraffin wax and 15-µm sections shall be cut and stained with gallocyanin. A single section, consisting of two hemisections, shall be cut from each block.

Sections shall be examined microscopically and numerical scores given to each hemisection of the lumbar and cervical cord enlargements and to each anatomical structure (see Appendix 1) within each hemisection of the brain blocks according to the following grading system:

1 (minimal): 1–3 small, focal inflammatory infiltrates. A few neurons may be changed or lost;
2 (moderate): more extensive focal inflammatory infiltrates. Neuronal changes or loss affects not more than one-third of neurons;
3 (severe): neuronal changes or loss of 33–90% of neurons, with moderate focal or diffuse inflammatory infiltration;
4 (overwhelming): more than 90% of neurons are changed or lost, with variable, but frequently severe, inflammatory infiltration.

Each brain block contains several anatomical structures which contribute in different ways to the assessment of a test sample. For

example, certain structures differentiate more reproducibly than others between acceptable and unacceptable yellow fever seed lots and vaccines (*20*). These are called discriminator areas, whereas structures that are more susceptible to yellow fever virus replication are called target areas. Though either rhesus or cynomolgus monkeys are acceptable, the discriminator and target areas are different for the two species. The major difference is that in cynomolgus monkeys the cervical and lumbar cord are target areas whereas in rhesus monkeys they are discriminator areas. The footnotes to the worksheets (Appendix 1) indicate in more detail discriminator and target areas for the two species. The worksheets also lists other anatomical structures that will be present in the brain sections but are not included in the evaluation of a test sample because they are rarely affected (spared areas).

Three separate scores shall be calculated for each monkey: discriminator areas only, target areas only, and discriminator plus target areas. These scores shall be calculated as shown in the example worksheets provided in Appendix 1 (pp. 55–57). Overall mean scores shall also be calculated for each group of monkeys as the arithmetic mean of individual monkey scores for discriminator areas only and for discriminator plus target areas. Both overall mean scores shall be considered in determining virus seed lot acceptability. For the histological criterion of the neurotropism test to be satisfied both overall mean scores for the test monkeys shall not be significantly greater (at the 5% significance level) than the overall mean scores for the monkeys injected with reference virus.

Both the clinical and histological criteria of the neurotropism test shall be satisfied for the virus seed lot to satisfy the requirement for neurotropism.

A.4.1.4 Identity test

An identity test shall be performed on at least one container from each master and working seed lot, using one of the techniques described in section A.6.1.

A.4.1.5 Sterility tests on primary and secondary seed lots

Each lot of seed virus in its final containers shall pass the tests described in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*21*).[1]

[1] See the 1995 amendment to the General Requirements for the Sterility of Biological Substances, section 5.3, *Sterility test for mycoplasmas* (Annex 3, p. 70 of this report).

A.4.2 *Tests on uninoculated eggs*

If monitoring of the flocks supplying embryonated eggs is not under the direct responsibility of the vaccine manufacturer, the following tests shall be performed.

A sample of 2% of, but in any case not less than 20 and not more than 50 uninoculated embryonated eggs from the batch used for vaccine production shall be incubated under the same conditions as the inoculated eggs. At the time of virus harvest, the uninoculated eggs shall be processed in the same manner as the inoculated eggs, and the extract from the control embryos shall be shown to be free from haemagglutinating agents and other extraneous agents by tests approved by the national control authority.

A.4.3 *Single harvests*

After inoculation and incubation at a controlled temperature, only living, typical chick embryos shall be harvested. The age of embryos at the time of harvest shall be reckoned from the initial introduction of the eggs into the incubator and shall be no more than 12 days. After homogenization and centrifugation, the embryonic extract shall be kept at –60 °C or below until further processing.

A.4.3.1 Sterility tests

The embryonic extract that forms a single harvest shall be tested for the presence of bacteria, fungi and mycoplasmas as described in Part A, section 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*21*).[1]

A.4.3.2 Virus titration

Live virus content shall be determined by titration in cell culture against a reference preparation of yellow fever virus as described in Appendix 3.

A.4.4 *Final bulk*

The final bulk shall be prepared from one or several single harvests and shall be submitted to the following tests, unless these tests have already been performed on each single harvest. The final bulk shall in any case be tested for sterility. Samples that are not tested immediately shall be stored at or below –60 °C.

[1] See the 1995 amendment to the General Requirements for the Sterility of Biological Substances, section 5.3, *Sterility test for mycoplasmas* (Annex 3, p. 70 of this report).

A.4.4.1 Sterility tests

The requirements concerning tests for bacteria, fungi and mycoplasmas as described in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*21*) shall apply to yellow fever vaccine.[1]

A.4.4.2 Adventitious agents

Mycobacterium avium shall be tested for by cell-culture methods approved by the national control authority.

> In some countries, the national control authority requires that the final bulk should be shown by suitable tests to be free from ALV or other agents.

A.4.4.3 Protein content

The level of protein nitrogen before the addition of any stabilizer shall be not more than 0.25 mg per human dose.

A.4.4.4 Addition of stabilizers and preservatives

No β-lactam antibiotics shall be added to the virus suspension at this or at any other stage during production. Added stabilizing agents shall be approved by the national control authority.

A.4.4.5 Virus titration

The live virus content of each final bulk shall be determined by titration in cell culture against a reference preparation of yellow fever virus as described in Appendix 3.

A.5 Filling and containers

The requirements concerning filling and containers given in Good Manufacturing Practices for Biological Products (*18*) shall apply to yellow fever vaccine.

Care shall be taken to ensure that the materials of which the container, and if applicable the closure, are made do not adversely affect the virus content of the vaccine under the recommended conditions of storage.

> Single- and multiple-dose containers may be used.

The vaccine shall be freeze-dried.

> Failure to achieve adequate drying will result in a product that is liable to rapid deterioration even at 0 °C.

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[1] See the 1995 amendment to the General Requirements for the Sterility of Biological Substances, section 5.3, *Sterility test for mycoplasmas* (Annex 3, p. 70 of this report).

Since yellow fever virus is extremely labile, unless the container is well sealed variations in virus content may occur during storage. The manufacturer should ensure that the seal is satisfactory.

The manufacturer shall provide the national control authority with adequate data to prove the stability of the vaccine under appropriate conditions of storage and shipping.

A.6 Control tests on final lot

A.6.1 *Identity test*

An identity test shall be performed on at least one container from each filling lot after reconstitution of the vaccine according to the indications of the manufacturer for preparing the vaccine for human administration. A high-titre, monospecific immune serum known to be free from neutralizing agents that react with other flaviviruses shall be used. Either of two methods may be used for the identity test.

A.6.1.1 Test in mice

Progressive dilutions not greater than fivefold each shall be made of the vaccine in a suitable diluent. Aliquots of each dilution shall be mixed with equal volumes of a reference immune serum and similar aliquots mixed with equal volumes of a reference non-immune serum. The serum–virus mixtures shall be incubated at 37 °C and each shall be injected into not less than 6 mice per dilution as described in Appendix 3. All mice shall be observed for 21 days, and all deaths shall be recorded. Only deaths considered to be specifically caused by yellow fever virus infection shall be taken into account. Mice paralysed on day 21 shall be counted as alive. The LD_{50} for vaccine mixed with immune serum shall be more than ten times the LD_{50} for vaccine mixed with non-immune serum.

A.6.1.2 Test in cell cultures (plaque reduction test)

The technique described in section A.4.1.3, *Immunogenicity test*, (pp. 37–38) shall be used, with dilutions of vaccine with immune and non-immune serum. If a 50% reduction in plaque number at the 1:10 dilution is not observed for the vaccine mixed with immune serum compared with vaccine mixed with non-immune serum, the vaccine shall be rejected.

A.6.2 *Potency test*

Three final containers shall be selected at random from each filling lot and shall be individually tested on the same day against a reference preparation of yellow fever vaccine approved by the national control

authority. The containers shall be tested by assay in cell cultures or mice by a technique demonstrated to be sensitive.

Before assay but after reconstitution of the vaccine in the volume and diluent recommended by the manufacturer for preparation for human administration, the vaccine shall stand at a temperature between 20 °C and 30 °C for 20 minutes before further dilution. This material shall be considered as undiluted vaccine.

The titre of the vaccine shall be not less than 1000 mouse LD_{50}, or its equivalent in PFU, in the dose recommended by the manufacturer for use in humans. Each laboratory shall establish to the satisfaction of the national control authority the relationship between mouse LD_{50} and PFU.

Each laboratory using a cell culture assay shall have the approval of the national control authority for this assay. Appropriate techniques for the potency test are given in Appendix 3.

A.6.3 *Thermal stability test*

Three final containers from the freeze-dried final lot shall be incubated at 37 °C for 2 weeks. These containers shall be titrated in parallel with three containers that have been stored at or below the recommended storage temperature. Results should be expressed in infectious units or their equivalent in PFU. A reference reagent for yellow fever virus approved by the national control authority shall be included in each assay. At the end of the incubation period, the geometric mean infectious titre in the incubated final containers shall not have decreased by more than 1.0 $\log_{10}$ unit and shall be at least equal to the required minimum number of infectious units per human dose.

Procedures for carrying out the above test and for the interpretation of results, including the specification of confidence limits, should be approved by the national control authority. A suitable test for determination of virus content (i.e. titre) is described in Appendix 3.

A.6.4 *Tests for bacteria and fungi*

The requirements concerning tests for bacteria and fungi as described in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*21*) shall apply to yellow fever vaccine.

A.6.5 *Tests for other agents*

In some countries the national control authority requires that the vaccine should be shown by suitable tests to be free from ALV or other agents. If

these tests have been performed on the final bulk they may be omitted for the final lot.

A.6.6 *General safety tests*

Each final lot shall be tested for the absence of abnormal toxicity in mice and guinea-pigs by appropriate tests approved by the national control authority.

A.6.7 *Residual moisture*

The residual moisture in a representative sample of each freeze-dried final lot shall be determined by a method approved by the national control authority. The upper limit of the moisture content shall be approved by the national control authority on the basis of stability tests.

Generally, moisture levels of 3% or less are considered satisfactory.

A.6.8 *Residual ovalbumin*

The content of residual ovalbumin shall be limited to a level approved by the national control authority.

A.6.9 *Test for endotoxin*

Each final lot shall be tested for endotoxin. The test and limits shall be approved by the national control authority.

A.6.10 *Inspection of final containers*

Every container in each final lot shall be inspected visually, and those showing abnormalities shall be discarded.

A.7 Records

The requirements in section 8 of Good Manufacturing Practices for Biological Products (*18*, pages 27–28) shall apply.

A.8 Samples

The requirements in section 9.5 of Good Manufacturing Practices for Biological Products (*18*, page 29) shall apply.

A.9 Labelling

The requirements in section 7 of Good Manufacturing Practices for Biological Products (*18*, pages 26–27) shall apply, with the addition of the following:

The label on the carton or the leaflet accompanying the container shall:

— state that the vaccine fulfils Part A of these Requirements;
— state the nature of the preparation, specifying the strain of yellow fever virus in the vaccine, the minimum number of infectious units per human dose, and the origin of the substrate used to prepare the vaccine (i.e. whether the flock was specific-pathogen free (SPF)).
— state the nature and quantity of any residual antibiotic present in the vaccine;
— indicate that the vaccine contains proteins derived from eggs;
— indicate that contact of the vaccine with disinfectants is to be avoided;
— indicate that the dose shall be the same for persons of all ages;
— indicate the volume and nature of the diluent to be added to reconstitute the vaccine, and specify that only the diluent supplied by the manufacturer should be used;
— state that the vaccine is not recommended for administration to children less than 6 months of age;
— state that the reconstituted vaccine shall be kept at 2–8 °C and in the dark and shall be used or discarded within 1 hour after the container is opened.

A.10 Distribution and shipping

The requirements in Good Manufacturing Practices for Biological Products (*18*, section 8) shall apply.

A.11 Storage and expiry date

The requirements in Good Manufacturing Practices for Biological Products (*18*) shall apply.

A.11.1 Storage conditions

Before being distributed by the manufacturing establishment, or before being issued from a depot for the maintenance of vaccine reserves, all vaccines shall be kept at all times at a temperature below –20 °C.

If possible, storage should be at a temperature below –25 °C.

The manufacturer shall recommend conditions of storage and shipping that will ensure the vaccine conforms to the requirements of potency until the expiry date stated on the label.

A.11.2 Stability of vaccine and expiry date

The stability of the vaccine shall be established at the recommended storage temperature and calculated from the date of a given satisfactory potency test. The expiry date shall be fixed with the approval of the national control authority and shall be no less than 2 years after the potency test.

Accelerated degradation studies at 37 °C may provide useful additional information.

Part B. National control requirements

B.1 General

The general requirements for control laboratories contained in the Guidelines for National Authorities on Quality Assurance for Biological Products (*22*) shall apply.

The national control authority shall give directions to manufacturers concerning the yellow fever virus strains to be used in vaccine production and concerning the recommended human dose.

The national control authority should take into consideration information available on strains before deciding on those permitted for vaccine production.

In addition, the national control authority shall provide or approve a reference preparation of live yellow fever virus (see section A.1.3) for tests for virus concentration (see sections A.4.4.5 and A.6.2) and shall specify the virus content required to achieve adequate immunization of humans at the recommended dose.

B.2 Release and certification

A vaccine lot shall be released only if it fulfils the national requirements and/or Part A of the present Requirements. A protocol based on the model given in Appendix 1, signed by the responsible official of the manufacturing establishment, shall be prepared and where appropriate submitted to the national control authority in support of a request for release of vaccine for use. For subsequent routine lot release, a less comprehensive document may suffice, a model for which is found in Annex 4 of this volume of the WHO Technical Report Series.

At the request of the manufacturing establishment, the national control authority shall provide a certificate that states whether the vaccine meets all national requirements and/or Part A of the present Requirements. The certificate shall be based on the model given in Appendix 2.

The purpose of the certificate is to facilitate the exchange of live yellow fever virus vaccine among countries.

Authors

The draft of these revised Requirements was prepared by Dr V. Grachev, Scientist, Biologicals; Dr D. Magrath, Chief, Biologicals (1987–1994); and Dr E. Griffiths, Chief, Biologicals, World Health Organization, Geneva, Switzerland.

Acknowledgements

Acknowledgements are due to the following experts for their comments and advice on the draft Requirements:

Professor I.D. Gust, Director, Research and Development, CSL Ltd, Victoria, Australia; Dr K.J. Healy, Head, Quality Assurance, CSL Ltd, Victoria, Australia; Professor J. L'age-Stehr, Robert Koch Institute, Berlin, Germany; Mr P. Lemoine, formerly Head, Biological Standardization, Institute of Hygiene and Epidemiology, Brussels, Belgium; Dr I. Levenbook, Center for Biologics Evaluation and Research, Division of Product Quality Control, Kensington, MA, USA; Mrs S. Marsden, Viral Division, National Institute for Biological Standards and Control, Potters Bar, Herts., England; Dr L. Palkonyay, Chief, Viral Products Division, Bureau of Biologics, Drugs Directorate, Ottawa, Ontario, Canada; Dr F. Reigel, Head, Section for Immunologicals, Swiss Federal Office of Public Health, Bern, Switzerland; Ms J. Sokhey, Director, Central Research Institute, Kasauli, India; Dr D. Wood, Division of Virology, National Institute for Biological Standards and Control, Potters Bar, Herts., England.

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Appendix 1
Example, for guidance, of a summary protocol for the production and testing of yellow fever vaccine[1]

Master virus seed lot

Source of 17D substrain _____

Master virus seed lot no. _____

Name and address of manufacturer _____

Passage level _____

Date of inoculation of embryos _____

Temperature of incubation _____

Date of harvest _____

Age of embryos (at harvest) _____

Number of containers _____

Conditions of storage _____

Production control (A.4)

Monkey safety test (A.4.1.3)

Species _____

Number inoculated _____

Master virus seed lot no. _____

Reference virus lot no. _____

Date of serology tests before inoculation _____

Dilution used for the inoculation _____

Volume and route of inoculation _____

Date of inoculation _____

Number of mouse LD_{50} or PFU inoculated _____

Date of end of the test _____

[1] Based on Requirements for yellow fever vaccine (Requirements for Biological Substances No. 3, revised 1995). In: *WHO Expert Committee on Biological Standardization. Forty-sixth Report.* Geneva, World Health Organization, 1997, Annex 2 (WHO Technical Report Series, No. 872).

Viscerotropism test (master virus seed lot)

Specify if mice or cells are used for virus titration.

Monkey no.	Titre of circulating virus on:			Maximum titre of circulating virus
	Day 2	Day 4	Day 6	
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Result (pass or fail) _____

Immunogenicity test (master virus seed lot)

Specify if mice or cells are used for virus titration.

Monkey no.	Seroneutralization titre:	
	Day 0	Day 30
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Result (pass or fail) _____

Neurotropism test (master virus seed lot)

Summary clinical results

Date of inoculation: _____

Master virus seed lot no.:		Reference virus lot no.:	
Monkey no.	Clinical score	Monkey no.	Clinical score
1		11	
2		12	
3		13	
4		14	
5		15	
6		16	
7		17	
8		18	
9		19	
10		20	
Group mean		Group mean	

Result (pass or fail) _____

Histological worksheet

The worksheets below are provided as an example of how the histological score would be calculated for a cynomolgus monkey with lesions graded as shown.

Species: cynomolgus
Pathology no:
Monkey no:

Corpus striatum & thalamus	Block I:		Block II:		Total
	L	R	L	R	
N. caudatus[a]	1	0	2	2	(5/4 =) 1.25
Globus pallidus[a,b]	0	1	2	0	(3/4 =) 0.75
Putamen[a,b]	2	0	1	1	(4/4 =) 1.00
N. ant./med. thalami[a,b]	1	1	0	1	(3/4 =) 0.75
N. lat. thalami[a,b]	1	2	1	1	(5/4 =) 1.25
Hypothalamus	0	1	0	0	(1/4 =) 0.25

[a] Discriminator area for rhesus.
[b] Discriminator area for cynomolgus.

Mesencephalon (Block III)	L	R	Total
Colliculi superior	0	0	0
Corpus geniculatum med.	0	0	0
N. oculomotorius	0	0	0
N. ruber	0	0	0
Substantia nigra[c]	2	2	(4/2 =) 2.00

[c] Target area for rhesus and cynomolgus.

Pons (Block IV)	L	R	Total
N. abducens	0	0	0
N. vestibularis	0	0	0
N. trigeminus	0	0	0
N. facialis	0	1	(1/2 =) 0.5
Formatio reticularis	1	0	(1/2 =) 0.5
Oliva superior	0	0	0

Medulla oblongata (Block V)	L	R	Total
N. hypoglossus	0	0	0
N. glossopharyngeus	0	0	0
N. vestibularis	0	0	0
N. trigeminus	0	0	0
N. ambiguus	0	0	0
Formatio reticularis	0	0	0
Oliva inferior	0	0	0

Cerebellum (Blocks IV and V)	L	R	Total
N. dentatus	0	0	0
Other nuclei	0	0	0

Spinal cord	I		II		III		IV		V		VI		Total		
	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L&R
Cervical enlargement[a,d]	2	3	2	3	2	2	2	2	1	2	2	2	11	14	(25/12 =) 2.08
Lumbar enlargement[a,d]	2	2	1	2	1	2	1	2	0	1	0	2	5	11	(16/12 =) 1.33

[a] Discriminator area for rhesus.
[d] Target area for cynomolgus.

Calculations:

Discriminator areas (globus pallidus, putamen, n. ant./med. thalami, n. lat. thalami):

$$\text{Lesion score} = \frac{(0.75 + 1.00 + 0.75 + 1.25)}{4} = 0.94$$

Target areas (s. nigra, cervical enlargement, lumbar enlargement):

$$\text{Lesion score} = \frac{(2.00 + 2.08 + 1.33)}{3} = 1.80$$

Discriminator plus target areas:

$$\text{Lesion score} = \frac{0.94 + 1.80}{2} = 1.37$$

Summary histopathology results

Date of inoculation:_____ Species:_____

Master virus seed lot no.:			Reference virus lot no.:		
Monkey no.	Discriminator area score	Discriminator plus target area score	Monkey no.	Discriminator area score	Discriminator plus target area score
1			11		
2			12		
3			13		
4			14		
5			15		
6			16		
7			17		
8			18		
9			19		
10			20		
Group mean			Group mean		

Result (pass or fail) _____

Identity test (A.4.1.4)

Comparison of virus titre in presence of normal serum with that in presence of immune serum

Method _____

Date _____

Results _____

Sterility tests (A.4.1.5)

Number of containers tested _____

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Media used	_____	_____	_____
Observation period	_____	_____	_____
Results	_____	_____	_____

Tests for avian and other viruses

Method _____

Date _____

Results _____

Working virus seed lot

Working virus seed lot no. _____

Name and address of manufacturer _____

Passage level _____

Date of inoculation of embryos _____

Temperature of incubation _____

Date of harvest _____

Age of embryos (at harvest) _____

Number of containers _____

Conditions of storage _____

Monkey safety test (A.4.1.3)

Species _____

Number inoculated _____

Working virus seed lot no. _____

Reference virus lot no. _____

Date of serology tests before inoculation _____

Dilution used for inoculation _____

Volume and route of inoculation _____

Date of inoculation _____

Number of mouse LD_{50} or PFU inoculated _____

Date of end of the test _____

Viscerotropism test (working virus seed lot)

Specify if mice or cells are used for virus titration. See p. 52 for an example of the viscerotropism test worksheet.

Result (pass or fail) _____

Immunogenicity test (working virus seed lot)

Specify if mice or cells are used for virus titration. See p. 53 for an example of the immunogenicity test worksheet.

Result (pass or fail) _____

Neurotropism test (working virus seed lot)

Please see pp. 54–58 for examples of the clinical and histological worksheets

Result (pass or fail) _____

Identity test (A.4.1.4)

Comparison of virus titre in presence of normal serum with that in presence of immune serum

Method _____

Date _____

Results _____

Sterility tests (A.4.1.5)

No. of containers tested _____

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Media used	_____	_____	_____
Observation period	_____	_____	_____
Results	_____	_____	_____

Tests for avian and other viruses

Method _____

Date _____

Results _____

Control of single harvests (A.4.3)

Name and address of manufacturer _____

Laboratory reference no. _____

Information on source materials

Virus used to inoculate embryos

Derived from master seed virus lot no. _____

Working virus seed lot, reference no. and source _____

Passage level of working virus seed lot _____

Source of eggs

Is the flock under direct control of manufacturer? _____

Is the flock monitored for compliance with these Requirements? _____

Results of tests on flock or control tissues

Tests for salmonella _____

Tests for fowl pox virus _____

Tests for avian mycobacteria _____

Tests for other microbial agents (give details) _____

Information on manufacture

Date of inoculation of embryos _____

Temperature of incubation _____

Date of harvest _____

Age of embryos (at harvest) _____

Results of sterility tests _____

Control of final bulk (A.4.4)

Sterility tests (A.4.4.1)

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Media used	_____	_____	_____
Observation period	_____	_____	_____
Results	_____	_____	_____

Protein content (A.4.4.3)

Method _____

Date _____

Result _____

Stabilizers and preservatives (A.4.4.4)

Name of stabilizer _____

Quantity or percentage _____

Name of preservative _____

Quantity or percentage _____

Date _____

Virus titration (A.4.4.5)

Method _____

Date _____

Result _____

Control tests on final lot (A.6)

Identity test (A.6.1)

Comparison of virus titre in presence of normal serum with that in presence of immune serum

Method _____

Date _____

Result _____

Potency test (A.6.2)

Date of test _____

Reference batch no. _____

Relationship between titre as determined by inoculation in mice, expressed in LD_{50} per human dose, and/or the titre as determined in cell culture, expressed in plaque forming units (PFU), per human dose is: _____

	Vaccine samples			*Reference samples*
	1	2	3	
Virus concentration in each container	____	____	____	_____
Mean virus titre per human dose, with 95% fiducial limits	_____			_____

Thermal stability test (A.6.3)

Method _____

Date _____

Results _____

Sterility tests (A.6.4)

	bacteria	*fungi*
Date of inoculation	_____	_____
Media used	_____	_____
Observation period	_____	_____
Results	_____	_____

Tests for other agents (if performed) (A.6.5)

Method _____

Date _____

Results _____

General safety test (A.6.6)

Tests in mice

Date of inoculation _____

No. of mice tested _____

Volume and route of injection _____

Observation period _____

Results (give details of deaths) _____

Tests in guinea-pigs

Date of inoculation _____

No. of guinea-pigs tested _____

Volume and route of injection _____

Observation period _____

Results (give details of deaths) _____

Residual moisture (A.6.7)

Method _____

Date _____

Result _____

Residual ovalbumin (A.6.8)

Method _____

Date _____

Result _____

Endotoxin (A.6.9)

Method _____

Date _____

Result _____

Inspection of final containers (A.6.10)

Method _____

Date _____

Result _____

Submission addressed to national control authority

Name (typed) and signature
of head of production laboratory _____

_____

Date _____

Certification by person taking overall responsibility for production and control of the vaccine:

I certify that lot no. ____ of yellow fever vaccine satisfies national requirements and/or Part A of the Requirements for Biological

Substances No. 3 (Requirements for Yellow Fever Vaccine, revised 1995).

Signature _____

Name (typed) _____

Date _____

Appendix 2
Model certificate for the release of yellow fever vaccine by national control authorities[1]

The following final lots of yellow fever vaccine produced by _____[2] in _____,[3] whose numbers appear on the labels of the final containers, meet all national requirements[4] and Part A[5] of Requirements for Biological Substances No. 3 (Requirements for Yellow Fever Vaccine), revised 1995[6] and comply with Good Manufacturing Practices for Pharmaceutical[7] and Biological[8] Products.

Lot no.	Date of last potency test by manufacturer	Expiry date	Lot no.	Date of last potency test by manufacturer	Expiry date

As a minimum, this certificate is based on examination of the manufacturing protocol.

The Director of the National Control Laboratory (or Authority as appropriate):[9]

Name (typed) _____

Signature _____

Date _____

[1] To be completed by the national control authority of the country where the vaccine has been manufactured, and to be provided by the vaccine manufacturer to importers.
[2] Name of manufacturer.
[3] Country.
[4] If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the national control authority.
[5] With the exception of provisions on distribution and shipping, which the national control authority may not be in a position to assess.
[6] WHO Technical Report Series, No. 872, 1998, Annex 2.
[7] WHO Technical Report Series, No. 823, 1992, Annex 1.
[8] WHO Technical Report Series, No. 822, 1992, Annex 1.
[9] Or his or her representative.

Appendix 3
Techniques for the potency evaluation of yellow fever vaccine

Cell-culture technique

Vero cell

Vero-cell seed and a description of a method for Vero-cell cultivation may be obtained from the World Health Organization. Monolayers of Vero cells are prepared in 35-mm Petri dishes, and 4 dishes are inoculated with each virus dilution (0.2 ml per dish). After incubation for 1 hour at 36 °C, the virus dilution is replaced by 3 ml of an overlay medium consisting of Leibovitz medium No. 15 or MEM medium at pH 7.1–7.2, bovine fetal serum (final concentration, 5%), and agarose (final concentration, 0.5%). Five days after inoculation, 0.1 ml of the overlay medium, with the addition of a 1:50000 dilution of neutral red dye, is added. Plaques are counted on day 6. When plaque-forming titre is calculated, all dilutions should be considered in which the average number of plaques per dish is between 1 and 30.

Alternatively, equal amounts (0.2 ml) of a Vero-cell suspension (approximately 6×10^5 cells/ml) and virus dilution in Leibovitz medium No. 15 or MEM medium are placed in each of the 16-mm flat-bottomed wells in sterile trays suitable for cell culture. The trays are sealed and incubated for 4 hours at 36 °C. After incubation, 0.4 ml of overlay medium, consisting of Leibovitz medium No. 15 or MEM medium, bovine fetal serum (3%), and carboxymethylcellulose (low viscosity sodium salt) (1.6%), is added to each well. The trays are resealed and incubated at 36 °C for 5 days. On the sixth day, the trays are drained, washed with saline, stained with 1% naphthalene black or any other suitable stain and thoroughly rinsed with tap water. The plaques are then counted. All dilutions in which the average number of plaques per well is between 1 and 30 should be considered in calculating titre.

PS cell[1]

This assay technique is a modification of that used by De Madrid & Porterfield (*1*). Equal amounts (0.2 ml) of a PS-cell suspension (approximately 6×10^5 cells/ml) and virus dilution in Leibovitz medium No. 15 (*2*) are placed in each of the 16-mm flat-bottomed wells

[1] These cells are latently infected with swine fever virus and their importation is prohibited in certain countries.

in sterile trays suitable for cell culture. The trays are sealed and incubated for 4 hours at 36°C. After incubation, 0.4 ml of overlay medium, consisting of Leibovitz medium plus bovine fetal serum (3%) and carboxymethylcellulose (low-viscosity sodium salt) (1.6%), is added to each well. The trays are resealed and incubated at 36°C for 5 days. On the sixth day, the trays are drained, washed with saline, stained with 1% naphthalene black and thoroughly rinsed with tap water. The plaques are then counted. All dilutions in which the average number of plaques per well is between 1 and 20 should be considered in calculating titre.

Mouse LD_{50} technique

The mouse LD_{50} is the quantity of virus suspension estimated to produce fatal, specific encephalitis in 50% of intracerebrally inoculated mice.

Appropriate serial dilutions of the reconstituted vaccine are made in diluent for yellow fever virus (0.75% solution of bovine albumin (fraction V) in phosphate-buffer which has been demonstrated to be free of yellow fever virus inhibitors).

Mice of a strain highly susceptible to yellow fever virus, aged 4–6 weeks, are injected intracerebrally under anaesthesia with 0.03 ml of vaccine dilution. Groups of at least 6 mice are used for each dilution, and the series of dilutions should result in mortality rates after inoculation spanning the range 0–100%. Inoculation of the mice should be performed immediately after the dilutions have been made. All deaths are recorded during a period of 21 days. Mice dying from unrelated causes are removed from both the numerator and denominator of mortality calculations. Mice paralysed on the twenty-first day are counted as alive.

References

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2. **Leibovitz A.** The growth and maintenance of tissue-cell cultures in free gas exchange with the atmosphere. *American journal of hygiene*, 1963, **78**:173–180.

H. TETANUS TOXOID VACCINE

Documents provided in this section include:

1. Sample Procurement Requirements Tetanus Toxoid Vaccine 557
2. TT Vaccine Model Package Insert (UNICEF) 564
3. Tetanus Toxoid, *USP 23 - NF 18*, 1994 565

SAMPLE

PROCUREMENT REQUIREMENTS

TETANUS TOXOID (TT) VACCINE

A. General Description of Intended Use

This vaccine is intended for use in a public-sector health programme for immunization of women of childbearing age in _____ [*place*] beginning on _____ [*date*].

The total requirement for this solicitation is _____ [*quantity*] doses. _____ [*name*] expects to procure additional quantities of vaccine annually beginning in _____ [*month, year*] and subsequent years as follows: _____ [*quantity*], _____ [*quantity*] and _____ [*quantity*].

B. Specific Requirements

Item: Tetanus Toxoid Vaccine, adsorbed.

Sterile suspension of purified tetanus toxoid. The toxoids are adsorbed onto _____ [*specify*]. _____ [*specify*] is used as a preservative. Intended for intramuscular injection.

The potency of the vaccine per single human dose shall be at least 40 (forty) infective units.

The vaccine shall meet _____ [*specify*] requirements (WHO Biological Requirements for Tetanus Vaccine Requirements for Biological Substances No. 10 or equivalent requirements of the USFDA as detailed in USP 23, or another fully equivalent set of biological requirements for Tetanus Toxoid Vaccine).

	Editors' note: It is recognized that equivalence does not mean identical, only that both provide the same minimum level of potency safety and efficacy.
	The vaccine shall be currently registered in the country of manufacture and shall meet all requirements of the licensing authority of the country of manufacture. The vaccine also shall be currently registered in the country of use and shall meet all requirements of the licensing authority of the country of use.
Quantity:	_____ doses from one production lot.
Dosage Size:	0.5 ml.
Dose Package:	Vials of 10 doses. Vials of 20 doses.
Filling Volume:	Each 10-dose vial should contain 5 ml plus 15% overfill. Each 20-dose vial should contain 10 ml plus 15% overfill.
Storage Temperature:	Between +2 and +8 degrees C[32]; vaccine must NOT be frozen.
Shelf Life:	30 months from beginning of last satisfactory test for potency; at least 18 months must remain after shipment.
Stability:	Stable for a minimum of _____ years at _____ to _____ degrees C; manufacturer to specify thermal stability characteristics.
Labelling:	The label on each vial shall conform to the requirements of the country of use and shall appear in the language of _____.

[32] This temperature range is more conservative than indicated in model package insert, page 564.

All labelling shall withstand immersion in water and remain intact.

All labels shall state the name of the vaccine, name of the manufacturer, place of manufacture, lot number, composition, concentration, dose and mode for administration, expiry date, storage temperature and any other marking that is appropriate.

Optional Labelling: If vaccine is destined for use in public-sector immunization programmes, the statement "Not for Sale; For Use Only in Government-Approved Immunization Programmes" shall appear on each label in the language of the purchasing country.

Closures: Vaccine vials shall be fitted with closures that conform to ISO standard 8362-2.

Printed Materials: Two (2) information sheets, printed in _____ [language], shall be included in each box of ___ vials (see Model Package Insert, attached)[33].

C. Quality Assurance

Compliance: The Supplier shall guarantee that the products as packed for shipment (a) comply with all provisions of the specification and related documents; (b) meet internationally recognized standards for safety, efficacy and quality; (c) are fit for the purposes made known to the Seller and (d) are free from defects in workmanship and in materials.

[33] See example, page 564.

Evidence:	The Supplier shall provide objective evidence, acceptable to the Purchaser, of the satisfaction of the requirements of this document for which no specific inspection has been mentioned. The Supplier shall provide a copy of the manufacturing record and procedures to the Purchaser for each lot intended for shipment. The Supplier shall provide a copy of the batch record and all quality assurance documentation to the Purchaser for each lot being supplied. The Supplier shall provide a copy of the Certificate of Analysis to the Purchaser for each lot intended for shipment. The Supplier shall provide to the Purchaser a copy of the approval of each source material, constituent material and component for each lot intended for shipment. The Supplier shall retain a sample of twenty (20) vials from each lot shipped for two years beyond the printed expiration date. Chemical, physical and microbiological test data for in-process and finished product testing must be on record for each lot shipped and must be available to Purchaser's representatives when requested.
Inspection:	The Purchaser may inspect and sample, or cause to be sampled, the product at the Supplier's factory and/or warehouse at a mutually agreeable time prior to shipment of the product.
Testing:	The Purchaser may cause independent laboratory testing to be performed as deemed necessary to assure that the goods conform to prescribed requirements. Said testing laboratory

shall be of the Purchaser's choice and suitably equipped and qualified to conduct quality assurance tests on biological products.

D. Packing

Storage:	Supplier shall state storage volume occupied per dose of vaccine (storage volume includes the vaccine vial, packet containing the vaccine vial and any intermediate packaging).
Inner Boxes:	_____ [*number*] individual vials will be contained in sturdy white cardboard boxes outfitted with individual segments for protecting and separating each vial.
Cold Chain Monitor Cards:	One cold chain monitor card (description attached)[34] in _____ language shall be packed with each ___ doses of vaccine. [_____ freeze watch indicators shall be included with each carton of vaccine.]
Overpacking:	Boxes shall be overpacked so that the vaccine remains refrigerated at between +2 and +8 degrees C and does not freeze. The containers must be suitable for export shipment in accordance with WHO/EPI Guidelines on International Packaging and Shipping of Vaccines. They must have adequate insulation and/or sufficient refrigerant to ensure that the warmest storage temperature of the vaccine does not rise above +8 degrees C in continuous outside ambient temperature of +43 degrees C nor fall below +2 degrees C in continuous outside ambient temperature of -20 degrees C during transit and for a period of at least 48 hours after arrival at the airport of destination.

[34] See example in Supplement VI.G.1.a., page 783.

Additional cushioning shall be provided, sufficient to protect the vials from breakage during transit and handling.

Exterior Shipping Cartons: Product and printed materials, packaged as specified above, shall be packed in weather-resistant, triple-wall corrugated fibreboard cartons with a bursting test strength of not less than 1,900 kPa.

The overall dimensions of the exterior shipping cartons should be such that the product does not become damaged during transportation and storage.

E. Markings

All containers and invoices must bear the name of the vaccine, expiry dates of the vaccine and appropriate storage temperature.

Inner Boxes: The inner boxes shall be marked with the following information in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Manufacturer's name and registered address
- Manufacturer's national registration number
- Lot or batch number
- Composition and concentration
- Number of vials contained in box
- Expiration date (month and year)
- Instructions for storage and handling†
- Place of manufacture (Made in _____)

Exterior Shipping Cartons: The following information shall be stencilled or labelled on the exterior shipping cartons on two opposing sides in bold letters at least __ mm

† To be provided by Purchaser.

high with waterproof ink in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Lot or batch number
- Expiration date (month and year)
- Manufacturer's name and registered address
- Manufacturer's national registration number
- Destination country license or registration number
- Consignee's address and emergency phone number
- Destination airport
- Contract number
- Number of vials contained in the carton
- Gross weight of each carton (in kg)
- Carton #___ of ___
- Instructions for storage and handling†
- Place of manufacture (Made in _____)

F. Documentation

Supplier shall provide to purchaser a copy of the batch record, including all quality assurance documentation for the vaccine being supplied.

† To be provided by Purchaser.

TT VACCINE

DESCRIPTION

The vaccine contains purified tetanus toxoid. The toxoid is adsorbed onto _____(specify). _____ is used as a preservative. One dose of 0.5 ml has a potency of at least 40 International Units. TT is used in the prevention of neonatal tetanus by immunizing women, especially of childbearing age, and also in the prevention of tetanus following injury.

ADMINISTRATION

The vaccine should be shaken before use in order to get a homogeneous suspension. It should be injected intramuscularly or deep subcutaneously.

A sterile needle and a sterile syringe should be used for each injection.

Opened vials of vaccine may be used in subsequent immunization sessions until a new shipment of vaccine arrives providing that the conditions described in WHO/EPI/LH15/95.1 are met.

IMMUNIZATION SCHEDULE

TT immunization of women for the prevention of tetanus and neonatal tetanus consists of two doses of 0.5 ml given intramuscularly or deep subcutaneously at least four weeks apart followed by the third dose 6 - 12 months later. To maintain the immunity against tetanus through the child bearing period, a fourth dose should be given at least one year after the third dose, and the fifth dose should be given at least one year after the fourth dose. TT immunization can be administered safely during pregnancy even during the first trimester.

SIDE EFFECTS

Rare and mild. Some transitional tenderness and redness at the site of the injection and occasional fever. Safe to give in pregnancy.

CONTRAINDICATIONS

A severe reaction to a previous dose of TT.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED PERSONS: HIV infected persons, both asymptomatic and symptomatic, should be immunized with TT vaccine according to standard schedules.

STORAGE

TT should be stored and transported between 0° and +8°C. IT MUST NOT BE FROZEN.

PRESENTATION

The vaccine comes in vials of _____ doses.

Tetanus Toxoid

» Tetanus Toxoid conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile solution of the formaldehyde-treated products of growth of the tetanus bacillus (*Clostridium tetani*). It meets the requirements of the specific guinea pig potency test of antitoxin production based on the U.S. Standard Tetanus Antitoxin and the U.S. Control Tetanus Test Toxin. It meets the requirements of the specific guinea pig detoxification test. It contains not more than 0.02 percent of residual free formaldehyde. It contains a preservative other than a phenoloid compound.

Packaging and storage—Preserve at a temperature between 2° and 8°.

Expiration date—The expiration date is not later than 2 years after date of issue from manufacturer's cold storage (5°, 1 year).

Labeling—Label it to state that it is not to be frozen.

Tetanus Toxoid Adsorbed

» Tetanus Toxoid Adsorbed conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile preparation of plain tetanus toxoid that meets all of the requirements for that product with the exception of those for potency, and that has been precipitated or adsorbed by alum, aluminum hydroxide, or aluminum phosphate adjuvants. It meets the requirements of the specific mouse or guinea pig potency test of antitoxin production based on the U.S. Standard Tetanus Antitoxin and the U.S. Control Test Tetanus Toxin. It meets the requirements of the specific guinea pig detoxification test.

Packaging and storage—Preserve at a temperature between 2° and 8°.

Expiration date—The expiration date is not later than 2 years after date of issue from manufacturer's cold storage (5°, 1 year).

Labeling—Label it to state that it is to be well-shaken before use and that it is not to be frozen.

Aluminum content—It contains not more than 0.85 mg per single injection, determined by analysis, or not more than 1.14 mg calculated on the basis of the amount of aluminum compound added.

Tetanus Toxoids, Diphtheria and—*see* Diphtheria and Tetanus Toxoids

Tetanus and Diphtheria Toxoids Adsorbed for Adult Use

» Tetanus and Diphtheria Toxoids Adsorbed for Adult Use conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile suspension prepared by mixing suitable quantities of adsorbed diphtheria toxoid and adsorbed tetanus toxoid using the same precipitating or adsorbing agent for both toxoids. The antigenicity or potency and the proportions of the toxoids are such as to provide, in each dose prescribed in the labeling, an immunizing dose of Tetanus Toxoid Adsorbed as defined for that product, and one-tenth of the immunizing dose of Diphtheria Toxoid Adsorbed as defined for that product for children, such that in the specific guinea pig antigenicity test it meets the requirement of production of not less than 0.5 unit of diphtheria antitoxin per mL and each immunizing dose has an antigen content of not more than 2 Lf (flocculating units) value as measured with the U.S. Reference Diphtheria Antitoxin for Flocculation Test. Each component meets the other requirements for those products. It contains not more than 0.02 percent of residual free formaldehyde.

Packaging and storage—Preserve at a temperature between 2° and 8°.

Expiration date—The expiration date is not later than 2 years after date of issue from manufacturer's cold storage (5°, 1 year).

Labeling—Label it to state that it is to be well-shaken before use and that it is not to be frozen.

Tetanus Toxoids Adsorbed, Diphtheria and—*see* Diphtheria and Tetanus Toxoids Adsorbed

Tetanus Toxoids and Pertussis Vaccine Adsorbed, Diphtheria and—*see* Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed

Tetanus Toxoids and Pertussis Vaccine, Diphtheria and—*see* Diphtheria and Tetanus Toxoids and Pertussis Vaccine

PART IV - QUALITY ASSURANCE

A. Role of the World Health Organization 568
B. Information for Procurement Personnel 568
1. Licensing or registration by NCA 569
2. Procurement procedures 569
3. Specifications 569
4. Pre-qualification of vaccines and suppliers 570
5. Pre-shipment inspection and testing 570
6. Government import controls 571
7. Receipt inspection 571
C. Donations and Special Offers of Vaccine 571
D. Pre-Shipment Compliance Programmes 573
1. Function 573
2. Sampling 573
3. Inspection 574
4. Testing 575
E. Guidelines for National Authorities on Quality Assurance for Biological Products 583
F. Regulation and Licensing of Biological Products in Countries with Newly Developing Regulatory Authorities 600
G. WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce 608
H. National Control Authority Guidelines for Assessment of Vaccine Quality in Non-Producing Countries 620
I. GVP Policy Statement - Vaccine Donations 639

PART IV - QUALITY ASSURANCE

A. Role of the World Health Organization

The World Health Organization (WHO) has published a number of guidelines and recommendations relating to manufacturing requirements and quality criteria for vaccines and invites countries to adopt these as a basis for their own national regulations concerning vaccines. In addition to biological requirements and detailed models of summary protocols for specific vaccines, WHO offers many useful technical reports. It provides advice on regulation and licensing of biological products in countries with newly created regulatory authorities. It also assesses specific vaccines from specific manufacturers and approves them in principle for purchase by UN bodies such as UNICEF. Furthermore, WHO recommends that each country participate in the WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce.

Part IV.G., page 608

If a country has not established an NCA with expertise in biologicals, WHO will provide guidance to the health authorities on the creation of such a body. Guidelines have also been established for the assessment of vaccine quality in non-producing countries in the document "National Control Authority: Guidelines for Assessment of Vaccine Quality in Non-Producing Countries" (WHO/VSQ/95.1) which appears in Part IV.H.

Part IV.H., page 620

B. Information for Procurement Personnel

From a manufacturing standpoint, controlling the quality of biological products such as vaccines is complex because biological processes are, by their nature, subject to variation; consequently the characteristics of each batch can vary slightly. Thus, quality cannot be established by tests on material in the final container alone.

While vaccines produced in industrialized countries are subject to adequate systems of quality assurance and meet agreed international standards, nearly half of the world's vaccine supply is presently produced in non-industrialised countries. Manufacturers in these countries do not always produce vaccines under conditions that ensure the safety, potency and efficacy of their products.

The importation of vaccines licensed by the NCA of the purchaser's country is the most satisfactory method of ensuring the use of safe and effective vaccine. If no NCA exists in the country, purchasing should not be undertaken by the government without appropriate guidance and supervision. In the absence of an NCA with appropriate expertise in biologicals, the guidance of WHO or a qualified national authority such as USFDA may be requested to advise on the best course of action. Advice from WHO will include information on vaccines produced by

manufacturers that have been reviewed by WHO and which produce vaccines of "Known Good Quality".

1. *Licensing or registration by NCA*

The licensing status of a pharmaceutical product or a vaccine governs its importation and distribution, and thus, its eligibility for purchase.

Vaccines used in immunization programmes must be licensed by the NCA in the country of manufacture and should also be licensed by the NCA in the purchaser's country. WHO has recommended that countries employ a mandatory process of licensing vaccines using an NCA created through appropriate legislation. Procurement personnel should bear in mind that at least six months usually are required to license a vaccine in an importing country.

2. *Procurement procedures*

Part I., page 11
Part II., page 27

By choosing appropriate procurement procedures and adopting carefully designed contractual requirements, the procurement entity often is able to circumvent delays and programme disruptions associated with rejection of a poor quality product at the point of delivery. These procedures and conditions include the use of detailed specifications[35], restrictive payment terms, pre-shipment inspection (and in specific circumstances, pre-shipment testing), well crafted packing and shipping instructions and insistence on adequate cold chain provisions. Using the pre-qualification and purchasing processes described in this manual will ensure that quality considerations do not impede the supply process by requiring expensive and time-consuming quality control procedures for each contract.

Part VI., page 705
Part IV.D., page 573
Part IV.C., page 572
Part II., page 153

3. *Specifications*

Detailed specifications are key to the receipt of products that meet all expectations and comply with quality standards. Technical specifications cover the physical properties of the vaccine, quality assurance norms and required storage temperatures. Programme specifications cover acceptable variations in vial size, packaging, packing, etc. and may call for a shelf life beyond the minimum established by the NCA.

a. Technical specifications

Technical specifications for vaccines are established by the NCA of the purchasing country and these define the vaccines that can be licensed for

[35] Part III includes WHO and 1996 USFDA requirements for the standard EPI vaccines (BCG, DTP, measles, OVP, yellow fever, hepatitis B), along with sample procurement requirements appropriate for soliciting offers from prospective suppliers.

import into that country. When establishing the technical specifications, the NCA may decide to take into account specifications already existing in producer countries and those developed by WHO for public-sector vaccination programmes. The responsibility for this rests with the NCA of the importing country, if an NCA has been established. Further information on the functions of an NCA is available from WHO publications.

Part IV.H., page 620

b. Programme specifications

Programme specifications for vaccines may differ from time to time depending upon how and where the vaccine will be stored, distributed and administered. For example, a programme manager may determine that it is more cost-effective to purchase vaccine for use in a mass immunization campaign in 20- or 50-dose vials, instead of the normal presentation for routine EPI. Five-dose or even single-dose vials may be required for specific populations. In another case, vaccine may be purchased with labels affixed on the packing boxes at the narrow ends rather than the wide sides, so expiration dates will be visible in a specific storage configuration at the central cold stores.

4. Pre-qualification of vaccines and suppliers

Vaccine is normally pre-qualified through licensing by the NCA of the purchaser's country. Suppliers may themselves be pre-qualified for sales of licensed vaccine in a country based on a review of technical credentials, previous performance, references and other attributes such as a verifiable legal relationship with the manufacturer of vaccine offered for sale. The manufacturer of a licensed vaccine is automatically pre-qualified as a supplier of that vaccine. The subject of pre-qualification is dealt with in more detail in Part II.B.

Part II.B., page 35

5. Pre-shipment inspection and testing

In the case of *licensed* vaccines purchased *directly* from well-known, internationally reputable manufacturers, a manufacturer's Certificate of Analysis and a Lot Release Certificate approved by the NCA of the manufacturer's country are usually considered adequate protection against receipt of substandard, ineffective or dangerous products. *Laboratory testing is not required except in special circumstances* (see Part IV.C., Donations). However, many purchasers and some lending institutions require a pre-shipment inspection to verify other aspects of the cargo before it leaves the seller's facility. In this case, the shipment is visually examined to verify that it has been prepared in accordance with the contract in all ways; e.g., the correct goods are being supplied in the correct presentation and quantity, the goods are correctly labelled, the package inserts are correct, there is no evidence of damage or deterioration, the manufacturer's certificate of analysis matches the specification, etc. There are a number of

Part IV.C., page 571
Part IV.D., page 573

specialist companies that undertake pre-shipment inspections. These are usually contracted at a relatively low cost based on a time and travel distance formula. A selection of inspection agents is included with additional information on pre-shipment compliance programmes at Part IV.D.

Part IV.D., page 573

6. *Government import controls*

Regulatory bodies such as the NCA assign certain enforcement activities to customs authorities, who must allow or disallow entry of a product based on documentary evidence of compliance with national licensing requirements.

In addition, some governments have adopted national import controls that require pre-shipment inspection by a specified agent for all products above a certain value. In these cases, a "Clean Report of Findings" must accompany commercial documents presented for customs clearance by the importing country. This certificate attests to the good condition of the merchandise immediately prior to shipment. It is not a substitute for the pre-shipment inspection of vaccine described above.

7. *Receipt inspection*

Quality control procedures in an importing country usually involve at least two levels of investigation:

- Examination of the documentation and certificates accompanying the batch of vaccine[36], to ensure the vaccine is licensed in the country and that details of identity, manufacture, dosage, remaining shelf life, etc. are in agreement with the contract requirements. The NCA also reviews the Lot Release Certificates issued by the regulatory authority of the manufacturing country before it releases each batch of vaccine for use in the country.
- Visual inspection of samples of the boxes, cartons and individual dosage units to establish that they are correctly labelled, are accompanied by the correct information and are free from defects, damage or signs of deterioration.

Some countries quarantine incoming medicines and vaccines until tests can be conducted and the results approved by their own health authorities. Once again, the licensing of the vaccine by the NCA will eliminate the need for all but the inspection of the physical characteristics and the satisfactory packaging and storage of the shipment.

C. Donations and Special Offers of Vaccine

An NCA with biologicals expertise in the recipient country is vital to the receipt of safe and effective donated vaccines. If no such NCA exists in the country benefiting from the offer of a donated or specially priced vaccine, WHO, through its head-

[36] Part VI includes examples of shipping and quality documents.

quarters and regional and country offices, is prepared to provide technical advice and guidance in the adoption and implementation of a donations policy. In providing such advice, particular attention is paid to advocacy with donors regarding the suitability of donations, training and monitoring. The provision of a rapid response to the resolution of reports of adverse events following the use of donated vaccines is also an important part of such advice.

Offers of free or very low cost vaccine are often made to public-sector immunization programmes, particularly in situations of emergency caused by a disease outbreak or an unforeseen stock shortage during supplementary immunization. If such offers are properly managed, they can be of considerable benefit; but if they are not properly managed, the risks to the immunization programme can be very high. Although special offers of vaccine are not usually made through the procurement entity, many of the purchasing guidelines in this manual, especially those detailed in the pre-qualification section, are applicable to the donation. *The procurement entity should therefore manage the delivery of such vaccines.*

WHO has recommended that governments establish a policy for the receipt of donations of drugs and vaccines and has issued guidelines on the receipt of donated drugs (WHO/DAP/96.2, available from WHO), which are based on four principles:

1. Maximum benefit to the recipient
2. Respect for the wishes and authority of the recipient
3. No double standards in quality
4. Effective communication between donor and recipient

For the most part, the guidelines are applicable to biologicals, but it may be useful to restate them with specific reference to vaccines:

- The vaccine must be helpful to, and consistent with, the goals of the immunization programme.
- The vaccine must be subject to the licensing and control procedures established by the government through the NCA.
- The vaccine must meet all the specifications and be consistent with the vaccines normally used in the programme. Particular attention must be paid to potency, liquid or freeze dried presentation, transport, shelf life, number of doses per vial, thermostability and labelling.
- The vaccine should be shipped only on the written request of the responsible national officials.

Additional discussion may be found in the GPV Policy Statement on Vaccine Donations found in Part IV.I.

Part IV.I., page 639

Before agreeing to accept special offers of vaccine for which the source is not clearly established, the recipient government should arrange for the vaccine to be tested. Such testing must be carried out before shipment to establish conformity with the vaccines licensed in the country, those in use in the immunization programme and

the objectives of the programme itself. Typical costs of laboratory tests are included at the end of Part IV.D.4. as a guide to concerned government officials.

Part IV.D.4., page 576

D. Pre-Shipment Compliance Programs

1. Function

Pre-shipment compliance programs are used to assure the purchaser that products, as prepared for shipment, meet all expectations and contractual requirements. Used in conjunction with a Letter of Credit, they are an especially effective means of contract enforcement since payment can be withheld until documentary proof of compliance has been presented to a specified bank.

Part VI.C.5., page 707

A pre-shipment compliance program might rely entirely on visual inspection of the products, packaging, packing, labelling and markings and examination of quality assurance documents or, *in exceptional circumstances*, it may require laboratory testing as well. A very simple sample compliance program appears at the end of this section.

Supplement IV.D.1., page 579

2. Sampling

Compliance activities can be based on a "sampling" of the product rather than 100 percent inspection. For purposes of this manual, "sampling" is defined as the process of selecting a small, representative quantity of materials from a much larger batch, shipment or consignment. Inspecting this representative sample enables judgement about the quality of the entire batch or shipment of products without having to inspect each individual unit. Although a sample should represent the population from which it comes, there is no guarantee that any sample will precisely mirror the population. Therefore, steps must be taken in the design of the plan to minimize the chance that the products chosen for inspection will be atypical.

If laboratory testing is included in the pre-shipment compliance program, the inspection agent should be directed to randomly select a pre-determined number of samples from each manufacturing lot and forward them to a designated laboratory. For any typical batch of products supplied by a reputable manufacturer, one set[37] of samples is sufficient for analytical tests. If any possibility exists that they are deteriorated or adulterated, it is recommended that at least one additional set of samples be available for confirmation of test results.

[37] See information on pages 576-577 regarding number of vials in a set of samples.

3. *Inspection*

Inspection criteria should outline exactly what is to be examined and what constitutes compliance. A sample inspection order is provided at the end of this section as part of the compliance program in Supplement IV.D.1. Test results mentioned in the sample inspection criteria are derived from the NCA dossier on the product.

Supplement IV.D.1., page 581

Pre-shipment inspection of the physical characteristics of vaccine consignments typically costs under $600. However, rates vary and are usually based on the inspector's time and travel distance, so firm quotations should be obtained. For pre-qualified vaccines it will normally only be necessary for the physical aspects of the shipment to be inspected for compliance with the contract requirements. These may include packing boxes and temperature insulation in addition to markings and the quantities of vaccine in the shipment.

Partial List of International Inspection Agents

The following organisations offer pre-shipment inspection and sampling services:

FRANCE
Bureau Veritas
16 bis Place Des Refletes
Courbevoie
F-92077 Paris, La Defence
France
Fax +33-142-91-52-94

UNITED KINGDOM
Lloyd's of London
Agency Department
One Lime Street
London LC3M 7HA
United Kingdom
Tel. +44-171-623 7100
Fax. +44-171-327 6777

UNITED STATES
Intertek
3741 Red Bluff Road
Pasadena, TX 77503
USA
Tel. +1-713-475 2082
Fax. +1-713-475 2083

SWITZERLAND
Societe Generale de Surveillance SA
Consumer Products Department
1, Place des Alps
CH-1211 Geneva 1
Switzerland
Tel. +41-22-739 9111
Fax. +41-22-731 1666

UNITED STATES
ABS Industrial Verification
263 North Belt East
Houston, TX 77060
USA
Tel. +1-713-873 5200
Fax. +1-713-874 9553

4. *Testing*

Laboratory testing of vaccine shipments is expensive, has limited value and is not required if the vaccine and the supplier have been pre-qualified, and there is no question about the integrity of the cold chain from the point of manufacture until delivery into the hands of the Purchaser.

Part IV.C., page 571

From time to time, however, donations or offers of very low-cost vaccine are made to government authorities without a clearly established source, quality and/or cold chain history (see Part IV.C.). In these cases, the government must arrange for pre-shipment testing by a competent, independent laboratory, usually at government expense. Before agreeing to accept such offers, the recipient government should consider the cost and the time required for the vaccine to be tested. Apart from the expense of sampling and carrying out the necessary tests, the time required can cause unacceptable delays in the implementation of immunization programmes.

Part IV.D.3., page 574

In addition to testing costs, it may be necessary to hire an international inspection agency for assistance with random selection of samples and transmittal to a qualified laboratory. Contact information for a selection of international inspection agencies is listed in Part IV.D.3.

A sample of estimated costs and the time required for vaccine testing are included on the following pages as a guide and decision-making tool. The tests listed are representative of those usually carried out, but do not include all of the common tests for each vaccine. Testing protocols and results should conform to the requirements of the relevant National Control Authorities and/or an appropriate internationally reputable pharmacopoeia or WHO Requirements for Biological Substance. For purposes of convenience, WHO Technical Reports have been used as a basis for the following estimates. Prices vary from laboratory to laboratory, so firm quotations should be obtained.

Vaccine Potency Tests

VACCINE	REFERENCE	TYPE OF TEST	SAMPLE	COST	DURATION
Measles	WHO Technical Report No. 840 Pages 100-168	Virus concentration using cell culture plaque	6 vials	$4200	5 weeks
IPV (Inactivated Polio Vaccine)	WHO Technical Report No. 673 Pages 40-86	Antigen content by ELISHA. Seroconversion ³3 groups 10 rats per group injected	1 vial 10 vials	$500 $900	2 days 6 weeks
DTP - Diphtheria	WHO Technical Report No. 800 Pages 87-179	Seroconversion mouse blood vs standard 3x16 mice or guinea pigs test 10x4 control	~10 vials	$3000	4 weeks +
DTP - Pertussis (not the same test)	WHO Technical Report No. 800 Pages 87-179	Seroconversion mouse blood vs standard 3x16 mice or guinea pigs test 10x4 control	~10 vials	$3000	4 weeks +
DTP - Tetanus (not the same test)	WHO Technical Report No. 800 Pages 87-179	Seroconversion mouse blood vs standard 3x16 mice or guinea pigs test 10x4 control	~10 vials	$3000	4 weeks +
BCG	WHO Technical Reports No. 745 Pages 60-92	Plate counts for Bacillus CFUs	10 vials	$1000	3 weeks
Hepatitis B (Plasma derived)	WHO Technical Report No. 858 Pages 61-92	Seroconversion and titre 20 test mice plus 20 control	20 vials	$3600	35 days
Hepatitis B (Recom-binant)	WHO Technical Report No. 786 Pages 38-71	Seroconversion and titre for "several" groups of 20 test mice and "several" groups of 20 control mice	~ 20 vials	$14400	42 days

Quality Tests Other Than Potency

TEST NAME	US$ COST (LAB 1)	US$ COST (LAB 2)	EUROPE COST
Animal General Safety	$405	$640	FF1600
Sterility (>200 products manufactured)	$175-300	$500	FF500
Karl Fischer Moisture Determination	$90	$75	FF300
Pyrogens - 3 rabbit - 5 rabbit repeat - 8 rabbit repeat	$185 $360 $545	$650	FF750 FF1200 FF1500

Vaccine Identity Tests

VACCINE	REFERENCE	TYPE OF TEST	SAMPLE	COST	DURATION
Measles	WHO Technical Report No. 840 Page 117	Virus concentration using cell culture plaque assays	2 Vials	$1400	5 weeks
Oral Polio (Live)	WHO Technical Report No. 800 Page 51	Serological Identity	1 vial	$70	1 week
IPV (Inactivated Polio)	WHO Technical Report No. 673 Page 60	Potency test serves as the identity test			
DPT – Diphtheria	WHO Technical Report No. 800 Page 147	Elute from carrier and centrifuge. Then test Vs anti-toxin	~1 vial	$100	1 week
DPT - Pertussis	WHO Technical Reports No. 800 Page 147	Agglutination of organisms with antipertussis OR Seroconversion of animals	1 vial ~1 vial	$100 $600	1 week 3 weeks
DPT - Tetanus	WHO Technical Report No. 800 Page 147	Elute from carrier and centrifuge. Then test versus tetanus antitoxin	~1 vial	$100	1 week
BCG	WHO Technical Report No. 745 Page 75	Stain or smear and positive ID	1 Vial	$50	1 week
Hepatitis B (Plasma derived)	WHO Technical Report No. 858 Page 77	ELISHA versus Anti-	1 Vial	$700	1 week
Hepatitis B (Recom-binant)	WHO Technical Report No. 858 Page 56	Potency test serves as identity test			

Partial List of Testing Laboratories

GERMANY:
BCG, DTP, DT and Td, TT, OPV
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Sample Compliance Programme

Prior to shipment, the Purchaser, or appointed representative, has the right to sample and inspect each consignment of vaccine at the factory or Supplier's warehouse in accordance with Technical Specification _____ of this contract.

Packaging, Packing and Marking

a. One hundred percent (100%) of the exterior shipping cartons will be examined for:
   - General physical characteristics and condition
   - Markings per Technical Specification _____

b. A representative sample of the inner boxes will be drawn from the exterior shipping cartons

c. The sample will be examined for:
   - General physical characteristics per Technical Specification _____
   - Markings per Technical Specification _____

d. All aspects of the samples, including the exterior shipping cartons, inner boxes and vials or ampoules will be further inspected and any defects classified as follows:

   Critical
   - The shipping documents do not coincide with the vial or ampoule information
   - Broken vials or ampoules
   - Illegible or missing text or markings on the vials or ampoules
   - Batch/lot number or expiration date incorrect or missing from vial or ampoule labelling
   - Product information sheet does not match the product
   - Package insert or information sheet missing
   - Shipping carton badly closed or broken
   - Inner boxes in bad condition, open, dirty or torn/broken
   - Individual boxes missing from the multiple-unit shipping cartons
   - Batch/lot number or expiration date incorrect or missing from inner boxes
   - Foreign matter in inner boxes

   Major
   - Manufacturer's national registration number missing on inner boxes
   - Vials or ampoules missing from the inner boxes
   - Manufacturer's name, address, or importing country's registration number missing from inner boxes

- Package insert or information sheet illegible, dirty or torn
- Labelling missing from inner boxes
- Instructions for storage missing from inner boxes

Minor

- Printing on vials or amouples defective but legible

Vaccine

At the discretion of the Purchaser, part of the selected sample may be sent to a qualified independent laboratory to confirm any or all of the manufacturer's test data on the final product.

A Certificate of Analysis for production lot(s) represented by test samples shall be made available to the inspector and/or Purchaser upon request. The certificate shall state all tests performed, their specifications and the actual test results obtained. In each case, test results shall meet Pharmacopoeia limits.

Resolution of Defects

a. Packaging, Packing and Markings

   Defects in exterior shipping carton markings must be corrected by the Supplier prior to shipment.

   All Critical Defects must be corrected and re-inspected at the Supplier's expense or all goods from the same production lot will be rejected.

   Major Defects must be corrected by the Supplier to the satisfaction of the Purchaser prior to shipment.

   Minor Defects will be resolved on a case-by-case basis to the satisfaction of the Purchaser.

b. Vaccine

   Any deviation from the manufacturer's Certificate of Analysis, product specification or Pharmacopoeia limits shall result in rejection of goods from the entire production lot.

Sample Inspection Order

To: Societe Generale de Surveillance (SGS)
Date: October 10, 1997
Purchase Order: 97-068

Vendor: XYZ Corporation
123 Main Street
Seoul, Korea

Consignee: Government of Indonesia
Capital Building
Jakarta, Indonesia

INSPECTION CRITERIA

1. Examine Certificate of Analysis and QC records for shipment of 5000 vials (0.5 ml, 0.5 dose) Hepatitis B Vaccine (Hepavax B).

   Certificate of Analysis must include:
   Commodity Name
   Lot Number
   Date of Manufacture
   Analysis Results

   Test results as indicated on Certificate of Analysis must fall within the limits of the following specification:

Appearance	Opalescent
pH	Value on determination
Thimerosal Content	NMT 0.012 W/V%
Formaldehyde Content	NMT 0.01 W/V%
Aluminium Content	NMT 1.25 mg/ml
Filling Volume	0.68 ml - 0.8 ml
Foreign Material	None
HBsAg Content	9-11 g/ml
Sterility	No growth in FTM media (31°, 36° C) No growth in SDB media 25° C)
Abnormal Toxicity	Freedom from abnormal toxicity
Potency	NLT Reference
QC/QA Records	must support Certificate of Analysis

2. Examine Labelling

   Provide a written report for approval by the Government of Indonesia on labelling prior to release of a clean bill of goods including:

   Photocopy of labelling found on vials
   Confirmation of consistent labelling on all vials
   Photocopy of any package inserts

(If photocopies are not possible, a written report of the information is acceptable.)

3. Examine Packing and Markings

Provide a written report for approval by the Government of Indonesia on packaging prior to release of a clean bill of goods including:

Description of cartons

Description of insulation material, quantity and type

Number of vials per container

Number of Infochem Freezewatch Indicators included and their physical location within the shipping container

Markings shall include:

Government of Indonesia

Letter of Credit Number: LC0001-003

Invoice Number

Date of Invoice:

Lot Number:

Expiry Date:

PERISHABLE MATERIAL, KEEP REFRIGERATED (+2 to +8 °C)

KEEP FROM FREEZING

Gross Weight:

Box # _____ of _____ Boxes

AIRPORT OF DESTINATION: JAKARTA, INDONESIA

Telephone consignee upon arrival. Telephone No.: 526 237

4. Examine Documentation

Refer to attached Shipping Instructions and confirm all documents listed are complete except the Air Waybill which is acceptable in draft form.

5. Unless otherwise specified in writing, the SGS representative is not authorised to sign the "Authorisation for Shipment" form.

E. Guidelines for National Authorities on Quality Assurance for Biological Products

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WHO Technical Report Series, No. 822, 1992

Annex 2
Guidelines for national authorities on quality assurance for biological products

1. Scope 31
2. Introduction 31
3. General considerations 33
4. Structure of a national control authority for biological products 34
5. Procedures for approval of manufacturers and products 35
6. The national control laboratory 37
7. Post-licensing monitoring of products 39
8. Procedures for approval of imported products 40
Authors 42
Acknowledgements 43
References 43
Appendix 1
Model certificate of approval of a biological product 45
Appendix 2
Model certificate for the release of a lot or lots of a biological product 46

1. Scope

This document describes measures for assuring the safety and efficacy of biological products intended for use in humans. Its aim is to provide general guidelines for national health authorities on quality assurance for biological products, except those used solely as *in vitro* diagnostic agents. The concepts and principles have been harmonized with those of the Requirements for Biological Substances and other related sets of recommendations published by WHO (*1*). Though it is difficult to provide a set of guidelines applicable to all national situations, an attempt has been made to cover a range of possibilities; the principles on which this document is based may assist in the development of national guidelines.

2. Introduction

Since the publication by WHO in 1981 of a document entitled "The national control of vaccines and sera" (*2*), it has become apparent that it would be helpful to provide updated guidance for countries wishing to develop or improve quality-assurance procedures for biological products. In addition, technological developments in the production of biologicals and the availability of new analytical methods for determining their quality

have made evident the need for a further revision of the Requirements for Biological Substances No.1 (General Requirements for Manufacturing Establishments and Control Laboratories), published in 1966 (*3*). The present guidelines reflect the decision to combine parts of these two documents into a single text.

National regulatory authorities have the duty to ensure that available pharmaceutical products, whether imported or manufactured locally, are of the required quality. This is particularly difficult for biological products, the quality of which cannot be established entirely by tests on the material in the final container. The national authority has the responsibility to confirm that the manufacturer is adhering to the approved standards of good manufacturing practice and to national and other requirements for manufacture and quality control specific to the product. The mechanism by which national authorities confirm the assurance of quality provided by the manufacturer may depend on the resources available and whether the product is manufactured locally or imported.

In general biological products are distinguished from other drugs by being derived from living organisms (ranging from normal or genetically modified microorganisms to humans), and frequently have a complex molecular structure. They require special quality considerations because of the biological nature of: (a) the starting materials, and/or (b) the manufacturing process, and/or (c) the test methods needed to characterize batches of the product.

Recognizing these difficulties and the need for international harmonization of quality standards, WHO, largely through its Biologicals unit, has a number of activities related to the quality assurance of biological products used for the diagnosis, prevention and treatment of diseases. These include: (a) publishing in the WHO Technical Report Series the reports of expert groups, which may include documents such as this one that constitute requirements or guidelines for use by national authorities; (b) organizing the distribution of International Standards and other reference materials for biological substances, which allow the characterization and assay of products in terms of internationally accepted units; (c) providing advice on the preparation of national reference materials; (d) advising and assisting Member States in the establishment and functioning of structures for national control purposes, e.g. by organizing visits by experts and assisting with programmes of training and research in relation to these activities; and (e) arranging scientific meetings to provide an up-to-date and scientifically valid consensus on topics relating to quality assurance that are generally applicable to Member States throughout the world.

Developments in biological products have been extremely rapid in recent years, and the potential of such products for improving health care on a global scale is immense. There is an urgent need to match technological advances with appropriate mechanisms for assuring the quality of the products.

General considerations

The quality, safety and efficacy of a biological product are the prime responsibility of the manufacturer; however, the national health authority of each Member State is responsible for establishing procedures for assuring that biological products intended for use in the country are of adequate quality, safety and efficacy. This responsibility should have a firm statutory basis backed by legislation. Marketing approval for a biological product should be granted by a national control authority (NCA), which should also be responsible for continued post-marketing monitoring. In carrying out these activities, the NCA should make use of expert committees and technical advisers, and have access to laboratory facilities.[1]

Individual Member States should have written standards, both general and product-specific, for biological products available for use in their countries. These should be based on contemporary standards, such as those available from WHO, and harmonized as far as possible with those of other Member States. For newly developed products, WHO, national and pharmacopoeial requirements may not have been developed and the NCA will need to agree on specifications with the manufacturer on a case-by-case basis.

In countries where biological products are manufactured, the NCA should have appropriate expertise to evaluate the adequacy of the manufacturer's establishment and facilities, starting materials, production processes, control-test procedures and product specifications, to determine whether they meet international and/or national requirements. These control activities should be fully independent of those of the manufacturer; ideally the national laboratory facilities should form a single administrative unit designated as the national control laboratory (NCL). The NCL may be administered directly by, or on behalf of, the NCA.

In countries where biological products are not manufactured, alternative approaches may be acceptable for assuring the safety and efficacy of these products (see section 8). However, an approval process limited to a mere listing of facilities and products would not be considered adequate.

In view of the complexity and cost of certain resources needed for control testing, it may be unavoidable in certain cases to share such resources with the manufacturer or an academic institution, or to rely on the resources of an NCL in another Member State.

National control authorities should, whenever appropriate, exchange information on safety issues, within the constraints of confidentiality, in accordance with the WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce (*4*).

[1] In this document reference is made only to the role of the NCA with respect to biologicals; in practice it is to be expected that the NCA will have responsibility for all pharmaceutical products.

4. Structure of a national control authority for biological products

In countries where biological products are manufactured, the health authorities should establish and maintain a competent NCA and NCL. It is recommended that the NCA should make use of independent expert advisers and advisory committees with appropriate expertise and have access to testing facilities, such as those of the NCL, to support decision-making. In some instances a multinational or regional structure may be more efficient.

4.1 *Personnel*

The personnel of the NCA and the NCL should include person(s) qualified and experienced in the control of biological products and experts in all appropriate disciplines. The qualifications and experience of the staff at all levels should be appropriate to the review and control activities required for the range of biological products to be controlled. It is advantageous for the director of the NCL to report directly to the director of the NCA.

Contact between NCA and NCL personnel and scientists working in related fields will be beneficial for exchanging ideas and discussing techniques, problems and analytical results.

All staff of the NCA and NCL should receive suitable training through active training programmes, covering both the technical and the administrative aspects of control procedures.

Encouragement of research activities within the NCL will both lead to the development of better control methods and help the laboratory to retain an interested, efficient and highly qualified staff.

4.2 *Administration*

The NCA should have established procedures for the receipt and review of manufacturers' submissions and for testing samples provided in support of applications. On completion of the review procedures, which include the evaluation of detailed reports (see sections 5 and 6), the NCA issues a notice of approval or disapproval to the manufacturer. It may also issue notices of suspension or revocation of approval. Consideration should be given to making available appropriate legal expertise in support of this activity.

The NCA should maintain adequate filing and archiving facilities, such that all submissions, evaluations, records and correspondence are available and kept up to date. Attention should be given to the need to maintain commercial confidentiality.

The NCA and NCL should possess, or have access to, library facilities appropriate to their fields of activity. The documents available should include: current national and international requirements for products that

are manufactured locally, relevant international requirements for biological substances, and other relevant specifications and recommendations published by WHO or other bodies.

4.3 *Good manufacturing practices (GMP) inspectorate*

The NCA should have access to suitably qualified inspectors who are independent of manufacturers. The purpose of inspections will be to ensure compliance of each manufacturer's facilities and procedures with the principles of GMP as described in WHO publications (*5*, *6*) and with the requirements and/or conditions for the approval for the product concerned. Guidelines are available for conducting inspections of manufacturers of both drugs and biologicals (*7*). Reciprocal arrangements for international inspection such as those of the Pharmaceutical Inspection Convention of the European Free Trade Association (*8*) may be used, as may the WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce (*4*).

5. Procedures for approval of manufacturers and products

Approval or licensing of a manufacturing establishment for production of biological products should be granted only if the manufacturer complies with the principles of GMP (*5*, *6*), as confirmed by inspection.

Approval or licensure of a given biological product will be given by the NCA when it is satisfied that the product conforms to the relevant national and/or international requirements, including the manufacturer's specifications applicable to the product. In order to evaluate the safety and efficacy of a biological product, the NCA should review the following detailed information before granting an approval.

5.1 *Information on the manufacturing establishment*

The manufacturer should provide sufficient information to demonstrate compliance with the principles of GMP (*5*, *6*), including the existence of adequate quality-assurance systems. Plans, diagrams, flow charts and texts may be used to convey the necessary information in relation to (but not limited to):

- personnel:
  - qualifications and experience,
  - organization and reporting relationships,
  - training schedules and recording systems;
- location and construction of the buildings used for manufacture and control;
- flow of raw materials, personnel and manufactured product through the facility;
- animal facilities;
- air, water and steam systems and power supply;
- drainage and effluent systems;

- segregation of operations;
- lists of major equipment;
- maintenance schedules for equipment and building services;
- cleaning schedules;
- quality-assurance and quality-control procedures;
- storage and quarantine facilities and procedures for:
  - raw materials,
  - packaging materials,
  - in-process and bulk materials,
  - final product;
- validation procedures;
- documentation and record-keeping systems;
- labelling and packaging facilities and procedures;
- recall and retrieval procedures.

To be certain that the buildings, facilities, personnel, procedures and practices comply with the description in the licence application, the NCA should arrange for the inspection of the manufacturer before granting the licence. The inspector(s) selected should be independent of the manufacturer and have sufficient expertise to conduct a meaningful review, in accordance with section 4.3, of the GMP system in use (including buildings, facilities, procedures, personnel and quality assurance).

5.2 *Information on the product*

The manufacturer should provide sufficient information to demonstrate the safety and efficacy of the product as manufactured and controlled in the establishment described above. The NCA should request from the manufacturer a critical evaluation of the procedures adopted for manufacture and control of the product and of preclinical and clinical studies relevant to its proposed use. The submission should include the following details, if appropriate:

- source materials (e.g. microorganisms, blood/plasma donations, cells/cell substrates, pollen), including their specifications and the tests used to demonstrate compliance with the specifications;
- raw materials and packaging materials, including their specifications and the tests used to demonstrate compliance;
- methods of manufacture, including a description of seed-lot and cell-substrate systems used, together with in-process, bulk and final product specifications and the tests employed to demonstrate compliance;
- demonstration of consistency of manufacture, which normally comprises the results of tests on a minimum of three satisfactory and consecutive production batches of a size corresponding to that contemplated for routine production;
- any proposal for reprocessing of the product;
- stability studies undertaken to justify the proposed validity period for the product under the indicated storage conditions;

- labels and package inserts;
- documentation used in the manufacturing and control procedures, including standard operating procedures and protocols containing details of production and quality-control testing;
- reports of preclinical studies;
- clinical trial data;
- a list of countries in which the product is approved for use.

The nature and extent of pre-licensing testing undertaken by the NCA should reflect any particular quality-assurance considerations relevant to the product; as a minimum, the NCA should undertake tests to evaluate its safety and efficacy. It may also perform chemical, physical and biological tests additional to those specified in national or international requirements.

6. The national control laboratory

The size of facilities and number of staff of the NCL will depend on the nature and extent of the quality control required. Some guidance is available in the *Manual for the design, equipping and staffing of facilities for production and quality control of bacterial vaccines* (*9*).

6.1 *Laboratory testing and evaluation*

The NCL (or equivalent) should be able to perform all necessary tests on samples of source materials and of intermediate, bulk and finished products. This will require specialized facilities and equipment. If the NCL is on a site where biological products are manufactured, national control activities should be independent of control activities associated with manufacture or production.

The NCL's responsibilities should include some or all of the following activities:

- provision of advice to the NCA on technical matters relevant to the approval of products and manufacturing establishments;
- evaluation of manufacturers' preparative and analytical procedures, standard operating procedures, validation experiments and batch protocols;
- pre-licensing control testing of samples of batches, in particular for ascertaining consistency of production, as well as testing for batch release;
- evaluation of shelf-life specifications and expiry dates of final lots on the basis of the manufacturer's thermal stability tests and stated conditions of storage, and experimental verification of stability;
- development, evaluation, establishment and implementation of testing procedures and release criteria;
- review of reports of quality defects in distributed material, retesting if appropriate, and provision of advice on whether the preparations should remain on the market or be withdrawn;

- undertaking of research in relation to the above activities (which may involve collaboration with manufacturers).

In order to fulfil its responsibilities, the NCL should have the authority to demand appropriate samples (e.g. of starting materials, intermediate products and finished biologicals) from manufacturers. The samples should be properly labelled and portions should be kept for future reference.

The control laboratory should maintain adequate analytical records of all samples examined, including:

- the results of tests performed, including original observations and calculations, relating to compliance with the established specifications;
- the date and signature(s) of the person(s) who performed the quality-control tests; and
- a final review, with a dated endorsement of the final decision by a responsible person.

6.2 *Establishment of national reference materials for biological substances*

WHO has published guidelines on the preparation, characterization and establishment of international and other reference materials (*10*), and a list of International Standards, International Reference Reagents and International Reference Preparations is available (*11*).

It may be desirable for appropriate national (secondary) reference materials, calibrated against international reference materials, to be established by the NCL and made available to manufacturers. However, a multinational or regional approach to the establishment and supply of secondary reference materials may be more efficient. The secondary reference materials should be used in the routine laboratory testing of biological products. Their use permits the expression of *potency* in International Units, and in some cases they may be useful in confirming the *identity* of biological products.

6.3 *Other activities of the national control laboratory*

The NCL, or the laboratories providing the services of an NCL, should devise effective internal control measures to permit the evaluation of their reliability in performing all tests. The inclusion of reference preparations and coded replicate samples in test procedures, simultaneous independent testing, and routine checks on the sensitivity and calibration of instruments are necessary as part of good laboratory practice for the NCL. The NCL should, if possible, participate in collaborative studies with other NCLs and other relevant laboratories to enhance its expertise.

7. Post-licensing monitoring of products

7.1 *Product release*

At the time a product is approved, the NCA should decide whether controls involving the NCA are to be applied to release of batches of the product. This decision will be influenced by the nature of the product. Controls will usually be imposed on complex products and on those obtained by complex manufacturing procedures. The control system may involve the following activities and may be reviewed and revised once satisfactory and consistent production has been demonstrated:

7.1.1 Testing of samples of intermediate, bulk or final product, to confirm compliance with the requirements and agreed specifications. The nature and frequency of the tests to be carried out are decided by the NCA.

7.1.2 Evaluation of the manufacturer's protocols for manufacture and control of each batch. Examples of model summary protocols are annexed to the individual Requirements for Biological Substances published by WHO. The critical review of batch protocols by the NCA is a most important part of the control of biological products. The information provided should make it possible to review the manufacture and testing of each batch of a particular product, including all required in-process controls and control tests on final products, to confirm compliance with the approved specifications.

7.2 *Inspections*

Periodic inspections of the manufacturing facility should be carried out on behalf of the NCA to assure continued compliance with GMP and with the specifications established for the product at the time of approval. Records of complaints and reports of adverse reactions should be examined.

7.3 *Post-marketing surveillance*

The procedures described in sections 7.1 and 7.2 above do not preclude the need for a post-marketing sampling and surveillance system. Countries should establish a national system for the post-marketing surveillance of biological products. Clinicians and other health workers should be encouraged to report to manufacturers and NCAs unexpected adverse events occurring after administration of biological products. The manufacturers and the NCAs should assess these reports and, in consultation with each other, attempt to evaluate their significance. This assessment may require the testing of products already released and inspection of production and control facilities. If an imported product is associated with adverse reactions, the manufacturer and, where appropriate, other NCAs and WHO should be notified.

Guidance for operating a monitoring system for adverse reactions is provided in a report published by the Council for International Organizations of Medical Sciences (*12*).

7.4 *Recall and revocation*

NCAs should have a system for enforcing the recall of batches, revoking approvals, and communicating such decisions to users and to the NCAs of any countries importing the product.

7.5 *Approval of manufacturing changes*

Any significant change to the manufacturing establishment, source materials, production process, quality-assurance procedures, product specifications or labelling should have the prior approval of the NCA.

7.6 *Approval of new indications*

NCAs should require that significant proposed changes in product indications or use be submitted by manufacturers for evaluation and approval.

8. Procedures for approval of imported products

The national authorities of countries wishing to import biological products could simplify the licensing formalities, and reduce the need for testing, by accepting certificates, issued by the responsible authorities in the country of manufacture, stating that the quality of the product meets a certain standard. The WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce (*4*) and GMP texts (*5*, *6*), with the inclusion of the procedures described in this section, provide a suitable basis for such a mechanism. For the purpose of this Certification Scheme, "biological product" refers to a product presented in its finished dosage form and to the bulk material that is processed to produce this dosage form.

8.1 *Participating Member States*

Each Member State participating in the Certification Scheme should communicate to WHO the name and address of the department of its NCA dealing with biological products and, if appropriate, any significant reservations relating to its participation. WHO would then notify all other Member States.

Exporting Member States participating in the Certification Scheme should ensure that:

- approval of biological products is subject to appropriate control testing by the NCA to assure safety and efficacy, and adequate facilities are available for such testing;
- the manufacturer conforms to requirements for GMP and quality assurance of biological products as recommended by WHO, or to equivalent national standards;
- the NCA conducts appropriate inspections, including, for example, examination of records and samples, to ensure that manufacturers conform to these requirements;
- the inspectors in the service of the NCA have appropriate expertise.

Exporting Member States participating in the Certification Scheme should, whenever possible, ensure that International Nonproprietary Names (INN) are used on certificates and for labelling the biological product.

8.2 *Certification of products*

Biological products exported under the Certification Scheme should be certified by the NCA of the exporting Member State by means of certificates to be sent to the NCA of the importing Member State. The importing Member State would then either license the product or make licensing conditional on the submission, and approval, of supplementary data.

The issue of certificates for a biological product would be subject to the conditions set by the NCA of the exporting Member State. Certificates would, however, be expected to state that:

- the product is approved for use within the exporting Member State (if not, the reason should be given); and
- the manufacturing establishment in which the product is produced is inspected at suitable intervals to check that the manufacturer conforms to the principles of GMP (*5, 6*) and quality assurance (as defined in the present guidelines).

For many biological products, certification on an individual lot basis is necessary because of the difficulty of controlling starting materials and ensuring that batch-to-batch variation is within acceptable limits.

Suggested model certificates for biological products are given in Appendices 1 and 2.

8.3 *Requests for additional information*

Additional information may be requested by the NCA of the importing Member State from the NCA of the exporting Member State. This information may be provided directly by the NCA, or through the manufacturer, and may include:

- details of the implementation of requirements for GMP and quality assurance of biological products;
- information on control tests performed on the product by the NCA of the exporting Member State;
- the names and functions of the persons officially designated to sign release certificates for individual batches of the product;
- copies of all documentation and labels that are supplied with the product on packaging materials and package inserts and that have been approved by the NCA in the exporting Member State, together with the date(s) on which such approval was accorded.

Information on general and specific standards for quality assurance of the biological product to be exported may also be requested if so required by

the legislative provisions of the importing Member State. The consent of the manufacturer to the provision of such information should be obtained by the NCA of the exporting Member State.

8.4 *Reporting of defects and adverse reactions*

Defects may occur in the quality of biological products imported under the Certification Scheme. If they are considered to be of a serious nature by the importing country, and are not attributable to local conditions of storage and transport, the NCA of the importing country should notify the NCA of the exporting Member State and provide the relevant data. Adverse reactions of unexpected severity or frequency should also be notified to the NCA of the exporting country. Similarly, if the NCA of the exporting Member State discovers quality defects or receives reports of unexpected adverse reactions, it should inform the NCA of the importing Member State of any action taken.

8.5 *Procedure for testing imported biological products*

Countries wishing not to rely solely on the certification scheme described in sections 8.1-8.4 may consider implementing an abridged version of the system of control applicable to the NCA of the country of manufacture, as described in sections 4-7. The system may include a review of information on the manufacturing establishment (as described in section 5.1) and on the biological product to be imported (as outlined in section 5.2), in order to assess its safety and efficacy. If adequate facilities and personnel of appropriate expertise are available, pre-licensing testing may be performed as described in section 7.1.1, although intermediate samples may not be available. Additionally, the NCA of the importing country may, if necessary, perform control tests on the biological product on a batch-to-batch basis, to obtain assurance regarding safety and efficacy, including the possibility of defects occurring during shipment.

Authors

The draft text of these guidelines was prepared at a meeting of a WHO Ad Hoc Committee on Guidelines for National Authorities and Manufacturers on Quality Assurance for Biological Products, held in Ottawa, Canada, 11-15 June 1990, supported by the Health Protection Branch, Bureau of Biologics, Ottawa, and attended by the following participants:

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Acknowledgements

Acknowledgements are due to the following experts for their comments and advice: Dr J. Cameron, North York, Ontario, Canada; Dr A. Fenyves, Paul Ehrlich Institute, Langen, Germany; Dr C. Guthrie, General Manager, Blood Products Division, CSL Ltd., Parkville, Australia; Dr M. Kantoch, Head, Virology Department, National Institute of Hygiene, Warsaw, Poland; Mr J. Lyng, State Serum Institute, Copenhagen, Denmark; Dr N. V. Medunitsin, Director, Tarasevich State Institute for the Standardization and Control of Medical Biological Preparations, Moscow, USSR.

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Appendix 1

Model certificate of approval of a biological product[1]

Name and dosage form of product: _____

Manufacturer and/or, when applicable, person responsible for placing the product on the market: _____

_____

Address(es): _____

_____

It is certified that:

☐ This product has been authorized to be placed on the market for use in this country.[2]
Number of licence and date of issue (if applicable):[2]

_____

_____

The enclosed documents constitute the complete text of all labelling and prescribing information authorized for use in this country.[2]

or

☐ This product has not been authorized to be placed on the market for use in this country for the following reasons:

_____

_____

It is also certified that (a) the manufacturing plant in which the product is produced is subject to inspections at suitable intervals, and (b) the manufacturer conforms to requirements for good manufacturing practices[3] and other relevant requirements published by WHO, in respect of products to be sold or distributed within the country of origin or to be exported.

Name of designated authorized person (typed) _____

_____ _____

(Signature of designated authorized person) (Place and date)

---

[1] This certificate is intended to define the status of the biological product and its manufacturer in the exporting and manufacturing country. It is issued by the competent national authority in the exporting country in response to a request by the competent national authority in the importing country. It may be required by the importing country at the time of the first importation and subsequently if confirmation or updating is required. The certificate is intended to be product-specific, since confusion will inevitably arise if information relating to different products, or even different dosage forms of the same product, is attached to the same certificate; whenever possible, International Nonproprietary Names (INN) or national nonproprietary names should be used.

[2] Delete as appropriate.

[3] Good manufacturing practices for pharmaceutical products. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-second Report.* Geneva, World Health Organization, 1992 (WHO Technical Report Series, No. 823), Annex 1; Good manufacturing practices for biological products. In: *WHO Expert Committee on Biological Standardization. Forty-second Report.* Geneva, World Health Organization, 1992 (WHO Technical Report Series, No. 822), Annex 1.

Appendix 2

Model certificate for the release of a lot or lots of a biological product

The following biological product _____, produced by _____[1] in _____,[2] whose lot numbers appear on the labels of the final containers, meets all national requirements (_____[3]) and is manufactured in accordance with the requirements for good manufacturing practices[4] and (if applicable) the product-specific requirements published by WHO.

Lot number	Expiry date
_____	_____
_____	_____
_____	_____
_____	_____

As a minimum this certificate is based on examination of the manufacturing protocol.

Address of the National Control Authority or Laboratory _____

_____

_____

Name of the Director of the National Control Authority or Laboratory (or representative)

_____

Signature _____

Date _____

[1] Name of manufacturer.

[2] Country.

[3] Reference to appropriate document.

[4] Good manufacturing practices for pharmaceutical products. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-second Report.* Geneva, World Health Organization, 1992 (WHO Technical Report Series, No. 823), Annex 1; Good manufacturing practices for biological products. In: *WHO Expert Committee on Biological Standardization. Forty-second Report.* Geneva, World Health Organization, 1992 (WHO Technical Report Series, No. 822), Annex 1.

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Annex 3
Guidelines for assuring the quality of monoclonal antibodies for use in humans

Introduction 48

Scope 48

General considerations 49

Part A. Manufacturing guidelines 50
A.1 Definitions 50
A.2 Reference materials 51
A.3 General manufacturing requirements 52
A.4 Production control 57
A.5 Distribution and shipping 61
A.6 Stability test and validity period 61

Part B. National control guidelines 62
B.1 General 62
B.2 Release certification 63

Authors 63

Acknowledgements 64

References 65

Appendix 1
Possible viral contaminants in hybridomas of murine origin 66

Appendix 2
Human tissues that should be considered for immunohistochemical investigations of the reactivity of monoclonal antibodies 67

Appendix 3
Model certificate for the release of monoclonal antibodies 68

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Annex 1

Regulation and licensing of biological products in countries with newly developing regulatory authorities

1. Introduction 22

2. Scope 22

3. General considerations 23

4. Structure and function of a national control authority for biological products 24
   - 4.1 Personnel 24
   - 4.2 Administration 25
   - 4.3 Registration documents 25
   - 4.4 Good manufacturing practices inspectorate 25

5. Aspects of the licensing process 26
   - 5.1 Establishing a registration system for existing medical (biological) products 26
   - 5.2 Screening of provisionally registered products 26
   - 5.3 New product licences 27
   - 5.4 Renewal and variation of licences 27
   - 5.5 Information on the manufacturing establishment 28
   - 5.6 Information on the product 29

6. Alternative procedures for approval of imported products – the WHO Certification Scheme 30
   - 6.1 Participating countries 30
   - 6.2 Certification of products 31
   - 6.3 Requests for additional information 31
   - 6.4 Reporting of defects and adverse reactions 32

7. Authorization of clinical trials 32

8. Post-licensing monitoring 32
   - 8.1 Product release 32
   - 8.2 Inspections 33
   - 8.3 Post-licensing surveillance 33
   - 8.4 Recall and revocation 34
   - 8.5 Approval of manufacturing changes 34
   - 8.6 Approval of new indications 34

9. Powers of enforcement 34

Authors 35

References 35

Other documents of interest 35

1. Introduction

National health authorities have the duty to ensure that available pharmaceutical products, whether imported or manufactured locally, are of good quality, safe and efficacious. This is particularly difficult for vaccines and other biological products, the quality of which cannot be established entirely by tests on the material in the final container. A national control authority should therefore be established that is responsible for ensuring that the manufacturer is adhering to approved standards of good manufacturing practice and quality assurance specific to the product. The procedures through which the national control authority confirms the assurance of quality provided by the manufacturer will depend on the resources available and whether the product is manufactured locally or imported.

In general, biological products are distinguished from other drugs by being derived from living organisms (ranging from normal or genetically modified microorganisms to fluids and tissues derived from various animal and human sources) and frequently have a complex molecular structure. They require special quality considerations because of the biological nature of: (a) the starting materials; and/or (b) the manufacturing process; and/or (c) the test methods needed to characterize batches of the product.

Developments in biological products have been extremely rapid in recent years, and the potential value of such products in improving health care on a global scale is immense. There is an urgent need to match technological advances with appropriate mechanisms for assuring the safety, quality and efficacy of the products.

2. Scope

The aim of this Annex is to provide guidance for newly developing national control authorities that may have limited resources to license and regulate biological products, including vaccines. It describes the responsibilities of such authorities and manufacturers and provides references to relevant WHO publications relating to their structure and activities. References to more detailed technical requirements published by WHO, specific for various products including vaccines, are also provided (*1*, Annex 7).

Much of the material included in this Annex is drawn from guidelines previously published by WHO in the reports of the WHO Expert Committee on Biological Standardization and the WHO Expert Committee on Specifications for Pharmaceutical Preparations (see, in particular, *1*, Annex 2; *2*, Annex 3; *3*, Annexes 5 and 6).

3. General considerations

The safety, quality and efficacy of a biological product are primarily the responsibility of the manufacturer; however, the national control authority of each country is responsible for establishing procedures for assuring that biological products intended for use in the country are of adequate quality, safety and efficacy. This responsibility should have a firm statutory basis backed by legislation. Marketing approval (licensing) for a biological product should be granted by a national control authority, which should also be responsible for continued monitoring after licensing. In carrying out these activities, the authority should make use of expert committees and technical advisers and have access to laboratory facilities for the testing of in-process and final product samples.

Countries should have written standards, both general and product-specific, for biological products available for use in them. These should be based on up-to-date standards, such as those available from WHO, and harmonized as far as possible with those of other countries. For new products, WHO, national and pharmacopoeial requirements may not have been established, and the national control authority will need to agree on specifications with the manufacturer on a case-by-case basis. In such cases, WHO or the authority in the country of origin could be consulted and their advice requested.

In general terms, the national control authority should be vested with legal powers to:

(a) issue, vary and revoke licences for biological products on grounds of quality, safety and efficacy;
(b) secure the subsequent safe and effective use of each product by controlling, under the terms of the product licence, the content of all labelling (including package inserts, associated prescribing information and advertising) and the channels through which the product may legitimately be supplied; and
(c) inspect and license all manufacturing premises and importing agents and, where applicable, wholesalers and distributors, hospital dispensaries, independent pharmacies and other retail outlets to ensure that they comply with the relevant regulations and guidelines.

In countries where biological products are manufactured, the national control authority should have the appropriate expertise to evaluate the adequacy of the manufacturer's establishment and facilities, starting materials, production processes, control-test procedures and product specifications, to determine whether they meet international and/or national requirements. Guidelines for national control authorities on quality assurance for biological products have been published by WHO (*1*, Annex 2).

Control (laboratory) activities should be fully independent of those of the manufacturer so as to ensure that the national control authority

underta ts tasks in an independent, authoritative and impartial manner. In some countries, the national laboratory facilities may constitute a separate entity called the national control laboratory. In this case, the laboratory should be administered directly by, or on behalf of, the national control authority.

In countries where biological products are not manufactured, alternative approaches can be defined, e.g. the WHO Certification Scheme (*2*, Annex 3) for assuring the safety, quality and efficacy of such products (see section 6). However, an approval process limited to a mere listing of facilities and products would not be considered adequate.

In view of the complexity and cost of certain facilities needed for control testing, it may be unavoidable in certain cases that the authority will have to share such facilities with the manufacturer or an academic institution, or to rely on those of an authority or laboratory in another country.

National control authorities should, whenever appropriate, exchange information on safety and other issues, within the normal legal constraints of confidentiality.

4. Structure and function of a national control authority for biological products

The health authorities should establish and maintain a competent national control authority with a defined organizational structure including, if relevant, a competent laboratory facility. The responsibilities, relationships, coordination, and legal status of employees should be specified in the light of their roles in a clearly defined decision-making process.

The authority can be either independent or part of the ministry of health. It is advisable to delegate decision-making responsibilities to those departments within the authority or laboratory with the necessary competence in the field of biologicals so as to facilitate the licensing/regulation process and ensure that it is carried out efficiently.

It is also recommended that the national control authority should make use of external expert advisers and advisory committees with appropriate expertise. Caution should be exercised when identifying such advisers to avoid conflicts of interest.

4.1 Personnel (see also *1*, p. 34)

The personnel of the authority or laboratory should include person(s) qualified and experienced in the control of biological products and experts in all appropriate disciplines. The qualifications and experience of the staff at all levels should be appropriate to the review and control activities required for the range of biological products to be controlled.

All staff members of the authority should undergo suital ining and should therefore attend "hands-on" training courses covering both the technical and administrative aspects of licensing and control procedures.

4.2 Administration (see also *1*, p. 34)

The national control authority should have established procedures for the receipt and review of manufacturers' submissions and, if applicable, for testing samples provided in support of applications. When the review procedures, which include the evaluation of detailed reports, have been completed, a notice of approval (licence) or disapproval is sent by the authority to the manufacturer. It may also issue notices of suspension or revocation of approval. Consideration should be given to making appropriate legal expertise available in support of this activity. It is of the utmost importance that proprietary and commercial information is kept confidential.

4.3 Registration documents (see also *1*, pp. 34–35)

The national control authorities should provide guidance on the information to be provided, the format to be used, and the acceptability of specific forms, and should maintain adequate filing and archiving facilities so that all submissions, evaluations, records and correspondence are available and kept up to date. A computer program for drug regulatory authorities can be obtained from WHO.[1]

The national control authority should possess, or have access to, library facilities appropriate to its fields of activity. The documents available should include current national and international requirements for biological substances, and other relevant specifications and recommendations published by WHO or other official bodies.

4.4 Good manufacturing practices inspectorate

The national control authority should have access either to suitably qualified inspectors who are independent of manufacturers or to recent inspection reports from qualified inspectors. The purpose of inspections is to ensure that each manufacturer's facilities and procedures comply with the principles of good manufacturing practice as described in national or WHO publications (*1*, Annex 1) and with the requirements and/or conditions for the approval of the product concerned. Guidelines are available on the conduct of inspections of manufacturers of drugs and biologicals (*1*, Annex 2; *2*, Annex 2).

[1] Available on request, together with a user manual, from the Division of Drug Management and Policies, World Health Organization, 1211 Geneva 27, Switzerland.

5. Aspects of the licensing process

5.1 Establishing a registration system for existing medical (biological) products (see also *3*, p. 71)

Before any system of control can be effective, it is necessary to identify and catalogue all the products already sold or otherwise supplied on the domestic market, in both the public and the private sectors, that qualify for control. Guiding principles for national drug regulatory authorities that have yet to introduce comprehensive legal provisions on drug regulation have been published by WHO (*3*, Annex 6). An appointed day should be established after which no existing biological products may be lawfully distributed or supplied unless they have been notified to the authority, and no new product may be introduced until a request for a product and establishment licence have been granted by it.

The effective administration of the provisional registration procedure depends on:

(a) the prior identification of all interested manufacturers and importers;
(b) a precise definition of a notifiable biological product based primarily on the claims made for it on the label and the indications for use;
(c) the issue of guidelines on the procedure to be followed.

Each notified product, as a minimum, must be identified by name, the names and full addresses of the manufacturer and of the responsible agent, if any, representing the manufacturer, the name and full address of an importing agent, a brief description of the manufacturing procedure including a declaration by the manufacturer that good manufacturing practices have been followed and a manufacturing flow chart, a description of the dosage form, its composition (including active and inactive ingredients (using International Nonproprietary Names where appropriate)), the therapeutic class, the indications, a copy of all labelling, including any package insert, a copy of any relevant certificates and warranties relating to the product or its components, and appropriate shipment conditions.

It is desirable for an updated compendium to be available containing information for the physician on approved products. This should distinguish those products given provisional approval from those given final approval (new product licences).

5.2 Screening of provisionally registered products (see also *3*, pp. 71–72)

A rapid screening of all provisionally registered products should be undertaken at the earliest opportunity with a view to securing the withdrawal from the market of any product which, simply on the basis of a review of its ingredients and indications, is judged not to meet appropriate safety standards.

After this preliminary review, a set of longer-term priorities needs to be set for the definitive assessment of provisionally registered products. Consideration needs to be given to the resources required, in terms of both personnel and information. The review should be adapted to a proposed time-schedule, depending on which, additional information may be requested by the national control authority from provisionally registered companies, e.g. periodic safety updates or evidence of lack of efficacy.

5.3 New product licences (see also *3*, pp. 73–74)

The licensing of a vaccine or other biological product requires the issue of licences for both the manufacturing establishment *and* the product. The approval or licensing of a manufacturing establishment for the production of biological products should be granted only if the manufacturer complies with the relevant international or equivalent national standards for good manufacturing practice.

A licence for a given biological product will be issued by the national control authority only when it is satisfied that the product is in conformity with the relevant national and/or international requirements, including the manufacturer's specifications, applicable to it.

The normal procedure for the issue of a product licence consists of the following three stages:

(a) the manufacturing establishment and product licence applications are received from the manufacturer, screened for completeness, and then reviewed for evidence of compliance with good manufacturing practices and for safety, quality and efficacy by the authority's technical staff;
(b) the authority may perform laboratory tests, review reports of or perform pre-licensing inspections, and seek the advice of external experts on specific technical questions when deciding whether or not to authorize the marketing of the product;
(c) the formal administrative action to grant or refuse a licence is then taken by the designated authorized person.

The assessment of the product must be based on its safety, quality and efficacy when used as intended. However, the availability of the product may be dictated by national policy considerations, such as the national need for comparative efficacy and/or safety, or cost-effectiveness. Advice on basic or essential drugs is published periodically by WHO (*4*).

5.4 Renewal and variation of licences (see also *3*, pp. 74–75)

The precise circumstances under which licence-holders are required to apply for a renewal or variation in a product licence differ from country to country and should be clearly defined by the national authority. In general, if a manufacturer wishes to vary the conditions of the approved

licence to [illegible] significant extent, the variations must be submitted to the authority for approval. Significant changes might include changes in aspects of the manufacturing procedures or the facility, or in the product specifications, dosage forms or labelling. The procedure for the renewal of licences is more variable. In many countries, reregistration, but not licence renewal, is required annually. In others, licences must be renewed every 5 or 7 years.

Licence-holders should be required, in all circumstances, to inform regulatory authorities immediately of unanticipated adverse effects that could possibly be associated with a licensed product and that might call for the licence to be made subject to certain restrictions or withdrawn (see section 8).

5.5 Information on the manufacturing establishment (see also *1*, pp. 35-36)

The manufacturer should provide sufficient information to demonstrate compliance with the principles of good manufacturing practices, including the existence of adequate quality-assurance systems. Plans, diagrams, flow charts, standard operating procedures and texts may be used to convey the necessary information in relation to (but not limited to):

- Personnel, and in particular their qualifications and experience, organization and reporting relationships, training schedules and record-keeping systems.
- The location and construction of the building used for manufacture and control.
- The flow of raw materials, personnel and manufactured product through the facility.
- The animal facilities.
- Air, water and steam systems and power supply.
- Drainage and effluent systems.
- The segregation of operations.
- Lists of major equipment.
- Maintenance schedules for equipment and building services.
- Cleaning procedures, schedules and control measures.
- Quality-assurance and quality-control procedures.
- Storage and quarantine facilities, and procedures for raw materials, packaging materials, in-process and bulk materials, and final product.
- Validation procedures.
- Documentation and record-keeping systems.
- Labelling and packaging facilities and procedures.
- Recall and retrieval procedures.

To be certain that the buildings, facilities, personnel, procedures and practices comply with the description in the licence application, the national control authority should review the availability of the

manufacturer's inspection reports. If a report is availab[illegible] on the country of origin, this should suffice. Alternatively, if no report is available, or no inspection has been performed, an inspection should be conducted before a licence is issued. The inspectors selected should be independent of the manufacturer and have sufficient expertise to conduct a meaningful review in accordance with the system of good manufacturing practices in use; this should include buildings, facilities, procedures, personnel and quality assurance.

5.6 Information on the product (see also *1*, pp. 36-37)

The manufacturer should provide sufficient information to demonstrate the safety, quality and effectiveness of the product as manufactured and controlled in the establishment described above, and should refer for guidance to the national requirements or, if these are not available, the relevant technical requirements published by WHO. The submission should include the following, if appropriate:

- Information on the source materials (e.g. microorganisms, blood/plasma donations, cells/cell substrate, pollen), including their specifications and the tests used to demonstrate compliance with the specifications.
- A description of the cold-chain procedures employed.
- Information on the raw materials and packaging materials, including their specifications and the tests used to demonstrate compliance.
- Information on the methods of manufacture, including a description of the seed lot and cell-substrate systems used, together with in-process, bulk and final product specifications, and the tests employed to demonstrate compliance.
- The demonstration of the consistency of manufacture, for which the results of tests on a minimum of three satisfactory and consecutive production batches, ideally of different bulk lots, of a size corresponding to that contemplated for routine production, are normally required.
- Any proposal for the reprocessing of the product.
- The results of stability studies undertaken to justify the proposed validity period for the product under the indicated storage conditions.
- The labels and package inserts.
- The documentation used in the manufacturing and control procedures, including standard operating procedures and protocols containing details of production and quality control testing.
- Reports of preclinical studies.
- Clinical trial data.
- A list of countries in which the product is approved for use.

The nature and extent of pre-licensing testing undertaken by the national control authority should reflect any particular considerations relevant to the product. Chemical, physical and biological tests additional to those specified in national or international requirements may also be performed.

For imported products, results of testing may be obtained under the WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce (see section 6).

6. Alternative procedures for approval of imported products – the WHO Certification Scheme

The national authorities of countries wishing to import biological products can simplify the licensing formalities, and reduce the need for testing, by accepting certificates issued by the responsible authorities in the country of manufacture, stating that the quality of the product meets a certain standard. The WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce, as summarized in this section, provides a suitable basis for the approval and release of imported products (*2*, Annex 3; *3*, Annex 5). For the purpose of this Certification Scheme, "biological product" refers to a product presented in its finished dosage form and to the bulk material that is processed to produce this dosage form.

6.1 Participating countries

Each country participating in the Certification Scheme should communicate to WHO the name and address of the department of its national control authority dealing with biological products and, if appropriate, any significant reservations relating to its participation. WHO would then notify all other countries.

Exporting countries participating in the Certification Scheme should ensure that:

- the approval of biological products is subject to appropriate control testing by the national control authority to guarantee safety and efficacy, and that adequate facilities are available for such testing;
- the manufacturer complies with the requirements for good manufacturing practices and quality assurance of biological products as recommended by WHO, or with equivalent national standards;
- the national control authority conducts appropriate inspections including, for example, the examination of records and samples, so as to ensure that manufacturers comply with these requirements;
- the inspectors in the service of the authority possess the appropriate expertise.

Exporting countries participating in the Certification Scheme should, whenever possible, ensure that International Nonproprietary Names are used on certificates and for labelling the biological product (*5*).

6.2 Certification of products

Biological products exported under the Certification Scheme should be certified by the national control authority of the exporting country by means of certificates to be sent to the corresponding authority of the importing country. The importing country can then either license the product or make licensing conditional on the submission and approval of supplementary data.

The issue of certificates for a biological product would be subject to the conditions set by the authority of the exporting country. Certificates would, however, be expected to state that:

- the product is approved for use within the exporting country or, if not, the reason for which approval has not been obtained;
- the manufacturing establishment in which the product is produced is inspected at suitable intervals to check that the manufacturer complies with the principles of good manufacturing practices and quality assurance (see *1*, Annex 2).

For many biological products, certification on an individual lot basis is necessary because of the difficulty of controlling the starting materials and ensuring that batch-to-batch variation is within acceptable limits.

6.3 Requests for additional information

Additional information may be requested by the national control authority of the importing country from the corresponding authority of the exporting country. This information may be provided directly by the latter, or through the manufacturer, and may include:

- Information showing that the requirements for good manufacturing practices and quality assurance of biological products have been satisfied.
- Information on control tests performed on the product by the authority of the exporting country.
- The names and functions of the persons officially designated to sign release certificates for individual batches of the product.
- Copies of all documentation and labels supplied with the product on packaging materials and package inserts and approved by the authority in the exporting country, together with the date(s) on which such approval was accorded.

Information on general and specific standards for quality assurance of the biological product to be exported may also be requested if so required under the legislation of the importing country. Of concern, in particular, is information that will ensure that the quality of the product will not be adversely affected by the storage and shipment conditions. The consent of the manufacturer to the provision of such information should be obtained by the national control authority of the exporting country.

6.4 Reporti f defects and adverse reactions

Defects may occur in biological products imported under the Certification Scheme. If they are considered to be of a serious nature by the importing country, and are not attributable to local conditions of storage and transport, the national control authority of the importing country should notify the corresponding authority of the exporting country and provide the relevant data. Adverse reactions of unexpected severity or frequency should also be notified to the authority of the exporting country. Similarly, if the authority of the exporting country discovers quality defects or receives reports of unexpected adverse reactions, it should inform the corresponding authority of the importing country of the problem and any action taken. Guidance on the reporting of adverse drug reactions is contained in the report of a Working Group of the Council for International Organizations of Medical Sciences (CIOMS) (*6*, Annex 1).

7. Authorization of clinical trials

An authority may occasionally need to consider an application to conduct a clinical trial of an unapproved product for the prevention or treatment of a condition. To provide for this contingency, the registration system should include provision for the importation of the necessary materials, subject to appropriate controls. Such trials should take place only after formal clearance has been obtained from the competent registration authority and after assurances have been obtained that they will be conducted in conformity with the principles contained in the World Medical Assembly's Declaration of Helsinki, the good clinical practices guidelines of national authorities, and the CIOMS guidelines (*7*, Annex 1).

8. Post-licensing monitoring

(see also *1*, pp. 39–40)

8.1 Product release

At the time a product is approved, the national control authority should decide what controls are to be applied to the release of batches of the product. This decision will be influenced by the nature of the product and the resources available for laboratory testing. Controls will usually be imposed on complex products, e.g. vaccines, and on those obtained by complex manufacturing procedures. The control system may involve the activities described below, and may be reviewed and revised once satisfactory and consistent production has been demonstrated:

The *testing of samples* of intermediate, bulk or final p ct should confirm compliance with the requirements and agreed spec ations (see section 5). The nature and frequency of the tests to be carried out are decided by the national control authority.

The *evaluation of the manufacturer's protocols* for the manufacture and control of each batch will be undertaken by the national control authority. Examples of model summary protocols are annexed to the individual requirements for biological substances published by WHO. The critical review of batch protocols by the authority is a most important part of the control of biological products. The information provided should make it possible to review the manufacture and testing of each batch of a particular product, including all required in-process controls and control tests on final products to confirm compliance with the approved specifications.

8.2 Inspections

Periodic inspections of the manufacturing facility should be carried out on behalf of the national control authority to assure continued compliance with good manufacturing practices and with the specifications established for the product at the time of approval. Records of complaints and reports of adverse reactions should be examined.

8.3 Post-licensing surveillance

The procedures described in sections 8.1 and 8.2 above do not preclude the need for a post-licensing sampling and surveillance system. Countries should establish a national system for the post-licensing surveillance of biological products. Clinicians and other health workers should be encouraged to report to national control authorities and manufacturers any unexpected adverse events occurring after the administration of biological products.

The mechanisms for reporting (e.g. standardized forms), the receiving body (e.g. the national control authority), the deadline for reporting (e.g. 48 hours), and the types of adverse events reportable need to be clearly defined by the authority and will depend on its structure and resources. The manufacturers and the authorities should assess these reports and, in consultation with each other, attempt to evaluate their significance. This assessment may require the testing of products already released and the inspection of production and control facilities and local distribution channels. If an imported product is associated with adverse reactions, the manufacturer and, where appropriate, other national control authorities and WHO should be notified.

Guidance on the operation of a monitoring system for adverse reactions is provided in a CIOMS report (*6*). The national control authority or another government body may decide to perform epidemiological studies to obtain further information on the performance of products in actual use.

8.4 Recall and revocation

National control authorities should have a system for enforcing the recall of batches, revoking approvals, communicating such decisions to users, and ensuring that the authorities of other receiving countries importing the product are notified accordingly.

8.5 Approval of manufacturing changes

Any significant change in the manufacturing establishment, source materials, production process, quality-assurance procedures, product specifications or labelling is subject to the prior approval of the national control authority. Significant changes in the manufacturing process may require major modifications to the existing licence, and the submission of new lots of product to demonstrate consistency of manufacture and new clinical data.

8.6 Approval of new indications

National control authorities should require manufacturers to submit significant proposed changes in product indications for use for evaluation and approval. An authority may request additional clinical data if changes in the dosage regimen, including the dose, route, frequency or timing, are proposed.

9. Powers of enforcement

In order to assume its responsibilities, an authority must possess powers of enforcement backed by legal provisions for imposing penalties for offences such as misbranding, adulteration and the sale of counterfeit products. It should also have the power to order the suspension of lot release, seizure or recall when necessary to prevent the release of suspect lots or to ensure that suspected hazardous materials are withdrawn.

In establishing administrative decision-making mechanisms, the authority should not lose flexibility. In particular, it should make provision for:

- implementing decisions regarded as urgent in the interest of public safety;
- formal consultation (usually through representative bodies) with pharmaceutical companies and other interested parties, including pharmacists, doctors, nurses and patients.

The authority should publish a list of licensed products and sources at regular intervals.

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1. *WHO Expert Committee on Biological Standardization. Forty-second Report* Geneva, World Health Organization, 1992 (WHO Technical Report Series, No. 822).
2. *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-second Report.* Geneva, World Health Organization, 1992 (WHO Technical Report Series, No. 823).
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Other documents

1. *International list of availability of vaccines.* Geneva, World Health Organization, (unpublished document under revision; available on request from Vaccine Supply and Quality, Global Programme for Vaccines and Immunization, World Health Organization, 1211 Geneva 27, Switzerland).
2. *List of national control authorities.* Geneva, World Health Organization, 1994 (unpublished document Pharm/86.39; available on request from Regulatory Support, World Health Organization, 1211 Geneva 27, Switzerland).

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WHO Technical Report Series, No. 863, 1996

Annex 10

Guidelines for implementation of the WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce

1. Provisions and objectives 155
2. Eligibility for participation 156
3. Requesting a certificate 157
4. Issuing a certificate 160
5. Notifying and investigating a quality defect 161

References 162

Appendix 1
Model Certificate of a Pharmaceutical Product 163

Appendix 2
Model Statement of Licensing Status of Pharmaceutical Product(s) 168

Appendix 3
Model Batch Certificate of a Pharmaceutical Product 170

Appendix 4
Glossary and index *[not intended to be a formal part of the Scheme]* 173

1. Provisions and objectives

1.1 A comprehensive system of quality assurance must be founded on a reliable system of licensing[1] and independent analysis of the finished product, as well as on an assurance obtained through independent inspection that all manufacturing operations are carried out in conformity with accepted norms referred to as "good manufacturing practices" (GMP).

1.2 In 1969, the Twenty-second World Health Assembly, by resolution WHA22.50, endorsed requirements for "Good practices in the manufacture and quality control of drugs"(*1*) (referred to henceforth as "GMP as recommended by WHO"). These comprise internationally recognized and respected standards that all Member States are urged to adopt and to apply. These requirements have since been revised twice. The first revision was adopted by the Health Assembly in 1975 in

[1] Throughout this document licensing refers to any statutory system of approval required at national level as a precondition for placing a pharmaceutical product on the market.

resolution WHA28.65 (*2*), and a second revision of the requirements is included in the thirty-second report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations (*3*).

1.3 These standards are fully consonant with those operative within the countries participating in the Convention for the Mutual Recognition of Inspection in Respect of the Manufacture of Pharmaceutical Products, and other major industrialized countries. They also provide the basis for the WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce (referred to henceforth as "the Scheme") recommended initially in resolution WHA22.50 (*1*). The Scheme is an administrative instrument that requires each participating Member State, upon application by a commercially interested party, to attest to the competent authority of another participating Member State that:

- a specific product is authorized to be placed on the market within its jurisdiction or, if it is not thus authorized, the reason why that authorization has not been accorded;
- the plant in which it is produced is subject to inspections at suitable intervals to establish that the manufacturer conforms to GMP as recommended by WHO; and
- all submitted product information, including labelling, is currently authorized in the certifying country.

1.4 The Scheme, as subsequently amended in 1975 (*2*) and 1988 (*4*) by resolutions WHA28.65 and WHA41.18, is applicable to finished dosage forms of pharmaceutical products intended for administration to human beings or to food-producing animals.

1.5 Provision for the certification of active ingredients is also included within the scope of the Scheme. This will be the subject of separate guidelines and certificates.

2. Eligibility for participation

2.1 Any Member State intending to participate in the Scheme may do so by notifying the Director-General of WHO, in writing, of:

- its willingness to participate in the Scheme;
- any significant reservations it intends to observe relating to this participation; and
- the name and address of its national drug authority or other competent authority.

2.2 These notifications are subsequently announced in the monthly *WHO pharmaceuticals newsletter.* An updated consolidated list will be published annually in the newsletter and will be available to governments at other times from the Division of Drug Management and Policies, WHO, 1211 Geneva 27, Switzerland. (See also section 3.3).

2.3 A Member State may opt to participate solely to control the *imp* of pharmaceutical products and active substances. This intention shou be stated explicitly in its notification to WHO.

2.4 A Member State intending to use the Scheme to support the *export* pharmaceutical products should first satisfy itself that it possesses:

- An effective national licensing system, not only for pharmaceuti products, but also for the responsible manufacturers and distributor
- GMP requirements, consonant with those recommended by WH to which all manufacturers of finished pharmaceutical products a required to conform.
- Effective controls to monitor the quality of pharmaceutical produ registered or manufactured within the country, including access to independent quality control laboratory.
- A national pharmaceuticals inspectorate, operating as an arm of t national drug regulatory authority, and having the technic competence, experience and resources to assess whether GMP a other controls are being effectively implemented, and the legal pow to conduct appropriate investigations to ensure that manufacture conform to these requirements by, for example, examining premis and records and taking samples.
- The administrative capacity to issue the required certificates, institute inquiries in the case of complaint, and to notify expeditious both WHO and the competent authority in any Member State know to have imported a specific product that is subsequently associat with a potentially serious quality defect or other hazard.

2.5 Each Member State assumes the responsibility to determine, throu a process of self-evaluation, whether it satisfies these prerequisites. T Scheme contains no provision for external inspection or assessme under any circumstances, either of a competent national authority or a manufacturing facility. However, should a Member State so wish, can approach WHO, or a well recognized drug regulatory authorit occasionally to delegate consultants to act as advisers in the course both national inspections and inspector training activities.

3. Requesting a certificate

3.1 Three documents can be requested within the scope of the Scheme

- a Certificate of Pharmaceutical Product (product certificate);
- a Statement of Licensing Status of Pharmaceutical Product(s); and
- a Batch Certificate of a Pharmaceutical Product.

3.2 Proposed formats for these documents are provided in Appendices 2 and 3 of these guidelines. To facilitate their use, they are presented forms suitable for generation by computer. All participating countries a henceforth urged to adopt these formats to facilitate the interpretation certified information. Requests for the provision of certificates offerir

more limited attestations – for instance, that the manufacturer complies with GMP or that the product is authorized for "free sale" within the country of export – are discouraged. Similarly, requests should not be made for the certification of information going beyond the scope of the Scheme. When manufacture takes place in a country other than that where the product certificate is issued, an attestation that such manufacture complies with GMP may still be provided as an attachment to the product certificate on the basis of inspections undertaken for registration purposes. The explanatory notes attached to the three documents referred to above are very important. While they are not part of the documents, they should always be attached to them.

3.3 A list of addresses of competent national regulatory authorities participating in the Scheme that are responsible for the registration of pharmaceutical and/or veterinary products, together with details of any reservations they have declared regarding their participation in the Scheme may be obtained from WHO as indicated in section 2.2.

3.4 The competent authority in each country participating in the Scheme should issue guidelines to all agents responsible for importing pharmaceutical products for human and/or veterinary use that operate under its jurisdiction, including those responsible for public sector purchases, to explain the contribution of certification of the drug regulatory process and the circumstances in which each of the three types of documents will be required.

Certificate of a Pharmaceutical Product

3.5 The Certificate of a Pharmaceutical Product (Appendix 1), issued by the exporting country, is intended for use by the competent authority within an importing country in two situations:

- when the product in question is under consideration for a product licence that will authorize its importation and sale;
- when administrative action is required to renew, extend, vary or review such a licence.

3.6 All requests for certificates should be channelled through the agent in the importing country (see section 3. 4) and the product-licence holder or other commercially interested party in the exporting country ("the applicant"). The applicant should submit the following information for each product to the authority issuing the certificate:

- the name and dosage form of the product;
- the name and the amount of active ingredient(s) per unit dose (the International Nonproprietary Name(s), where such exist(s), should be used);
- the name and address of the product-licence holder and/or manufacturing facility;
- the formula (the complete qualitative composition including all exci pients); this is particularly important when no product licence exists o when the formulation differs from that of the licensed product;
- product information for health professionals and for the publi (patient information leaflets) as approved in the exporting country.

For product information to be attached to the certificate, see section 4.7

3.7 The certificate is a confidential document. As such, it can be issue by the competent authority in the exporting country ("the certifyin authority") only with the permission of the applicant and, if different, o the product-licence holder.

3.8 The certificate is intended to be incorporated into a product-licenc application in the importing country. Once prepared, it is transmitted t the requesting authority through the applicant and, when applicable, th agent in the importing country.

3.9 When any doubt arises about the status or validity of a certificate, th competent authority in the importing country should request a cop directly from the certifying authority, as provided for in section 4.9 o these guidelines.

3.10 In the absence of any specific agreement, each certificate will b prepared exclusively in the working language(s) of the certifyin authority. The applicant will be responsible for providing any notarize translation that may be required by the requesting authority.

3.11 Since the preparation of certificates imposes a significar administrative load on certifying authorities, the service may need to b financed by charges levied upon applicants.

3.12 Supplementary attestations are obtainable only at the discretion o the certifying authority and with the permission of the applicant. Th certifying authority is under no obligation to supply additional infor mation. Requests for supplementary information should consequently b referred to the applicant, and only in exceptional circumstances to th certifying authority.

Statement of Licensing Status

3.13 The Statement of Licensing Status of Pharmaceutical Product(s (Appendix 2) attests only that a licence has been issued for a specific product, or products, for use in the exporting country. It is intended fc use by importing agents when considering bids made in response to a international tender, in which case it should be requested by the agent a a condition of bidding. It is intended only to facilitate the screening an preparation of information. The importation of any product that i provisionally selected through this procedure should be determined o the basis of a Certificate of a Pharmaceutical Product.

Batch Certificate

3.14 A Batch Certificate of a Pharmaceutical Product (Appendix 3) refers to an individual batch of a pharmaceutical product, and is a vital instrument in drug procurement. The provision of a Batch Certificate is usually a mandatory requirement in tender and procurement documents.

3.15 A Batch Certificate is normally issued by the manufacturer and only *exceptionally,* as in the case of vaccines, sera and some other biological products, by the competent authority of the exporting country. The Batch Certificate is intended to accompany and provide an attestation concerning the quality and expiry date of a specific batch or consignment of a product that has already been licensed in the importing country. The Batch Certificate should include the specifications of the final product at the time of batch release and the results of a full analysis undertaken on the batch in question. In most circumstances these certificates are issued by the manufacturer to the importing agent (i.e. the product-licence holder in the importing country), but they must be made available at the request of – or in the course of any inspection made on behalf of – the competent national authority.

4. Issuing a certificate

4.1 The certifying authority is responsible for assuring the authenticity of the certified data. Certificates should not bear the WHO emblem, but a statement should always be included to confirm whether or not the document is issued in the format recommended by WHO.

4.2 When the applicant is the manufacturer of the finished dosage form, the certifying authority should satisfy itself, before attesting compliance with GMP, that the applicant:

(a) applies identical GMP standards to the production of *all* batches of pharmaceutical products manufactured within the facility, *including those destined exclusively for export;*

(b) consents, in the event of identification of a quality defect consonant with the criteria set out in section 5.1, to relevant inspection reports being released, in confidence, to the competent authority in the country of import, should the latter so require.

4.3 When the applicant is not the manufacturer of the finished dosage form, the certifying authority should similarly satisfy itself – in so far as it has authority to inspect the records and relevant activities of the applicant – that it has the applicant's consent to release relevant reports on the same basis as described in section 4.2 (b) above.

4.4 GMP as recommended by WHO assigns to the manufacturer of the finished dosage form responsibility for assuring the quality of active ingredients. National regulations may require that suppliers of active ingredients be identified in the product licence, but the competent authority may have no power to inspect them.

4.5 Notwithstanding this situation, a certifying authority may agree, on a discretionary and voluntary basis, and at the request of a manufacturer, to undertake an inspection of a manufacturer of active ingredients to satisfy specific requirements of a requesting authority. Alternatively, pending the development of specific guidelines for active pharmaceutical ingredients, the certifying authority may be able to attest that the manufacturer is an established supplier of the substance in question to manufacturers of finished dosage forms licensed for marketing under its jurisdiction.

4.6 Whenever a product is purchased through a broker or another intermediary, or when more than one set of premises has been involved in the manufacture and packaging of a product, the certifying authority should consider whether it has received sufficient information to satisfy itself that those aspects of the manufacture of the product for which the applicant is not directly responsible have been undertaken in compliance with GMP as recommended by WHO.

4.7 The certifying authority should officially stamp and date all copies of product information submitted to it in support of an application for a certificate and intended to be appended to the certificate. Every effort should be made to ensure that certificates and all annexed documentation are consonant with the version of the product licence operative on the date of issue. When available, the certifying authority will add a summary basis of approval or any other material that it may deem relevant. Translation by an applicant of these materials into a widely used language, preferably English, shall be deemed to satisfy the provisions of section 3.10.

4.8 Any additional attachment to a certificate submitted by the applicant, such as price lists of products for which bids are offered, should be clearly identified as not forming part of the attestation made by the certifying authority.

4.9 To avert potential abuse of the Scheme, to frustrate attempts at falsification, to render routine authentication of certificates by an independent authority superfluous, and to enable the certifying authority to maintain comprehensive records of countries to which specific products have been exported, each certificate should identify the importing country and be stamped on each page with the official seal of the certifying authority. If requested by the importing country, an identical copy, clearly marked as duplicate, should be forwarded by the certifying authority directly to that country's authority.

5. Notifying and investigating a quality defect

5.1 Each certifying authority undertakes to institute enquiries into any quality defect reported in a product exported in accordance with the provisions of the Scheme, on the understanding that:

– the complaint is transmitted, together with the relevant facts, through the competent authority in the importing country;

– the complaint is considered to be of a serious nature by the latter authority; and
– the defect, if it appeared after delivery of the product into the importing country, is not attributable to local conditions.

5.2 In the case of obvious doubt, a participating national authority may request WHO to assist in identifying an independent quality control laboratory to carry out tests for the purposes of quality control.

5.3 Each certifying authority undertakes to inform WHO and, as far as is possible, all competent national authorities, of any serious hazard newly associated with a product exported under the provisions of the Scheme or of any criminal abuse of the Scheme directed, in particular, to the export of falsely labelled, spurious, counterfeited or substandard pharmaceutical products. On receipt of such notification, WHO will transmit the message immediately to the competent national authority in each Member State.

5.4 WHO stands prepared to offer advice should difficulty arise in implementing any aspect of the Scheme or in resolving a complaint, but it cannot be a party to any resulting litigation or arbitration.

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4. WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce. In: *Forty-first World Health Assembly, Geneva, 2-13 May 1988. Resolutions and decisions, annexes.* Geneva, World Health Organization, 1988: 53-55 (document WHA41/1988/REC/1).

Appendix 1
Model Certificate of a Pharmaceutical Product

Certificate of a Pharmaceutical Product[1]

This certificate conforms to the format recommended by the World Health Organization (*general instructions and explanatory notes attached*).

No. of Certificate: _____

Exporting (certifying) country: _____

Importing (requesting) country: _____

1. Name and dosage form of product:

_____

1.1 Active ingredient(s)[2] and amount(s) per unit dose:[3]

_____

_____

_____

For complete qualitative composition including excipients, see attached.[4]

1.2 Is this product licensed to be placed on the market for use in the exporting country?[5] yes/no (*key in as appropriate*)

1.3 Is this product actually on the market in the exporting country? yes/no/unknown (*key in as appropriate*)

If the answer to 1.2 is yes, continue with section 2A and omit section 2B.

If the answer to 1.2 is no, omit section 2A and continue with section 2B.[6]

2A.1 Number of product licence[7] and date of issue:

_____

2A.2 Product-licence holder (name and address):

_____

_____

_____

2A.3 Status of product-licence holder:[8] a/b/c (*key in appropriate category as defined in note 8*)

2A.3.1 For categories b and c the name and address of the manufacturer producing the dosage form are:[9]

_____

2A.4 Is Summary Basis of Approval appended?[10] yes/no (*key in as appropriate*)

2A.5 Is the attached, officially approved product information complete and consonant with the licence?[11] yes/no/not provided (*key in as appropriate*)

2A.6 Applicant for certificate, if different from licence holder (name and address):[12]

_____

_____

2B.1 Applicant for certificate (name and address):

_____

_____

2B.2 Status of applicant: a/b/c (*key in appropriate category as defined in note 8*)

2B.2.1 For categories b and c the name and address of the manufacturer producing the dosage form are:[9]

_____

2B.3 Why is marketing authorization lacking?

not required/not requested/under consideration/refused (*key in as appropriate*)

2B.4 Remarks:[13] _____

_____

3. Does the certifying authority arrange for periodic inspection of the manufacturing plant in which the dosage form is produced?

yes/no/not applicable[14] (*key in as appropriate*)

If no or not applicable proceed to question 4.

3.1 Periodicity of routine inspections (years): _____

3.2 Has the manufacture of this type of dosage form been inspected? yes/no (*key in as appropriate*)

3.3 Do the facilities and operations conform to GMP as recommended by the World Health Organization?[15]

yes/no/not applicable[14] (*key in as appropriate*)

4. Does the information submitted by the applicant satisfy the certifying authority on all aspects of the manufacture of the product?[16]

yes/no (*key in as appropriate*)

If no, explain: _____

_____

Address of certifying authority:

_____

_____

Telephone number: _____ Fax number: _____

Name of authorized person:

_____

Signature:

_____

Stamp and date:

_____

General instructions

Please refer to the guidelines for full instructions on how to complete this form and information on the implementation of the Scheme.

The forms are suitable for generation by computer. They should always be submitted as hard copy, with responses printed in type rather than handwritten.

Additional sheets should be appended, as necessary, to accommodate remarks and explanations.

ed to exclude excipients not approved for use
e country of import;
ed to meet a different maximum dosage limit

.

ıfacture is taking place in a country other than
nd inspection is conducted under the aegis of

s in the manufacture and quality control of
e those included in the thirty-second report of
ons for Pharmaceutical Preparations (WHO
992, Annex 1). Recommendations specifically
ve been formulated by the WHO Expert
ation (WHO Technical Report Series, No. 822,

n the product-licence holder or applicant
ribed in note 7 above. It is of particular
s are involved in the manufacture of the
applicant should supply the certifying
the contracting parties responsible for each
dosage form, and the extent and nature of
these parties.

vailable on diskette in WordPerfect from the
ies, World Health Organization, 1211 Geneva

Appendix 2

Model Statement of Licensing Status of Pharmaceutical Product(s)

No. of Statement _____

Exporting (certifying) country:

Importing (requesting) country:

Statement of Licensing Status of Pharmaceutical Product(s)[1]

This statement indicates **only** whether or not the following products are licensed to be put on the market in the exporting country.

Applicant (name/address):

Name of product	Dosage form	Active ingredient(s)[2] and amount(s) per unit dose	Product-licence no. and date of issue[3]

The certifying authority undertakes to provide, at the request of the applicant (or, if different, the product-licence holder), a separate and complete Certificate of a Pharmaceutical Product in the format recommended by WHO, for each of the products listed above.

Address of certifying authority:

Telephone/fax numbers:

Name of authorized person:

Signature:

Stamp and date:

This statement conforms to the format recommended by the World Health Organization (*general instructions and explanatory notes below*).

General instructions

Please refer to the guidelines for full instructions on how to complete this form and information on the implementation of the Scheme.

The forms are suitable for generation by computer. They should always be submitted as hard copy, with responses printed in type rather than handwritten.

Additional sheets should be appended, as necessary, to accommodate remarks and explanations.

Explanatory notes

1 This statement is intended for use by importing agents who are required to screen bids made in response to an international tender and should be requested by the agent as a condition of bidding. The statement indicates that the listed products are authorized to be placed on the market for use in the exporting country. A Certificate of a Pharmaceutical Product in the format recommended by WHO will be provided, at the request of the applicant and, if different, the product-licence holder, for each of the listed products.

2 Use, whenever possible, International Nonproprietary Names (INNs) or national nonproprietary names.

3 If no product licence has been granted, enter "not required", "not requested", "under consideration" or "refused" as appropriate.

The layout for this Model Statement is available on diskette in WordPerfect from the Division of Drug Management and Policies, World Health Organization, 1211 Geneva 27, Switzerland.

Appendix 3

Model Batch Certificate of a Pharmaceutical Product

Manufacturer's/Official[1] Batch Certificate of a Pharmaceutical Product

This certificate conforms to the format recommended by the World Health Organization (*general instructions and explanatory notes attached*).

1. No. of Certificate: _____
2. Importing (requesting) authority: _____
3. Name of product: _____

3.1 Dosage form: _____

3.2 Active ingredient(s)[2] and amount(s) per unit dose: _____

_____

_____

3.2.1 Is the composition of the product identical to that registered in the country of export? yes/no/not applicable[3] (*key in as appropriate*)

If no, please attach formula (including excipients) of both products.

4. Product-licence holder[4] (name and address):

_____

_____

_____

4.1 Product-licence number:[4] _____

4.2 Date of issue:[4] _____

4.3 Product licence issued by:[4] _____

4.4 Product-certificate number :[4, 5] _____

5.1 Batch number: _____

5.2 Date of manufacture: _____

5.3 Shelf-life (years): _____

5.4 Contents of container: _____

5.5 Nature of primary container: _____

5.6 Nature of secondary container/wrapping: _____

_____

5.7 Specific storage conditions: _____

_____

5.8 Temperature range: _____

6. Remarks:[6]

_____

7. Quality analysis

7.1 What specifications apply to this dosage form? Either specify the pharmacopoeia or append company specifications.[7]

_____

7.1.1 In the case of a product registered in the exporting country, have the company specifications[7] been accepted by the competent authority? yes/no *(key in as appropriate)*

7.2 Does the batch comply with all parts of the above specifications?

yes/no (*key in as appropriate*)

7.3 Append certificate of analysis.[8]

It is hereby certified that the above declarations are correct and that the results of the analyses and assays on which they are based will be provided on request to the competent authorities in both the importing and the exporting countries.

Name and address of authorized person:

_____

_____

Telephone number: _____ Fax number: _____

Signature of authorized person: _____

Stamp and date: _____

General instructions

Please refer to the guidelines for full instructions on how to complete this form and information on the implementation of the Scheme.

These forms are suitable for generation by computer. They should always be submitted as hard copy, with responses printed in type rather than handwritten.

Additional sheets should be appended, as necessary, to accommodate remarks and explanations.

Explanatory notes

Certification of individual batches of a pharmaceutical product is only undertaken exceptionally by the competent authority of the exporting country. Even then, it is rarely applied other than to vaccines, sera and biologicals. For other products, the

responsibility for any requirement to provide batch certificates rests with the product-licence holder in the exporting country. The responsibility to forward certificates to the competent authority in the importing country is most conveniently assigned to the importing agent.

Any inquiries or complaints regarding a batch certificate should always be addressed to the competent authority in the exporting country. A copy should be sent to the product-licence holder.

[1] Strike out whichever does not apply.

[2] Use, whenever possible, International Nonproprietary Names (INNs) or national nonproprietary names.

[3] "Not applicable" means that the product is not registered in the country of export.

[4] All items under 4 refer to the product licence or the Certificate of a Pharmaceutical Product issued in the exporting country.

[5] This refers to the Certificate of a Pharmaceutical Product as recommended by the World Health Organization.

[6] Indicate any special storage conditions recommended for the product as supplied.

[7] For each of the parameters to be measured, the specifications give the values that have been accepted for batch release at the time of product registration.

[8] Identify and explain any discrepancies from specifications. Government batch release certificates issued by certain governmental authorities for specific biological products provide additional confirmation that a given batch has been released, without necessarily giving the results of testing. The latter are contained in the manufacturer's certificate of analysis.

The layout for this Model Certificate is available on diskette in WordPerfect from the Division of Drug Management and Policies, World Health Organization, 1211 Geneva 27, Switzerland.

Appendix 4
Glossary and index

In order to facilitate understanding, terms used in the guidelines are explained here and/or reference is made to relevant sections. This appendix provides supplementary information and is not a formal part of the Scheme.

For the sake of clarity, all definitions taken from the glossary of "Good manufacturing practices for pharmaceutical products" (*1*) are preceded by an asterisk.

abuse of Scheme
See sections 4.9 and 5.2 of the guidelines.

active ingredients
See sections 1.5, 4.4 and 4.5 of the guidelines.

addresses of competent authorities
See sections 2.2 and 3.3 of the guidelines.

applicant
The party applying for a Product Certificate. This is normally the product-licence holder. Because certain data are confidential for commercial reasons, the competent authority in the exporting country must always obtain permission to release these data from the product-licence holder or, in the absence of a product licence, from the manufacturer.

authentication of certificates
See section 4.9 of the guidelines.

** batch (or lot)*
A defined quantity of a starting material, packaging material, or product processed in a single process or series of processes so that it can be expected to be homogeneous. In the case of continuous manufacture, the batch must correspond to a defined fraction of the production, characterized by its intended homogeneity. It may sometimes be necessary to divide a batch into a number of sub-batches, which are later brought together to form a final homogeneous batch.

batch certificate
A document containing information, as set out in Appendix 3 of the guidelines, will normally be issued for each batch by the manufacturer. Furthermore, a batch certificate may exceptionally be validated or issued by the competent authority of the exporting country, particularly for vaccines, sera and other biological products. The batch certificate accompanies every major consignment (see also section 3.14 of the guidelines).

** batch number*
A distinctive combination of numbers and/or letters which specifically identifies a batch on the labels, the batch records, and the certificates of analysis, etc.

** bulk product*
A product that has completed all processing stages up to, but not including, final packaging.

certifying authority
The competent authority that issues product certificates. It must ensure that it possesses the capacities listed in section 2.4 of the guidelines.

charges for product certificates
See section 3.11 of the guidelines.

competent authority
The national authority as identified in the formal letter of acceptance in which each Member State informs WHO of its intention to participate in the Scheme. The extent of its participation should be indicated in the letter of acceptance (see section 2.1 of the guidelines). The competent authority can issue or receive certificates.

WHO makes available on request a continuously updated list of addresses of competent authorities and, when applicable, the specific conditions for participation.

competence and evaluation of national authority
See sections 2.4, 2.5 and 4.2 of the guidelines.

dosage form
The form of the completed pharmaceutical preparation, e.g. tablet, capsule, elixir, suppository.

drug regulatory authority
An authority appointed by the government of a Member State to administer the granting of marketing authorizations for pharmaceutical products in that country.

** finished product*
A product that has undergone all stages of production, including packaging in its final container and labelling.

free sale certificate
See section 3.2 of the guidelines.

GMP certificate
See section 3.2 of the guidelines.

importing agents, guidelines for
See section 3.4 of the guidelines.

language of product certificate
See section 3.10 of the guidelines.

licence holder
An individual or a corporate entity possessing a marketing authorization for a pharmaceutical product.

licensee
An individual or corporate entity responsible for the information and publicity on, and the pharmacovigilance and surveillance of batches of, a pharmaceutical product and, if applicable, for their withdrawal, whether or not that individual or corporate entity is the holder of the marketing authorization.

limits of certificate by competent authority
See sections 3.12 and 4.8 of the guidelines.

lot
See *batch*.

** manufacture*
All operations of purchase of materials and products, production, quality control, release, storage, shipment of finished products, and related controls.

** manufacturer*
A company that carries out at least one step of manufacture. (For the different categories of manufacturer, see Appendix 1, explanatory note no. 7.)

marketing authorization
See *product licence*.

pharmaceutical product
Any medicine intended for human use or administered to food-producing animals, presented in its finished dosage form or as an active ingredient for use in such dosage form, that is subject to control by pharmaceutical legislation in both the exporting state and the importing state.

product
See *pharmaceutical product*.

product certificate
A document containing the information as set out in Appendix 1 of the guidelines that is validated and issued for a specific product by the competent authority of the exporting country and intended for use by the

competent authority in the importing country or – in the absence of such an authority – by the drug procurement authority (see also section 3.5 of the guidelines).

Transmission of product certificate: see sections 3.8 and 4.9 of the guidelines.

Validity of product certificate: see section 3.9 of the guidelines.

When to request a product certificate: see section 3.5 of the guidelines.

product information
The approved product information referred to in section 4.7 of the guidelines and item 2A.5 of the Product Certificate. It normally consists of information for health professionals and the public (patient information leaflets), as approved in the exporting country and, when available, a data sheet or a Summary of Product Characteristics (SPC) approved by the regulatory authority.

product licence
An official document issued by the competent drug regulatory authority for the purpose of the marketing or free distribution of a product. It must set out, *inter alia*, the name of the product, the pharmaceutical dosage form, the quantitative formula (including excipients) per unit dose (using International Nonproprietary Names or national generic names, where they exist), the shelf-life and storage conditions, and packaging characteristics. It also contains all the information approved for health professionals and the public (except promotional information), the sales category, the name and address of the licence holder, and the period of validity of the licence.

product-licence holder
See *licence holder*.

** production*
All operations involved in the preparation of a pharmaceutical product, from receipt of materials, through processing and packaging, to completion of the finished product.

registration
Any statutory system of approval required at national level as a precondition for introducing a pharmaceutical product on to the market.

registration certificate
See *product licence*.

specifications
See Appendix 3, explanatory note 7.

statement of licensing status
See section 3.13 of the guidelines and Appendix 2.

Summary Basis of Approval
The document prepared by some national regulatory authorities that summarizes the technical basis on which the product has been licensed (see section 4.7 of the guidelines and explanatory note 9 of the Product Certificate contained in Appendix 1).

Summary of Product Characteristics (SPC)
Product information as approved by the regulatory authority. The SPC serves as the basis for production of information for health personnel as well as for consumer information on labels and leaflets of medicinal products and for control of advertising (see also *Product information*).

tenders and brokers
See section 4.6 of the guidelines.

WHO responsibility
See section 5.4 of the guidelines.

Reference

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H. National Control Authority: Guidelines for Assessment of Vaccine Quality in Non-Producing Countries (WHO/VSQ/95.1)

WHO/VSQ/95.1
Original: English
Dist: limited

National Control Authority

GUIDELINES FOR ASSESSMENT OF VACCINE QUALITY IN NON-PRODUCING COUNTRIES

WORLD HEALTH ORGANIZATION
Geneva

NATIONAL CONTROL AUTHORITY

Ordering Code: WHO/VSQ/95.1

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January 1995

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GUIDELINES FOR ASSESSMENT OF VACCINE QUALITY IN NON-PRODUCING COUNTRIES

1. Introduction 1

2. Objectives 2

3. National Control Authority (NCA) 3

   3.1 Terms of reference 3
   3.2 Personnel 3
   3.3 Technical evaluation procedures 3

4. Product Specifications 4

5. Procurement 4

6. Monitoring of Vaccine Receipt and Distribution 4

   6.1. Stock control 4
   6.2. Assessment of vaccine quality during breaks in the cold chain 5
   6.3. Points of intervention for assurance of quality in the cold chain 5

7. Development of Procedures for Destruction of Vaccines 6

8. The Inspectorate 6

9. Postmarketing Surveillance 6

10. Conclusions 7

11. REFERENCES 9

12. Appendices 10

GUIDELINES FOR ASSESSMENT OF VACCINE QUALITY IN NON-PRODUCING COUNTRIES

1. Introduction

The vast majority of developing countries do not produce the vaccines they use in their immunization programmes, but instead import them from producing countries. Although the responsibility of assuring the quality and consistency of these products lies with the manufacturers and is guaranteed by a licensing authority in the country of manufacture, the importing country still has a duty to ensure that the potency, safety and efficacy of these products meet national requirements.

To effectively control vaccine quality each country needs to establish a National Control Authority (NCA) backed by appropriate legislation. Assurance of vaccine quality is a complex phenomenon that starts with the control of raw materials, continues through production monitoring and strict adherence to Good Manufacturing Practice (GMP) to release of vaccine (1). Thus, quality is built into the product. Testing of final product parameters such as potency, sterility, adjuvant content and stability cannot in itself guarantee quality. Further, quality needs to be safeguarded and confirmed until the vaccine is administered to the end user.

The NCA has the responsibility to review and evaluate information on all vaccine products to be used in the country before granting marketing authorization. In reviewing available clinical, safety, potency and efficacy data, the NCA should take the opportunity to obtain and use information provided by NCAs in other countries. In other words, small control agencies with limited financial and human resources should not attempt to re-evaluate all information emanating from more mature control authorities.

However, it is incumbent upon the importing country's NCA to review the adequacy of the data supporting safety, potency and efficacy before issuing a license. Rapid and recent developments have identified new approaches to developing vaccines that may ultimately prove safer than those used historically. This trend calls for different strategies of vaccine assessment and licensing and places added stress on the regulatory system. There is an urgent need to match technological advances with appropriate mechanisms for assuring the quality of these products. Networking among NCAs is one approach to facilitate assurance of quality.

Once the products are in distribution, national immunization programmes should establish mechanisms of routine monitoring of the cold chain and, in collaboration with the NCA, ways to deal with situations where the products might be compromised as a result of excessive exposure to extremes of temperature.

Through the activities of a postmarketing surveillance committee the NCA should organize channels for reporting and investigating adverse reactions following immunization. The NCA should also ensure that a written procedure exists for speedy and

immunization. The NCA should also ensure that a written procedure exists for speedy and efficient recall of a batch of a particular vaccine product, should it be found to be compromised in any way.

Table 1 indicates how the critical functions of the national control authorities should be allocated in countries depending on the source of vaccines used for national immunization programmes. The present guidelines deal with countries in the last two categories: those which procure vaccines from independent sources and those who receive vaccines through UNICEF.

Table 1. Critical national control functions, depending on vaccine source

Function	Production	Production Sharing	Independent Tender	Procured through UNICEF
Licensing				
Clinical evaluation		access	access	access
Lot release				
Lot testing			access	access
Inspections				
Postmarketing surveillance				

Shaded box means function is essential, "access" means that it can be assured by another entity

2. *Objectives*

The purpose of these guidelines is to provide a tool that:

(i) can be used by countries not producing vaccines in the assessment of quality of vaccines procured for use in national immunization programmes;

(ii) can be used by consultants assisting countries in the assessment of their vaccine demand, supply and financing as part of the development of National Vaccine Supply Plans. The specific indicators to be used during assessment of the national control functions are shown in Table 2.

3. *National Control Authority (NCA)*

First and foremost, it is important to verify if the country being assessed has a National Control Authority established under appropriate legislation. The law should spell out the *modus operandi* of the authority. In assessing the capacity of national authorities to carry out evaluation and licensing of vaccine products, it is important to look closely at the following:

3.1 Terms of reference

The terms of the NCA should be established by the relevant statutes. The authority should have a mandate to:

(a) define criteria on which license applications are to be assessed;

(b) issue, vary and revoke licenses for vaccines and other biological products on grounds of safety, potency and efficacy;

(c) secure the subsequent safe and effective use of each vaccine product by controlling, through the terms of the license, the content of all labelling (including package inserts, associated prescribing information and advertising) and the channels through which the product may legitimately be supplied.

3.2 Personnel

An NCA may have as few as one or two professionals plus support staff. The professional staff must have a thorough understanding of and practical experience in the different facets of the work. Generally the same skills as for evaluation of general pharmaceutical product applications will be needed.

The responsible officer is accountable for the professional validation and assessment of license applications and for the administrative aspects of licensing, determining priorities and developing timetables for implementation of controls.

3.3 Technical evaluation procedures

The NCA should have clearly defined procedures on how it will proceed with the actual evaluation process. Since the quality of vaccines is subject to stringent international standards, it is recommended that the NCA use the WHO published guidelines for vaccine production as a point of departure. The NCA can obtain information on products manufactured in other countries through application of the WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce (2).

Under the Certification Scheme the manufacturer is required to furnish to the

importer (through his NCA) information that stipulates that:

(a) the vaccine is registered by the National Control Authority of the manufacturer's country;

(b) the premises under which it is manufactured meet Good Manufacturing Practice standards (as defined by WHO) and are subject to regular inspection by the NCA; evidence of such recent inspection should be provided in the form of a GMP certificate in the format prescribed by WHO (Appendix A).

The NCA should request this information and process it speedily.

4. *Product Specifications*

Operating within the framework of a National Drugs Policy, and in consultation with the NCA, the immunization programme should draw up a list of recommended vaccines to be used in its immunization activities.

This process can be simplified by referring to the WHO global EPI recommendations on recommended vaccines; reference to the products on the National Essential Drugs and Vaccines List; and local epidemiologic disease data that may demand inclusion of a particular vaccine in certain settings. For example, Japanese encephalitis B and yellow fever vaccines might be included in some countries.

5. *Procurement*

A significant step in the assurance of quality of externally procured vaccines is achieved if the importing country uses vaccine sources licensed and released by competent and independent national licensing and control authorities. UNICEF, with technical advice from WHO, has built considerable experience in this field; countries meeting eligibility criteria may procure vaccines through UNICEF. Member States may choose to use the UNICEF procurement mechanisms if they meet eligibility criteria, or procure from the same sources.

6. *Monitoring of Vaccine Receipt and Distribution*

6.1. Stock control

The staff of the immunization programme should develop systematic stock control procedures that allow vaccines to be used on a FIRST-TO-EXPIRE-FIRST-OUT basis. Strict adherence to this system should be enforced at all levels of the distribution network, through appropriate training of supervisory personnel and store-keepers.

The monitoring of vaccines by expiry date and batch/lot number throughout the distribution system is useful in event of a manufacturer's recall for reasons of potency, toxicity, adverse event or contamination.

6.2. Assessment of vaccine quality during breaks in the cold chain

It is almost impossible to fool-proof breakdown in the cold chain system at all times. Accidents can occur, exposing vaccine to extremes of temperature not recommended by the manufacturer. The assistance of a control laboratory may be requested in the case of cold chain breakdown, if the quantity of vaccine involved is sufficiently large (Appendices B and C). Cold chain staff should be able to rapidly detect and respond to cold chain failures. There should be written instructions on how to proceed.

6.3. Points of intervention for assurance of quality in the cold chain

- At the port of entry
- On unpacking vaccine
- On storage at the central store
- On reception at the health facility
- On storage at the health facility

The most important initial point for comprehensive quality assurance intervention is after the vaccine arrives at the central store. The following questions should be answered:

i) Is the vaccine in the shipment the correct one ordered?
ii) Is the vaccine labelled and packaged according to the appropriate guidelines?
iii) Is the vaccine temperature within labelled guidelines?
iv) Are there any 3M vaccine monitors and Freeze-Watch indicators in the shipment if included in the procurement specifications?
v) Can it be assured that the vaccine was not exposed to high temperatures (above 8°C) for prolonged periods before it arrived?
vi) Were aluminum adsorbed (tetanus toxoid, DTP, hepatitis B) vaccines protected against freezing? These vaccines are rapidly damaged by a single freezing event.
vii) Was the shipment transported without delay anywhere on its way to the central store?

During storage further quality assurance interventions should be planned on the basis of answering the following questions:

i) Has the vaccine storage temperature stayed between 2-8°C?
ii) Has the vaccine been protected from exposure to high temperatures during storage?
iii) Can it be assured that aluminum-adsorbed vaccines have never been frozen as

per recording thermometers or Freeze-Watch monitors?

iv) Is there sufficient shelf-life remaining, as indicated by the expiry date, to allow for distribution time?

v) Are the vaccine labels still on the vials?

vi) Is the vaccine free from visible signs of contamination?

vii) Is the vaccine still fit for intended use?

If the answer to any of these questions is no, intervention must proceed.

7. *Development of Procedures for Destruction of Vaccines*

The NCA should have written protocols for destruction of and accountability of doses of vaccine judged to be unfit for use after an assessment following cold chain failure, suspected damage during transportation or upon expiry. Adequate records should be maintained.

8. *The Inspectorate*

The NCA should have a functional inspectorate section for regular inspection of vaccines at the port of entry into the country. Spot checks in the cold chain would assist in strengthening of the quality monitoring system.

9. *Postmarketing Surveillance*

Postmarketing surveillance of vaccines serves to monitor and assess safety and efficacy during widespread use of the products. To date this aspect has received little attention in immunization programmes around the world.

Adverse events, though rare, occasionally follow immunization with vaccines. Some of these events are due to the intrinsic characteristics of the vaccines. Some can be traced to programme problems such as poor handling or administrative technique. Some occur coincidentally after immunization due to other causes, and some may be due to changes in vaccine strain. An example of this is the case of BCG lymphadenitis in Zimbabwe in the late 1980s (8); cases declined on reverting to the original supplier. Some serious events have been recorded as a result of mishandling of vaccines and poor administrative techniques. Indeed, in rapidly expanding programmes reactions due to programmatic causes may predominate (3). As many of these impact injection safety, thorough investigation, analysis and action is required.

The detection of serious adverse events following immunization is important to the success of a programme since the occurrence of such events can influence the community's acceptance of immunization. The system should be built on the concept of spontaneous reporting and should motivate reporters through feedback on the outcome of specific case investigations.

The responsibility for monitoring product safety and efficacy in the field rests with the NCA, but the immunization programme needs to participate actively in the process, being the best source of data. Thus, investigation of reports should be a collaborative effort.

Each NCA should develop its own system of postmarketing surveillance. The system adopted should be able to detect, register and analyze unusual clustering of serious adverse events or clinical syndromes (which could indicate a lack of efficacy) after immunization. Prompt reporting is crucial to trigger timely responses from the NCA and manufacturers. The NCA needs to define the type of adverse events related to immunization that require monitoring and prepare protocols for response to these events and the action to be taken. The basic principles of field investigations of cases of infectious diseases are followed.

Postmarketing surveillance requires mobilization of personnel in different parts of the health care system. Collaboration with other sectors of the Ministry of Health (Disease Surveillance; Health Information System) is mandatory if the adverse events system is to be effective.

Prior to taking action in relation to reported specific cases, it is important to:

- examine the clinical data to confirm the diagnoses and outcome;
- search whether additional similar vaccine-associated cases can be detected;
- examine which specific vaccine errors in handling or administration techniques could be implicated by the reports; and
- suggest the need for further data collection, such as whether similar problems are being observed in children not recently vaccinated, or if there is a need for laboratory data.

Many records may have to be accumulated before conclusions regarding causation of event by the vaccine in question can be drawn.

10. Conclusions

National Control Authorities in countries procuring vaccines are responsible for the review of data and for making decisions on the safety and efficacy of vaccines used in immunization programmes. Working in collaboration with the national immunization programme, the NCA assures that vaccine quality is maintained until the product reaches the end user. To achieve these goals each vaccine-importing country needs to first establish a National Control Authority whose scope depends on its particular needs. That NCA must then address a number of priority activities:

i) Adoption of licensing requirements and a licensing system for vaccines.

ii) Development of a list of vaccines (specifications) which takes into account epidemiological need and demonstrated product efficacy. This should be undertaken in reference to national documents such as the Essential Drugs List and the WHO/EPI recommendations.

iii) Provision of the necessary elements to the NCA for assurance of the quality of vaccines during procurement.

iv) Recognition of the conditions affecting vaccine quality (the potency of vaccines declines rapidly with exposure to ultraviolet light, extreme temperatures and to disinfectants), and taking the necessary preventive action through control of the cold chain to avoid compromise of quality.

v) Assuring access to a testing laboratory should the need to use this facility arise (See Appendices B and C).

vi) Establishment of a system for postmarketing surveillance to monitor and assess safety during widespread use, and in particular to investigate reported cases of post-immunization problems.

vii) Establishment of a system that takes action to withdraw questionable vaccines from use when the need arises.

viii). Establishment of collaborative mechanisms between the NCA and the national immunization programme for achievement of the above activities.

All countries regardless of their vaccine source need to assume responsibility for product licensing and postmarketing aspects (see Table 1). In addition all countries need to be able to access laboratory testing facilities and clinical data on efficacy to assure the quality of vaccines used nationally. Countries that are procuring independently (not through UNICEF) by tender need to set up mechanisms for lot release of vaccines.

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7. *Assurance of Vaccine Quality: Will International Laboratory Networks Help?* Task Force on Situation Analysis, Children's Vaccine Initiative, April 1994, CVI/TFSA/94.8.

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9. *The Quality Control of Vaccines*; an integral part of the WHO Expanded Programme on Immunization, UNDP/WHO.

Table 2. **Indicators for monitoring of vaccine quality in nonproducing countries**

Parameter/Objective	Indicators	Status
National Control Authority (NCA)	NCA established by law Written procedures on requirements for vaccine registration Availability of adequate resources for the efficient functioning of the NCA Adequate staff and technical support from national experts Effective and meaningful communication with other NCAs Procedures for recall of vaccines from the field Procedures for destruction of non-useable vaccines Evidence of liaison with WHO when quality problems arise	
Vaccine product specifications	List of registered EPI and other vaccines Evidence of the use of a simple, accessible and manageable system for evaluation of clinical data Evidence of the link between EPI epidemiological data and the national health information system Updates of specifications as information on newer and improved vaccines becomes available	

Parameter/Objective	Indicators	Status
Procurement	Written procedures for vaccine procurement Rules for restricted or open tender procedures Up to date information on sources and prices of vaccines Procedures for handling of vaccine donations	
Preshipment assurance of quality	Evidence of product registration in producing country Summary of manufacturers' protocols defining manufacturing process Evaluation of manufacturers' procedures and protocols Batch/lot Quality Control Certificates from NCA that vaccine meets WHO requirements Evaluation of shelf-life specifications and stability Evidence of GMP compliance by the manufacturers	
Distribution/Use	Written procedures for quality monitoring in the cold chain Written procedures on how to handle cold chain failures	

Parameter/Objective	Indicators	Status
Inspectorate	Availability of vaccine production inspectors to the NCA Spot checks on vaccine license holders to ensure compliance with the operating terms of licenses	
National Control Laboratory	Access to a laboratory in case testing is necessary	
Postmarketing surveillance	Establishment of a surveillance systems for adverse reactions to vaccines post-immunization Inclusion of case investigations in this system Monitoring by the system of -BCG lymphadenitis -injection site abscesses -severe local reactions Availability of reporting forms to health facilities Existence of written guidelines on responses and action to confirmed adverse events following immunizations	

Model Certificate of a Pharmaceutical Product

No. of Certificate.....

Certificate of a Pharmaceutical Product[1]

Exporting (certifying) country:
Importing (requesting) country:

Proprietary name (if applicable) and dosage form:
Active ingredient(s)[2] and amount(s) per unit dose:[3]

1. Is this product **licensed** to be placed on the market for use in the exporting country?[4] If yes, complete **box A**. If no, complete **box B**.

A

Product-licence holder:
Status of licence holder:[5] a ☐ b ☐ c ☐ d ☐
Number of product licence[6] and date of issue:
Is an approved technical summary appended?[7] yes ☐ no ☐
Is the attached product information complete and consonant with the licence?
yes ☐ no ☐ not provided ☐

Applicant for certificate **if different** from the licence holder:[8]

B

Applicant for certificate:
Status of applicant:[5] a ☐ b ☐ c ☐ d ☐

Why is authorization lacking?
not required ☐ not requested ☐ under consideration ☐ refused ☐

Remarks:[9]

2. Does the certifying authority arrange for periodic inspection of the manufacturing plant in which the dosage form is produced? yes ☐ no ☐ If no, proceed to question 3

Periodicity of routine inspections (years):
Has the manufacture of this type of dosage form been inspected? yes ☐ no ☐
Do the facilities and operations conform to GMP as recommended by the World Health Organization?[10] yes ☐ no ☐

3. Does the information submitted by the applicant satisfy the certifying authority on all aspects of the manufacture of the product undertaken by another party?[11]
yes ☐ no ☐ - If no, explain:

Address of certifying authority:

Telephone/fax numbers:

Name of authorized person:
Signature:
Stamp and date:

This certificate conforms to the format recommended by the World Health Organization *(General instructions and explanatory notes overleaf)*

General Instructions

Please refer to the guidelines for further information on how to complete this form and on the implementation of the Scheme.

Forms should be completed using a typewriter to ensure legibility.

A cross should be placed in boxes as appropriate to indicate which options apply.

Additional sheets should be appended, as necessary, to accommodate remarks and explanations.

Explanatory notes

[1] This certificate, which is in the format recommended by WHO, establishes the status of the pharmaceutical product and of the applicant for the certificate in the exporting country. It is for a single product only since manufacturing arrangements and approved information for different dosage forms and different strengths can vary.

[2] Use, whenever possible, International Nonproprietary Names (INNs) or national nonproprietary names.

[3] A qualitative listing of other ingredients contained in the dosage form should be appended.

[4] When applicable, append details of any restriction applied to the sale, distribution, or administration of the product that is entered on the product licence.

[5] Specify whether the person responsible for placing the product on the market:
(a) manufactures the active ingredients and the finished dosage form;
(b) manufactures the finished dosage form;
(c) packages and/or labels a finished dosage form manufactured by an independent company; or
(d) is involved in none of the above.

[6] Indicate, when applicable, if the licence is provisional, pending technical review.

[7] This refers to the document, prepared by certain national regulatory authorities, that summarizes the technical basis on which the product has been licensed.

[8] In this circumstance, permission for issuance of the certificate is required from the product-licence holder.

[9] Please indicate the reason the applicant has provided for not requesting registration:
(a) the product has been developed exclusively for the treatment of conditions — particularly tropical diseases — not endemic in the country of export;
(b) the product has been reformulated with a view to improving its stability under tropical conditions;
(c) the product has been reformulated to exclude excipients not approved for use in pharmaceutical products in the country of import;
(d) the product has been reformulated to meet a different maximum dosage limit for an active ingredient;
(e) any other reason, please specify.

[10] The requirements for good practices in the manufacture and quality control of drugs referred to in the certificate are those adopted by the Twenty-eighth World Health Assembly in its resolution WHA 28.65 (see WHO Official Records, No. 226, 1975, Annex 12, Part 1). Proposals for the amendment of these requirements are included in the Thirty-second Report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations (WHO Technical Report Series, No. 822, 1992, Annex 1). Recommendations specifically applicable to biological products have been formulated by the WHO Expert Committee on Biological Standardization (WHO Technical Report Series, No. 822, 1992, Annex 1).

[11] This section is to be completed when the product-licence holder or applicant conforms to status (c) or (d) as described in note 5 above. It is of particular importance when foreign contractors are involved in the manufacture of the product. In these circumstances the applicant should supply the certifying authority with information to identify the contracting parties responsible for each stage of manufacture of the finished dosage form, and to indicate the extent and nature of any controls exercised over each of these parties.

APPENDIX B

The National Control Laboratory (NCL)

National Drugs Control Laboratories are fairly common in most Member States. Although these laboratories can analyze general pharmaceutical products, the capability to handle biological products is rare. Therefore, the role of the NCL in the assurance of the quality of vaccines in nonproducing countries is limited. Indeed the establishment of facilities dedicated to vaccine testing cannot be justified in most countries, until they have reached a level of development where they are moving towards local production (9).

Where local testing facilities have been developed, the laboratory may wish to devote its resources to critical areas during cold chain failures such verification of identity and potency testing for oral polio vaccine, which is particularly heat-labile.

Appendix C shows the number of doses involved during cold chain failure justifying a potency test and the number of doses needed for the test as recommended by WHO. Vaccine potency testing is a lengthy and costly procedure and therefore is justified only rarely.

In any case, facilities for potency testing will rarely be available in most National Control Laboratories of developing countries, and some collaborative arrangements may have to be made with external laboratories.

Whenever laboratories exist in the same geographical region, it may be useful to assess their ability to network and the move towards harmonization of vaccine test requirements and protocols. Exchange of information on vaccine testing to ensure that countries are using similar protocols should also be encouraged.

APPENDIX C

NUMBER OF DOSES OF VACCINE JUSTIFYING POTENCY TEST

Vaccine	Number of doses involved	Number of doses for test	Lead time for results
OPV	20,000	20	1 month
Measles (freeze-dried)	20,000	20	1 month
Yellow Fever (freeze-dried)	20,000	20	1 month
BCG (freeze-dried)	20,000	20	3 months
Diphtheria-pertussis-tetanus	200,000	20	3 months
Tetanus toxoid	50,000	20	3 months
Hepatitis-B			
Poliomyelitis (inactivated)	Until potency test is established Do not retest		

Source: WHO/EPI/MLM/91.5

WHO/VSQ/97.05
ORIGINAL: ENGLISH
DISTR.: GENERAL

GPV POLICY STATEMENT

Vaccine donations

Introduction

A vaccine donation is defined as a shipment of vaccine for which a government does not pay. Properly managed, vaccine donations may be useful to immunization programmes. However, accepting a vaccine donation could leave a country vulnerable to problems. For example, if there is no control over the specifications of the vaccine, or if the donated vaccine does not meet the needs of the government's immunization programme, the donation could actually disrupt the programme.

The aim of these guidelines is to improve the management of donated vaccines, and not to hinder donations. Vaccines shipped through UNICEF Supply in Copenhagen may technically be considered as donations, but as their specifications are developed by a collaborative effort between country officials and UNICEF country staff, such vaccines are not included in these guidelines.

The need for a policy on vaccine donations

The benefits of vaccine donations cannot be over-emphasized. There are many different reasons for vaccine donations. These include emergencies, such as disease outbreaks, or sudden shortages of stock during supplementary immunization activities, corporate donations, bilateral aid, or assistance in cases where the entire health infrastructure has been put at risk, such as during conditions of armed combat. Although these scenarios differ, there are some basic principles which should be considered whenever a donation is to be made which will ensure the optimization of the benefits of the donated vaccines for both the donor and the recipient. These guidelines aim to describe the approach of Good Donation Practice on the part of both donor and recipient.

While vaccine donations are designed to benefit the recipient, they do not always achieve this. Some examples are given below of situations where donations actually harmed the recipient country immunization programme.

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Vaccine Supply and Quality • World Health Organization
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In one country counterfeit meningitis vaccine was donated by a neighboring country at the peak of a meningitis outbreak. The counterfeit vaccine was ineffective at best, and at worst caused harm to the recipient. In either case, public confidence in the immunization programme was threatened.

BCG vaccine with no remaining shelf life was donated to an African country by an nongovernmental organization. Vaccines donated which are at the end of their shelf life generally cannot be used. If they are sent to immunization centers, staff will either have to ignore their training which tells them to follow the expiration date, or not use the vaccine. The result either undermines training and good immunization practices or a large amount of vaccine must be destroyed.

A large donation of OPV suffered a cold chain break in transport. In this case, the vaccine was donated at the request of WHO, by a manufacturer for a polio National Immunization Day. In this particular case, normal precautions had not been taken to guard the potency of the vaccine throughout transport to the recipient country. The result was that the cold chain was broken, the vaccine could not be used, and the NID had to be postponed.

Needed infrastructure

Most recipients of vaccine donations are countries dependent on UNICEF and other donors for their supply of vaccines. Many lack infrastructure to handle donations adequately. WHO recommends that all countries, including those which receive all their vaccines from UNICEF, exercise at least two essential national control functions: a published set of requirements for licensing, and surveillance of vaccine field performance (monitoring of adverse events following immunization). In the case of a problem in the field which may be vaccine related, or of doubt of vaccine potency because of a cold chain break in transport, UNICEF can be notified and, if vaccine testing is needed, WHO laboratories can be used.

But countries still have a responsibility to develop these two basic systems. To date, of 88 countries dependent on UNICEF for their vaccines, only 12 are assuring these two functions.

WHO drugs donations guideline

All countries which are likely to receive donated vaccine need a policy on donations. WHO has already published guidelines for receipt of donations of drugs (WHO/DAP/96.2). These are based on four core principles:

(1) Maximum benefit to the recipient;
(2) Respect for wishes and authority of the recipient;
(3) No double standards in quality; and
(4) Effective communication between donor and recipient.

These four principles have been expanded into 12 guidelines that apply to vaccines as well as pharmaceuticals.

1. All drug donations should be based on an expressed need and be relevant to the disease pattern in the recipient country. Drugs should not be sent without prior consent by the recipient.

2. All donated drugs or their generic equivalents should be approved for use in the recipient country and appear on the national list of essential drugs, or, if a national list is not available, on the WHO Model List of Essential Drugs, unless specifically requested otherwise by the recipient.

3. The presentation, strength, and formulation of donated drugs should, as much as possible, be similar to those commonly used in the recipient country.

4. All donated drugs should be obtained from a reliable source and comply with quality standards in both donor and recipient country. The WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce should be used.

5. No drugs should be donated that have been issued to patients and then returned to a pharmacy or elsewhere, or were given to health professionals as free samples.

6. After arrival in the recipient country all donated drugs should have a remaining shelf life of at least one year.

7. All drugs should be labelled in a language that is easily understood by health professionals in the recipient country; the label on each individual container should at least contain the International Nonproprietary Name, batch number, dosage form, strength,, name of manufacturer, quantity in the container, storage conditions, and expiry date.

8. As much as possible, donated drugs should be presented in larger quantity units and hospital packs.

9. All drug donations should be packed in accordance with international shipping regulations, and be accompanied by a detailed packing list which specifies the contents of each numbered carton by INN, dosage form, quantity, batch number, expiry date, volume, weight, and any special storage conditions. The weight per carton should not exceed 50 kilograms. Drugs should not be mixed with other supplies in the same carton.

10. Recipients should be informed of all drug donations that are being considered, prepared, or actually underway.

11. In the recipient country the declared value of a drug donation should be based upon the wholesale price of its generic equivalent in the recipient country, or, if such information is not available, on the wholesale world-market price for its generic equivalent.

12. Costs of international and local transport, warehousing, port clearance and appropriate storage and handling should be paid by the donor agency, unless specifically agreed otherwise with the recipient in advance.

Application to vaccine donations

For the most part, these guidelines are applicable for vaccines as well. Four proposed minimum specifications for vaccine donations restate these guidelines in a manner applicable to vaccines:

- the vaccine is helpful to the immunization programme; that is, the donated vaccines are consistent with the goals of the immunization programme;

- the vaccine is subject to prescribed licensing and control procedures set up by the recipient government;

- the vaccine meets all specifications consistent with other vaccines in the programme, including potency, liquid or freeze-dried presentation, transport, shelf life, number of doses per vial, thermostability, and labeling;

- the vaccine should be shipped only on request of the responsible national officials.

Policy implementation

Implementation of the policy will require some infrastructure within the country. However, the needs are not essentially different from those already identified as basic national control functions. There is a need for a focal point to check vaccines upon receipt and the ability to refuse vaccine donations not meeting the criteria. This implies a need for criteria, such as a published set of requirements for licensing, and their application. There is also a need for a system to detect and investigate complaints from the field, essentially the second critical control function, surveillance of vaccine field performance. Thus, developing a vaccine donations policy will reinforce the need for and functioning of the basic components of a national control system for vaccines.

WHO, through its headquarters, regional and country offices, is prepared to provide technical advice and advocacy for the adoption of a donations policy and its implementation, particularly in the areas of information dissemination, policy adoption, advocacy with donors as to the suitability of donations, training, monitoring of implementation, and to provide a rapid response for investigation, analysis, and resolution of reports of adverse events reported in conjunction with the use of donated vaccines.

PART V - THE UNICEF OPTION

A. UNICEF Standard Procurement Services 644

B. Vaccine Independence Initiative 645

C. UNICEF Purchase Agreement/General Conditions 645

D. Minimum Requirements for Vaccines to be Supplied for UNICEF and Model Inserts 653

E. UNICEF Quality Assurance Programme 662

1. Procedure for Assessing the Acceptability, in Principle, of Vaccines for Purchase by UN Agencies (WHO/VSQ/97.06) 663

F. UNICEF Application of Vaccine Vial Monitors 677

G. UNICEF Pricing 677

PART V - THE UNICEF OPTION

In 1995 UNICEF supplied over 1.1 billion doses of EPI vaccines globally, managing over 2,000 orders and shipments with a value exceeding $80 million. About 70 percent of the vaccine UNICEF purchases is for UNICEF-assisted country programmes. UNICEF finances the purchase of vaccines and related products from its own regular budget or supplementary funds contributed by donor governments and agencies.

The remaining 30 percent of the vaccine purchased by UNICEF is for government immunization programmes using UNICEF's Standard Procurement Services, including the Vaccine Independence Initiative (VII).

Part V.B., page 645

A. UNICEF Standard Procurement Services

The public-sector client indicates type, quantity and delivery date requirements for the products required and requests a price estimate. The request can be initiated through a local UNICEF representative or by direct inquiry to the Customer Services and Support Centre of the UNICEF Supply Division in Copenhagen. UNICEF will respond with a non-binding estimate which, if accepted, will result in a purchase agreement that is executed, using UNICEF General Conditions.

Part V.C., page 645

The UNICEF Supply Division tries to respond to all price request queries within one week, usually within two work days.

In order to comply with its financial regulations and rules, UNICEF usually requires payment in advance for procurement services transactions. The payment must be in a fully convertible currency unless the client is a government that has a Vaccine Independence Initiative agreement with UNICEF. In this case, local currency is acceptable up to a level that UNICEF can absorb locally in the administration of its office and the development programmes it is supporting. The amount to be deposited is based on the cost of the supplies required plus estimated shipping costs and a 6 percent service fee. A contingency deposit equal to 10 percent of the value of the transaction is also required to cover unforeseen additional expenses that might impact UNICEF's finances. Unused funds are returned after UNICEF has processed all invoices, including freight bills.

Vaccine is ordered from the manufacturer having the requisite capacity and the consignment is shipped directly from the manufacturer to the consignee. UNICEF monitors the vaccine shipments from supplier to arrival at the consignee's designated airport as part of its normal supply service.

B. Vaccine Independence Initiative

The Vaccine Independence Initiative functions in a manner similar to UNICEF's standard procurement services. The major difference is that the client government does not pay UNICEF for the vaccine until after it has been received. In order to offer this option, UNICEF establishes a revolving fund, capitalised by a donor. UNICEF holds the revolving fund on deposit as a payment guarantee in lieu of pre-payment by the government. The government reimburses the local UNICEF office in local currency. Upon receipt of the local currency payment, UNICEF reimburses the revolving fund the equivalent amount in hard currency, calculated at the appropriate United Nations rate of exchange. This initiative will be employed only when the local currency requirements of UNICEF are sufficient to absorb the amounts required for the purchase of the vaccines. Currency requirements and limitations are the same for the initiative as for standard procurement. An important feature of the initiative is that the participating government is encouraged, as part of the process, to make firm and accurate estimates of vaccine requirements and to place the requirement for vaccines and related supplies into the national health budget. This is often the first step toward self-sufficiency in vaccination needs.

C. UNICEF Purchase Agreement/General Conditions

See next page.

(Text resumes on page 662)

PURCHASE AGREEMENT

BETWEEN THE

UNITED NATIONS CHILDREN'S FUND

AND

_____
_____
_____

THIS AGREEMENT is entered into between the UNITED NATIONS CHILDREN's FUND (UNICEF) and

_____

("Customer") in accordance with UNICEF Financial Regulation 5.2 and Rules 105.5 to 105.8 to extend to the Customer the facilities and procurement services ("the Services") provided by the UNICEF Supply Division, whose prime purpose is to purchase and/or stock, set pack (and dispatch) supplies in support of UNICEF programme activities.

WITNESSETH

UNICEF and the Customer hereby agree as follows:

ARTICLE I

Type of Services

1. The Customer recognizes that the Services provided by UNICEF under its Financial Regulations and Rules are limited to assisting governments, UN agencies or other organizations to obtain supplies, equipment and other materials, at a nominal fee, for purpose consistent with the aims and policies of UNICEF.

2. Requests for Services by the Customer may be made for standard warehouse items as listed in the UNICEF Copenhagen Warehouse Catalogue and Price List or non-warehouse items if specifications for such items are clear and complete.

3. Requests for purchases will only be considered by UNICEF where supplies, equipment and services are required for use in projects related to UNICEF programme activities or are consistent with UNICEF's goals and objectives.

4. UNICEF reserves the right to accept or reject any Request for Services under this Agreement.

ARTICLE II

Request for Services

1. The Customer shall, for each request for Services under this Agreement, utilize to the extent possible the same UNICEF forms and follow similar steps to those for UNICEF programme implementation. The request shall be accepted by UNICEF subject to the UNICEF General Conditions.

2. Upon acceptance of the Request, UNICEF will notify the Customer thereof by mailing to the Customer the original of the Request, duly countersigned by an authorised official of UNICEF.

3. Once accepted by UNICEF, the Request and its Annexes shall constitute a contract between UNICEF and the Customer, falling under this Agreement.

ARTICLE III

Payment

1. The customer shall, immediately on notification by UNICEF of its acceptance of the Request, deposit an amount covering the total costs of the services in the currency of payment and in the bank account specified by UNICEF.

2. No Request shall be acted upon by UNICEF until the payment due has been made, except where reciprocal arrangements already exist or have been concluded by UNICEF with the Customer with the approval of the UNICEF Comptroller.

ARTICLE IV

Terms of Delivery

1. All supplies shall be delivered by UNICEF on C.I.F. (Cost-Insurance-Freight) terms, unless otherwise specifically agreed in writing, and the Customer produces proof satisfactory to UNICEF that the shipment will be covered by insurance.

2. UNICEF shall make provision for inspection of purchases prior to shipment and the cost of such inspection, if any, shall be billed to the Customer as part of the cost of the Services provided under the Request.

ARTICLE V

Settlement of Disputes

1. Any controversy or claim arising out of or in connection with the interpretation or execution of this Agreement or any Request accepted by UNICEF under this Agreement or any breach thereof, shall, unless it is settled by direct negotiations, be settled in accordance with the UNCITRAL Arbitration Rules as at present in force. UNICEF and the Customer agree to be bound by any arbitration award rendered as a result of such arbitration, as the final adjudication of such controversy or claim.

2. Nothing contained in or relating to this Agreement shall be deemed a waiver, express or implied, of any of the privileges and immunities of the United Nations, including UNICEF.

ARTICLE VI

Termination

1. The Agreement may be terminated for cause by either Party upon 90 days written notice to the other.

2. Upon termination of this Agreement, the Parties shall take all reasonable and necessary measures to conclude the Services already commenced in accordance with this Agreement.

3. The provisions of this Agreement shall survive any termination to the extent necessary to permit an orderly settlement of accounts between the Parties.

ARTICLE VII

General Provisions

1. This Agreement shall enter into force upon signature by the Parties.

2. This Agreement may be altered, modified or amended only by written instrument duly executed by both Parties with the approval of the Comptroller of UNICEF.

IN WITNESS WHEREOF, the Parties hereto have executed this Agreement.

on _____ at _____

_____

(for the CUSTOMER)

on _____ at _____

Director, UNICEF Supply Division

_____

(for UNICEF)

UNICEF GENERAL CONDITIONS

INTENDED PURPOSE

1. The Customer undertakes to ensure that all purchases requested from UNICEF under these arrangements are for purposes consistent with the Purchase Agreement and will be used solely in projects indicated in the Request.

COST OF PURCHASES

2. Purchases supplied by UNICEF under this Request shall be billed at the price stated by UNICEF. Any price increase resulting from price changes by the Supplier or Manufacturer, currency exchange fluctuations on non-warehouse items, or other incidental costs, as may be notified to the Customer, shall be billed and paid by the Customer as part of the total cost of the Services provided by UNICEF.

   For warehouse items the prices invoiced will be those valid at the time of the receipt and confirmation by Supply Division, Copenhagen of the firm order.

PAYMENT

3. The Customer shall, as a condition precedent to any action by UNICEF on a Request, pay the amount covering the total cost of the service in a currency and to the bank account agreed. If a price increase is notified to the Customer prior to shipment, the Customer shall immediately arrange for deposit of the amount as provided herein above. If a price increase is notified to the Customer after shipment, the Customer shall pay such price increases within ninety (90) days of such notification.

DELIVERY OF PURCHASES

4. Delivery of purchases shall be on C.I.F. (Cost-Insurance-Freight) terms, except where otherwise agreed, upon proof by the Customer that insurance has been effected. The Customer shall be responsible for the purchases from the time of shipment.

5. Upon shipment of the purchases, UNICEF shall forward to the Customer copies of the relevant shipping documents.

WARRANTY

6. UNICEF shall pass on any warranty offered by the Manufacturer or Supplier used by UNICEF to the Customer. Where no such warranty is available, UNICEF offers no warranty, express or implied, that supplies are merchantable or fit for any particular purpose. UNICEF will not accept the return of any purchases procured on behalf of the Customer. Only in

exceptional circumstances and with the prior agreement of UNICEF may standard warehouse items be returned to UNICEF in Copenhagen, provided that this shall be at the Customer's full expense.

CUSTOMS CLEARANCE

7. The Customer shall be fully responsible for reception, customs clearance and distribution of all purchases shipped. The Customer or its nominated representative shall be the consignee of such purchases.

AMENDMENTS, CANCELLATION

8. Amendments or cancellation or reduction of quantities already accepted by UNICEF, and where procurement action has been initiated, may only be effected with the agreement of UNICEF. The Customer shall be responsible for payment in advance of any resulting costs.

9. In case of cancellation or reduction of quantities before shipment, the customer shall pay any penalties imposed by Suppliers.

FINAL ACCOUNT

10. After shipment of all purchases and recording of all expenses incurred, UNICEF shall prepare a statement of account to be forwarded to the Customer, covering the total cost of the Services, and shall include any increases in cost, including penalties and credits due to decrease in price or quantities.

11. The statement of account shall be expressed in U.S. dollars. The United Nations operational rate of exchange shall apply to all currency conversions under this Request.

12. In the event that the statement of account indicates a balance of funds in favour of the Customer, these funds shall, unless otherwise requested by the Customer, be returned to the Customer.

13. In the event that the statement of account indicates a deficit in the funds deposited with UNICEF, the Customer shall, within ninety (90) days of notification by UNICEF, make the additional payments to the same bank account and in the same currency as the original deposit was made in order to settle the account.

LIABILITY AND CLAIMS

14. UNICEF shall not be responsible for delay of delivery or loss or damage to any purchases. UNICEF shall under no circumstances be liable for any indirect or consequential damages arising from acceptance or execution by UNICEF of a Request. UNICEF's total liability in any event shall not exceed the purchase price of the particular supply (or supplies) with respect to which a claim is made.

15. All claims relating to any defect in quality or quantity or for any loss or damage shall be handled directly by the Customer with the Supplier. UNICEF shall provide to the Customer any assistance that the Customer may reasonably request in handling such claims.

16. UNICEF accepts no liability for any third party claims arising out of or in connection with the acceptance or execution by UNICEF of the Request. The Customer shall indemnify, deal with, defend and hold UNICEF harmless in connection with any third party claim or other cause of action arising from the acceptance or execution by UNICEF of any Request under the Purchase Agreement.

SETTLEMENT OF DISPUTES

17. Any controversy or claims arising out of this Request shall be settled as provided in Article V of the Purchase Agreement between UNICEF and the Customer.

D. Minimum Requirements For Vaccine To Be Supplied for UNICEF and Model Inserts[38]

8.1 PRODUCTION AND TESTING

The vaccine shall be produced and tested in conformity with the requirements of national legislation and the following requirements established by the World Health Organization (WHO).

8.1.1 Good Manufacturing Practices for Biological Products (WHO Technical Report Series No. 822, 1992) and Guideline for National Authorities on Quality Assurance for Biological Products (WHO Technical Report Series No. 822, 1992).

8.1.2 General Requirements for the Sterility of Biological Substances (WHO Technical Report Series No. 530, 1973).

8.1.3 Requirements for Diphtheria, Tetanus, Pertussis and Combined Vaccines (Revised 1989) (WHO Technical Report Series No. 800, 1990).

8.1.4 Requirements for Poliomyelitis Vaccine (Oral) (Revised 1989) (WHO Technical Report Series No. 800, 1990 except that the minimum infectious units per human dose for type 3 should be 600,000. This is specified by WHO EPI following the recommendation of the Global Advisory Group of its meeting in Cairo in 1990).

8.1.5 The requirements for Measles, Mumps, and Rubella Vaccines and Combined Vaccine (Live). WHO Technical Report Series No. 840, 1994. The minimum quantity of vaccine virus in one human dose is 1000 viral infective units. Additionally, as specified by WHO-EPI following the recommendation of the Global Advisory Group at its meeting in Jakarta in 1992 for the maximum viral infective units per human dose in an assay that has been adequately validated against the International Reference Reagent for measles vaccine.

8.1.6 Revised Requirements for Dried BCG Vaccine (Revised 1985) (WHO Technical Report Series No. 745, 1987), (Amendment 1987) (WHO Technical Report Series No. 771, 1988).

8.1.7 If there is a change in formulation of vaccines from those supplied to UNICEF in 1994-95 against previous long-term arrangements, manufacturing protocols plus data on thermostability where appropriate and final container samples from five consecutive bulk lots shall be provided through the National Control Authority to the Chief, VSQ, WHO Geneva before the bid is considered.

8.1.8 Regarding BCG vaccine, freeze-dried, nominal 10 and 20 dose vials should contain actual 10 and 20 doses respectively for children under one year old, i.e., reconstituted vaccine of 0.5 ml and 1.0 ml respectively (0.05 ml/dose x 10 and 20 doses respectively) as described in page 14, BCG Vaccine Model Insert. Therefore, any

[38] Contract from the UNICEF Invitation to Bid for Vaccines (1997).

supplier's nominal vials that do not fulfil the above requirement may either be rejected or the offer adjusted to equivalent vial sizes.

8.1.9 It is recognised that, because of the special needs for vaccines for the EPI in developing countries, the specifications prepared for UNICEF by WHO may be more detailed than those given in the WHO Requirements, although they are not in conflict with them. If these specifications are not in accord with national requirements, provision is made for this to be stated in the model certificate for the release of vaccines acquired by United Nations agencies (TRS 786, Annex 1, Appendix 2).

8.2 SHELF LIFE

Vaccine shall be supplied with the maximum shelf life possible consistent with current vaccine production technology. Unless separately authorised by UNICEF, the remaining shelf life at the time of dispatch shall not be less than the figures given below:

Oral polio vaccine, if stored at -20°C:	18 months
Freeze-dried BCG, Freeze-dried Measles, DTP, TT and DT	20 months

8.3 PACKING AND SHIPPING

In addition to the specifications contained in paragraph. 7.1 Packing/ Shipping requirements must conform to the attached GUIDELINES ON THE INTERNATIONAL PACKAGING AND SHIPPING OF VACCINES FOR THE EXPANDED PROGRAMME OF IMMUNIZATION, WHO EPI/CCIS/ 81.4/Rev. 5 (Appendix 1).

8.4 LABELS AND INSERTS

8.4.1 Vial labels shall be affixed with water resistant adhesive so that the labels do not become loose or fall off. Labels shall state the name of vaccine, name of manufacturer, place of manufacture, lot number, composition, concentration, dose and mode of administration, expiry date, storage temperature, any marking that is appropriate. Expiry date and lot number shall be printed on each vial in indelible ink.

8.4.2 BCG, DTP, TT, DT, Td and measles vaccines must be colour coded on the paper label of the vial as follows:

BCG	..	PMS 300 BLUE
DTP	..	PMS PANTONE YELLOW
TT	..	PMS 327 GREEN
DT	..	PMS 376 LIME
Measles	..	PMS 151 ORANGE

8.4.3 Inserts shall be printed in English, French, Portuguese and Russian (Spanish and Arabic are optional). Separate inserts in the language appropriate for the country of destination will be welcome. In all inserts the following should be inserted under "Description of vaccines".

"The vaccine fulfils WHO requirements for _____ (name of vaccine)".

8.4.4 Inserts shall contain at least the information in the WHO Model Insert enclosed for that vaccine. Any additional information provided by the manufacturer must not confuse or contradict WHO policy on the use of that vaccine. It should also be noted that current model inserts attached will be revised and replaced during this year.

8.5 CLOSURES

Vaccine vials shall be fitted with closures that conform to ISO standards 8362-1 and 8362-2.

8.6 VACCINE VIAL MONITORS (VVM)

Vaccine vial monitors should comply with WHO specifications dated 9 September 1996 or later.

8.7 MODEL INSERTS

BCG VACCINE

DESCRIPTION
It is a live freeze-dried vaccine made from an attenuated strain of Mycobacterium bovis. It is used for the prevention of tuberculosis.

ADMINISTRATION
For children under one year 0.05 ml and for others 0.1 ml of reconstituted vaccine is given intradermally. Reconstitute only with the diluent supplied by the manufacturer. Special syringes allow administration of the exact dose. A sterile syringe and a sterile needle should be used for each injection. The skin should not be cleaned with antiseptic. Jet injectors do not generally provide a reliable dose of BCG and should not be used. Special care is needed in opening the vials and reconstituting the vaccine so that the vaccine is not blown out of the vial. Because of sensitivity to daylight, the vaccine must be kept in the dark. When withdrawals are made from the vial, the vaccine must be exposed to the light for the minimum period of time, and never for longer than four hours. If not used immediately after reconstitution, the vaccine should be kept on ice and protected from light, and any opened container remaining at the end of a session (maximum 4 hours) must be discarded. Skin testing with tuberculin is not generally carried out before giving BCG, but when performed, those who are found to be positive reactors need not be immunized.

IMMUNIZATION SCHEDULE
BCG should be given routinely to all infants at risk of early exposure to the disease. For maximum protection, this vaccine should be given as soon after birth as possible. It can be given at the same time as DTP, DT, TT, measles, polio vaccines (OPV and IPV), Hepatitis B and Yellow Fever vaccine.

SIDE EFFECTS
A local reaction is normal after BCG. A small tender red swelling appears at the site of the injection which gradually changes to a small vesicle and then an ulcer in 2 - 4 weeks. The reaction usually resides within two to five months and in practically all children leaves a superficial scar 2-10 mm in diameter. Rarely, the nodule may persist and ulcerate.

Occasionally, enlargement of axillary lymph nodes may appear in 2 - 4 months following immunization. Inadvertent subcutaneous injection produces abscess formation and may lead to ugly retracted scars.

CONTRAINDICATIONS
The vaccine is contraindicated in those with cell-mediated immune deficiency. Keloid and lupoid reactions may occur at the site of injection and such children should not be revaccinated.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED INFANTS: HIV-infected, non symptomatic infants should be immunized with BCG vaccine according to standard schedules. Infants with clinical (symptomatic) AIDS should not receive BCG vaccine (but should receive other EPI vaccines).

STORAGE
BCG vaccine should be stored and transported between 0° and +8°C. It is even more stable if stored in temperatures as low as -20°C. The diluent should not be frozen but kept cool. Protect the vaccine from the light.

PRESENTATION
The vaccine comes in boxes of ____ ampoules/vials each containing ____ doses.

8.7 MODEL INSERTS (continued)

DTP VACCINE

DESCRIPTION
The vaccine contains purified diphtheria and tetanus toxoids and inactivated whooping cough organisms. The toxoids are adsorbed onto ___(specify). _____ is used as a preservative. The potency of the vaccine components per single human dose is at least _____ (specify in International Units for three components).

ADMINISTRATION
The vaccine should be shaken before use in order to get a homogeneous suspension. It should be injected intramuscularly or deep subcutaneously. The anterolateral aspect of the upper thigh is the preferred site of injection. (An injection into a child's buttocks may cause injury to the sciatic nerve and is not recommended.)

It must not be injected into the skin as this may give rise to a local reaction. 1 dose is 0.5 ml. A sterile needle and sterile syringe should be used for each injection. Once opened, multi-dose vials should be kept between 0° and +8°C. Opened vials of vaccine may be used in subsequent immunization sessions until a new shipment of vaccine arrives providing that the conditions described in WHO/EPI/LH15/95.1 are met.

IMMUNIZATION SCHEDULE
In countries where pertussis is of particular danger to young infants, DTP immunization should be started as soon as possible with the first dose given as early as 6 weeks and two subsequent doses given at 4-week intervals.

DPT vaccine can be given safely and effectively at the same time as BCG, measles, polio vaccines (OPV and IPV), Hepatitis B and Yellow Fever Vaccine.

SIDE EFFECTS
Some transitional swelling, tenderness and redness at the site of injection together with fever occur in a large proportion of cases. Occasionally severe reactions of high fever, irritability and screaming develop within 24 hours of administration. Very rare neurological complications allegedly due to the pertussis component have been observed but are rare in comparison with those observed in the course of the disease.

CONTRAINDICATIONS
There are few contraindications to the first dose of DTP. Fits or abnormal cerebral signs in the newborn period or other serious neurological abnormality are contraindications to the pertussis component. DT should be given instead. A second or subsequent dose of DTP should not be given to a child who suffered a severe reaction to the previous dose. The pertussis component should be omitted and DT given for the remainder of the course.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED INFANTS: HIV-infected infants, both asymptomatic and symptomatic, should be immunized with DTP vaccine according to standard schedules.

STORAGE
DTP vaccine should be stored and transported between 0° and +8°C. IT MUST NOT BE FROZEN.

PRESENTATION
The vaccine comes in vial of ____ doses.

TT VACCINE

DESCRIPTION
The vaccine contains purified tetanus toxoid. The toxoid is adsorbed onto _____(specify). _____ is used as a preservative. One dose of 0.5 ml has a potency of at least 40 International Units. TT is used in the prevention of neonatal tetanus by immunizing women, especially of childbearing age, and also in the prevention of tetanus following injury.

ADMINISTRATION
The vaccine should be shaken before use in order to get a homogeneous suspension. It should be injected intramuscularly or deep subcutaneously.

A sterile needle and a sterile syringe should be used for each injection.

Opened vials of vaccine may be used in subsequent immunization sessions until a new shipment of vaccine arrives providing that the conditions described in WHO/EPI/LH15/95.1 are met.

IMMUNIZATION SCHEDULE
TT immunization of women for the prevention of tetanus and neonatal tetanus consists of two doses of 0.5 ml given intramuscularly or deep subcutaneously at least four weeks apart followed by the third dose 6 - 12 months later. To maintain the immunity against tetanus through the child bearing period, a fourth dose should be given at least one year after the third dose, and the fifth dose should be given at least one year after the fourth dose. TT immunization can be administered safely during pregnancy even during the first trimester.

SIDE EFFECTS
Rare and mild. Some transitional tenderness and redness at the site of the injection and occasional fever. Safe to give in pregnancy.

CONTRAINDICATIONS
A severe reaction to a previous dose of TT.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED PERSONS: HIV-infected persons, both asymptomatic and symptomatic, should be immunized with TT vaccine according to standard schedules.

STORAGE
TT should be stored and transported between 0° and +8°C. IT MUST NOT BE FROZEN.

PRESENTATION
The vaccine comes in vial of _____ doses.

ADSORBED DT VACCINE FOR CHILDREN

DESCRIPTION
The vaccine contains purified diphtheria and tetanus toxoids. The toxoids are absorbed onto _____ (specify). _____ is used as a preservative. One dose of 0.5 ml contains _____ LF of diphtheria toxoid and _____ LF of tetanus toxoid. The potency of vaccine components per single human dose is: at least 30 IU (International Units) of potency for diphtheria toxoid and at least 40 IU of potency for tetanus toxoid.

ADMINISTRATION
The vaccine should be shaken before use in order to get a homogeneous suspension. It should be injected intramuscularly or deep subcutaneously.

A sterile syringe and a sterile needle should be used for each injection.

DT vaccine is recommended for children below 8 years of age. For persons 8 years and older, a special absorbed vaccine for adults, Td, is recommended.

Opened vials of vaccine may be used in subsequent immunization sessions until a new shipment of vaccine arrives providing that the conditions described in WHO/EPI/LH15/95.1 are met.

IMMUNIZATION SCHEDULE
Recommended for use in childhood immunization instead of DTP when contraindications to pertussis component exist. To complete the course, two intramuscular injections of 0.5 ml at least four weeks apart for children. It may be given at the same time as BCG, measles, polio vaccines (OPV and IPV), Hepatitis B and Yellow Fever vaccine.

SIDE EFFECTS
Some transitional tenderness and redness at the site of the injection and occasional fever.

CONTRAINDICATIONS
A second or subsequent dose of DT should not be given to a child who suffered a severe reaction to the previous dose.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED CHILDREN: HIV-infected children, both asymptomatic and symptomatic, should be immunized with DT vaccine according to standard schedules.

STORAGE
DT should be stored and transported between 0° and +8°C. IT MUST NOT BE FROZEN.

PRESENTATION
The vaccine comes in vial of _____ doses.

8.7 MODEL INSERTS (continued)

MEASLES VACCINE

DESCRIPTION
An attenuated live virus vaccine. Each dose contains not less than _____ (specify) infective units of viral vaccine strain _____(specify), and not more than _____ gm _____ residual antibiotic (specify the antibiotic). This vaccine is a freeze-dried product which must be reconstituted only with 0.5 ml of the sterile diluent provided separately by the manufacturer for that purpose.

ADMINISTRATION
The immunization consists of a single dose of 0.5 ml injected SUBCUTANEOUSLY, preferably in the upper arm. A sterile needle and sterile syringe must only be used for each injection. Once the vaccine has been reconstituted, it should be used the same day, and only then if the vial has been maintained between 0° and +8° and protected from light in the meantime. The diluent MUST BE kept cool prior to use.

IMMUNIZATION SCHEDULE
A single dose of measles vaccine is sufficient to provide prolonged immunity to infection. In countries where the incidence and mortality from measles is high in the first year of life, the recommended age for immunization against measles is a soon as possible after 9 months of age (270 days). Countries where measles is less of a problem may decide on a later date for immunization. Measles vaccine can be safely and effectively given simultaneously with DT, Td, TT, BCG, polio vaccines (OPV and IPV), Hepatitis B and Yellow Fever vaccine.

SIDE EFFECTS
A mild fever and rash lasting one to three days may occur in up to 15% of vaccinees 8 to 12 days after vaccination. Encephalitis has been reported following measles vaccination, approximately one case per million doses administered.

CONTRAINDICATIONS
There are few contraindications to the administration of measles vaccine. On the contrary, it is particularly important to immunize children with malnutrition. Low grade fever, mild respiratory infections or diarrhoea and other minor illnesses should not be considered as contraindications.

It is contraindicated in patients with a known severe allergy to _____ (specify the antibiotic used as a preservative). Since the effect of the live measles virus vaccine on the fetus is not known, it is also contraindicated in pregnancy.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED PERSONS: The vaccine is contraindicated in those with immune deficiency disease or supposed immune response from leukaemia, lymphoma or generalized malignancy. However, HIV-infected persons, both asymptomatic and symptomatic, should be immunized with measles vaccine according to the standard schedule.

STORAGE
Freeze-dried measles vaccine should be kept below +8°C (and if possible below 0°C) until used. Stability is even better if the vaccine (not the diluent) is kept at -20°C. The diluent should not be frozen but kept cool immediately prior to use. The vaccine should be protected from light.

PRESENTATION
The vaccine comes in vial of _____ doses.

ORAL POLIO VACCINE

DESCRIPTION
The live oral poliomyelitis vaccine (OPV) is a trivalent vaccine containing suspensions of types 1, 2 and 3 attenuated poliomyelitis viruses (Sabin strains). Each dose contains not less than _____ (specify) infective units of type 1, and _____ of type 2, and _____ of type 3. _____ (specify) is used as a stabilizer.

ADMINISTRATION
OPV must only be administered orally. Two drops are delivered directly into the mouth from the multi-dose vial through dropper or dispenser. For older children it may be preferred to avoid the possible bitter taste by first placing the drops on a sugar lump or in syrup. Care should be taken not to contaminate a multi-dose dropper with saliva of the vaccinee. Opened vials of vaccine may be used in subsequent immunization sessions until a new shipment of vaccine arrives providing that the conditions described in WHO/EPI/LH15/95.1 are met.

IMMUNIZATION SCHEDULE
Infants should receive at least three doses of OPV at minimum intervals of 4 weeks. In non-endemic areas the first dose can be given from 6 weeks with the first dose of DTP. In endemic areas, an extra dose should be given as soon as possible after birth.

OPV can be given safely and effectively at the same time as measles, DTP, DT, Td, TT, BCG, Hepatitis B and Yellow Fever vaccine.

SIDE EFFECTS
In the vast majority of cases there are no side effects. Very rarely, there may be vaccine-associated paralysis (less than one case per 3 million doses administered).

CONTRAINDICATIONS
The vaccine should not be administered to those with an immune deficiency. No adverse effects are produced by giving OPV to a sick child. However, if in doubt, for instance during diarrhoea, a repeat dose can be given after recovery.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED INFANTS: HIV-infected infants, both asymptomatic and symptomatic, should be immunized with OPV according to standard schedules.

STORAGE
Vaccine is potent if stored at not higher than -20°C until the expiry date indicated on the vial. It should not be stored for a period of longer than six months at a temperature not higher than +5°C. Protect from light.

PRESENTATION
The vaccine comes in vial of _____ doses.

E. UNICEF Quality Assurance Programme

The Vaccine Supply and Quality (VSQ) section of the Global Programme for Vaccines and Immunization (GPV) at WHO Geneva Headquarters acts as an adviser to UNICEF on matters related to the quality of vaccines and has formulated criteria for evaluating the acceptability of vaccines for purchase by UN agencies.

All quality assurance functions are provided to UNICEF by WHO. These include random lot testing, inspection of the supplier's facility and the National Control Authority Laboratory by qualified experts, periodic re-inspection and follow-up investigation of adverse events reported. Additional information on quality assurance measures may be found in Part IV.B. - Quality Assurance.

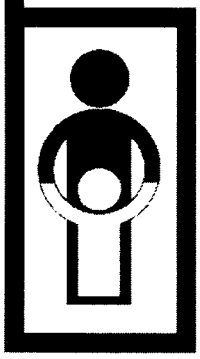
Part IV.B., page 568

The quality assurance procedures established by the NCAs in the countries where vaccine is produced facilitate international export and import by guaranteeing their quality and acceptability for human use. Another example of such guidelines is the following WHO document "Procedure for Assessing the Acceptability, in Principle, of Vaccines for Purchase by United Nations Agencies" (WHO/VSQ/97.06), which includes a "Model Certificate for the Release of Vaccines Acquired by United Nations Agencies". It is currently being applied to the procurement of vaccines by UNICEF for the Expanded Programme on Immunization.

(Text resumes on page 677)

WHO/VSQ/97.06
ORIGINAL: ENGLISH
DISTR.: LIMITED

Procedure for assessing the acceptability, in principle, of vaccines for purchase by United Nations agencies



GLOBAL PROGRAMME FOR VACCINES AND IMMUNIZATION
VACCINE SUPPLY AND QUALITY

World Health Organization
Geneva
1997

The Vaccine Supply and Quality Unit of the Global Programme for Vaccines and Immunization thanks the donors whose unspecified financial support in 1996 has made the production of this document possible.

Ordering code: WHO/VSQ/97.06
Printed : August 1997

GPV Catalogue available on the Internet at:
http://www.who.ch/programmes/gpv/gEnglish/avail/gpvcatalog/catlog1.htm

Copies may be requested from:
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Table of contents

Introduction 1

Steps of the procedure 2

1. Product File 2

2. Initial testing of vaccine samples 2

3. WHO site visits 3

4. Report and outcome of the assessment 3

5. Supply 4

6. Reassessments 4

7. Random testing of samples 5

8. Monitoring of complaints from the field 5

9. Recommendations for action 5

10. Costs 5

11. Confidentiality 6

12. No conflict of interest 6

Annex 1: Provisions for team members participating in WHO missions to assess acceptability, in principle, of vaccines for purchase by United Nations agencies 9

Annex 2: Model certificate for the release of vaccines acquired by United Nations Agencies 11

Procedure for assessing the acceptability, in principle, of vaccines for purchase by United Nations agencies

Introduction

The World Health Organization, through its Global Programme for Vaccines and Immunization (GPV), will provide advice to UNICEF and other United Nations agencies on the acceptability, in principle, of vaccines considered for purchase by UN agencies.

The purpose of this assessment is to verify that the vaccines (a) meet the specifications of the relevant UN agency and (b) the requirements recommended by WHO, including those for good manufacturing practices (GMP). This is to ensure that vaccines used in national immunization programmes in different countries are safe and effective and that they meet particular operational specifications for packaging and presentation.

The assessment procedure established by WHO is based on the following principles:

- Reliance on the National Control Authority (NCA) of the country of manufacture;
- General understanding of the production process and quality control methods;
- Assessment of production consistency through compliance with GMP specifications;
- Random check-testing of vaccines to monitor compliance with specifications on a continuing basis;
- Monitoring of complaints from the field.

Since reliance on the NCA's effective and independent quality assurance plays a critical role in the system, WHO recommends that manufacturers (a) inform their NCA of their application for the assessment procedure; (b) at the same time request the NCA to participate in the process; and (c) provide the NCA with the necessary authorization to discuss the relevant files with WHO representatives.

WHO can only advise UNICEF and other UN agencies whether vaccines effectively meet WHO recommended requirements if the vaccines have been assessed through this procedure. ***Other vaccines that have not gone through this process may, however, be as safe and effective as those that have actually been assessed.***

Steps of the procedure

WHO requires general information related to the manufacturing company and the product itself. The manufacturer will provide this information in part in the Product File and also during the site visit. If the manufacturer is not willing to deliver the required information, WHO and the manufacturer will conduct discussions with a view to trying to resolve the situation in a mutually acceptable manner. However, WHO reserves the right to terminate the assessment if at any time it feels that it has not been provided with adequate information to complete the assessment effectively.

1. Product File

A manufacturer for which the procedure is initiated will provide WHO with a Product File containing information related to the following aspects:

1) Composition of the vaccine (formulation).
2) Layout of the production facilities.
3) Organizational chart of the company.
4) Copy of the relevant national requirements for production.
5) Staffing of production, quality control and quality assurance units, plus information on the staffs' professional qualifications.
6) Copy of the original regulatory approval by the NCA of the country of origin.
7) List of countries where the vaccine is registered and is currently distributed by the applicant or any other authorized entity.
8) Clinical data showing the safety and efficacy of the vaccine in the target population, at the dosage and schedules intended to be used in national immunization programmes.
9) Additional data on reactogenicity or lack of efficacy detected through post-marketing surveillance.
10) Flowchart of the production process with a detailed description of the relevant steps involved. For recombinant vaccines, an adequate description of the construction of the recombinant vector should be provided.
11) Detailed description of the quality control methods used (a) during the production and (b) applied to the final product; this should include an adequate characterization of starting materials and gene products (if applicable).
12) Quality specifications set at different stages of the production process and for the final product.

2. Initial testing of vaccine samples

The manufacturer will include, with the Product File, summary protocols of five lots produced from five consecutive bulk lots, and will send separately twenty

multidose vials or ampoules of each of these final lots to WHO. WHO will send the vaccine samples to one of its collaborating laboratories where they will be tested for potency and toxicity; in special cases other tests may be performed.

The list of WHO collaborating laboratories will be kept confidential. Neither the manufacturer, nor any other party who may have requested vaccines to be tested through this system, will be informed where the testing is actually performed. On request, each manufacturer and the relevant NCA will, however, receive a report of the test results.

3. WHO site visits

When the review of the Product File and the testing has been satisfactorily completed, WHO will put together a team to visit the manufacturing facility. UNICEF may elect to participate in the team if the vaccine in question is under consideration for supply to UNICEF. Otherwise the team will be composed of a group of experts, selected by WHO, in three main areas: **production, quality control and GMP.** A WHO staff member will lead the team and the team members will act, on a temporary basis, as expert advisers to WHO. The team will perform the site visit and report its findings in accordance with the terms outlined in this document.

It is preferable for the visit to start at the National Control Authority/Laboratory for discussions on the procedures in place for regulatory approval, lot release, testing and inspection of vaccine manufacturers, as well as the post-marketing surveillance system. Although the laboratory facilities of the NCA may be visited, staff discussions will be of primary importance.

A representative of the National Control Authority involved in control and release of the vaccine would be normally expected to accompany the team to the manufacturing facility to review the manufacturing process, in-process testing, personnel qualifications and practices, animal facilities, compliance with GMP, packaging and shipping processes, and post-marketing surveillance activities.

4. Report and outcome of the assessment

The team will write a report, describing its findings and conclusions and giving its recommendations to the manufacturer and the NCA.

If any adjustments need to be made by the manufacturer, WHO will postpone its final recommendations to UNICEF or the other UN agency involved until such adjustments have been incorporated and verified by WHO.

Once WHO is satisfied that the process is complete, it will send a letter to UNICEF or the other UN agency involved, advising on (a) whether or not it finds the vaccine to meet both the WHO recommended requirements and the specifications of the relevant UN agency, and (b) the role of the NCA in certifying this.

WHO will send a copy of the letter to the manufacturer and the NCA.

5. *Supply*

All lots shipped in response to orders placed by a UN agency must have been released beforehand by the NCA. Lot-release certificates will be kept by the manufacturer and sent, on request, to the UNICEF Supply Division or to the Chief, Vaccine Supply and Quality Unit, Global Programme for Vaccines and Immunization, World Health Organization, Geneva (VSQ/GPV/WHO). In addition, at least twenty samples of each lot of vaccine supplied will be retained by the manufacturer, to be provided to VSQ/ GPV/WHO for testing on request.

The manufacturer should inform VSQ/GPV/WHO of any changes notified to the NCA in the formulation, in methods of manufacturing, in facilities, or in any other aspects which might (a) result in a change of safety and/or efficacy of the vaccine or (b) change the basis of the regulatory approval by the NCA. Such changes may necessitate a further assessment by WHO to assure continued compliance with WHO recommended requirements.

6. *Reassessments*

Reassessments will be done in the following situations:

(i) Before every new agreement for purchase.

(ii) If vaccine fails to meet WHO recommended requirements and/or the specifications of the offer to bid.

(iii) If suspension of supply for a period is equal to, or greater than, two years.

(iv) In case of a suspension of production.

(v) If, in the opinion of WHO, changes made in the formulation, manufacturing methods, facilities or other production aspects require that a reassessment be made.

Reassessments before every new agreement for purchase, item (i) above, require a joint site visit, which may also include a visit to the NCA/NCL, by a WHO team and a representative of the NCA; UNICEF may elect to be represented in the team if it is considering purchase of the vaccine in question. The purpose of the visit will be primarily to verify that the vaccine continues to meet WHO recommended requirements and the specifications of the relevant UN agency, and that it complies with GMP standards. Furthermore, these site visits provide an opportunity for the manufacturer and the team members to discuss any changes which may be foreseen in production and/or quality control methods, as well as any new specifications and/or issues regarding introduction of new policies or strategies proposed by WHO.

The characteristics of the reassessment will vary depending on the actual circumstances and, for items (ii) to (v) above, may require provision of special written information (amendments made to the original regulatory approval, Standard Operating Procedures, validation data, etc.), testing of samples (from lots derived from consecutive bulk lots to verify consistency of production), a site visit and possibly a review of product samples and circulars.

7. *Random testing of samples*

Random samples of lots will be selected, every six months, for independent testing of final product characteristics. Lot summary protocols and information on lot release will be provided by the manufacturer or NCA as appropriate, for review by WHO upon request.

In the event of failure to meet the established criteria for reassessment or testing, WHO will investigate the problem and provide UNICEF or the UN agency with written information, copied to the manufacturer and the NCA, on the actions that need to be taken.

8. *Monitoring of complaints from the field*

Complaints from the field concerning vaccines supplied by UNICEF will be communicated via the field officers to the UNICEF Supply Division in Copenhagen. The Supply Division will then request the intervention of WHO to investigate the complaint and ensure that, if necessary, a further in-depth investigation is performed. Information communicated by any other route should also be relayed immediately to WHO, through the UNICEF Supply Division in Copenhagen, to allow for the investigation procedure to begin.

In the case of vaccines purchased through other UN agencies, the information should be communicated through the relevant Supply Division to WHO so that the investigation process can be started.

After investigation, WHO will provide UNICEF or the other UN agency involved with a written report of the problem and include recommendations for action, if any. WHO will then be available as a technical resource while UNICEF or the other UN agency implements the recommendations.

WHO will make a copy of its report available to the manufacturer and the NCA/NCL.

9. *Recommendations for action*

In the event of the situations described in points 7 and 8 above, WHO may include a recommendation that manufacturers' lots of vaccines are more closely monitored during a probationary period, or that purchase of the vaccine be kept pending until formal reassessment has been completed. Depending on the nature of the failure to meet the established criteria, WHO may recommend a suspension of purchase pending investigation and resolution of the problem. WHO will generally route communications relating to problems in the field or failure to meet established criteria through the NCA.

10. *Costs*

Any costs incurred by the manufacturer in the assessment or reassessment of a vaccine, as described in this document, will be borne exclusively by the manufacturer. Moreover, the manufacturer will cover any costs borne by WHO in respect of reassessments or site visits related to problems in meeting specifications, or as a follow-up on implementation of recommendations made during a previous assessment.

11. *Confidentiality*

Information, to which WHO requires access for the purpose of assessing or reassessing the acceptability in principle of a vaccine for purchase by UN agencies, will not, as a general rule, include confidential information. However, if, in the opinion of the manufacturer, any information to be submitted to WHO and its expert team members in the course of the (re)assessment procedure includes confidential information, the manufacturer must advise WHO thereof in writing, prior to, or at the same time as the disclosure duly identifying the confidential information in question. Notwithstanding the foregoing, WHO and its expert team members will treat all information submitted to them during site visits as confidential, in accordance with the terms set forth below.

WHO will treat information so identified and information disclosed during site visits as confidential and proprietary to the manufacturer and, in this connection, take all reasonable measures to ensure (a) that such information ("the Confidential Information") is not used for any other purpose than the (re)assessment procedure described in this document, and (b) that it is not disclosed or provided to any person who is not bound by similar obligations of confidentiality and non-use as contained herein.

WHO and/or its expert team members will not, however, be bound by any obligations of confidentiality and non-use to the extent they are clearly able to demonstrate that any part of the Confidential Information:

- was known to them prior to any disclosure by the manufacturer; or
- was in the public domain at the time of disclosure by the manufacturer; or
- has become part of the public domain through no fault of WHO and/or any of its expert team members; or
- has become available to WHO and/or any of its expert team members from a third party not in breach of any legal obligations of confidentiality to the manufacturer.

12. *No conflict of interest*

The team for site visits referred to in point 3 above, includes experts in the field of production, quality control and GMP. These experts are selected by WHO and act as WHO temporary advisers or consultants. In this connection, the agreement between WHO and such experts will include similar obligations of confidentiality and non-use as contained in point 11 above, as well as a conflict of interest undertaking. Through this conflict of interest undertaking, the aforesaid experts agree to discharge their functions exclusively as advisers to WHO. They also confirm that they have no financial interest and/or other relationship with a party, which:

(i) may have a vested commercial interest in obtaining access to any ConfidentialInformation disclosed by the manufacturer in the course of the (re)assessment procedure described in this document; and/or

(ii) may have a vested interest in the outcome of the (re)assessment procedure,including, but not limited to, parties such as the manufacturer or manufacturers of competing vaccines.

At the manufacturer's request, WHO will advise the manufacturer in advance of the composition of the team performing the site visit, and provide *curricula vitae* of the temporary expert advisers included in the team. The manufacturer will then have the opportunity to express possible concerns regarding any of the expert team members to WHO prior to the site visit. If such concerns cannot be resolved in consultation with WHO, the manufacturer may reject an expert team member, within, at the latest, ten days of receipt of the proposed team composition.

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Annex 1

Provisions for team members participating in WHO missions to assess acceptability, in principle, of vaccines for purchase by United Nations agencies

In the course of discharging your functions as an expert adviser under this Agreement, you will gain access to certain information, which is proprietary to WHO or to the manufacturers of the vaccine(s) which need to be assessed for purchase by UN Agencies. You undertake to treat such information (hereinafter referred to as "the Information") as confidential and proprietary to WHO or the aforesaid manufacturer(s). In this connection, you agree to:

(a) not use the Information for any other purpose than discharging your obligations under this Agreement; and

(b) not disclose or provide the Information to any person who is not bound by similar obligations of confidentiality and non-use as contained herein.

However, you will not be bound by any obligations of confidentiality and non-use to the extent that you are clearly able to demonstrate that any part of the Information:

(i) was known to you prior to any disclosure by WHO and/or the manufacturer(s); or

(ii) was in the public domain at the time of disclosure by WHO and/or the manufacturer(s); or

(iii) has become part of the public domain through no fault of your own; or

(iv) has become available to you from a third party not in breach of any legal obligations of confidentiality to WHO and/or the manufacturer(s).

You also undertake not to communicate the deliberations and findings of the team(s) of experts in which you will participate, as well as any resulting recommendations and/or decisions of WHO, to any third party, except as explicitly agreed by WHO.

You will discharge your responsibilities hereunder exclusively in your capacity as an expert adviser to WHO. By signing this Agreement, you furthermore confirm that you have no financial interest and/or other relationship with a party, which:

(i) may have a vested commercial interest in obtaining access to any part of the Information referred to above; and/or

(ii) may have a vested interest in the outcome of the assessment of the vaccine(s), in which you will participate.

In this regard, it should be noted that the manufacturer(s) of the aforesaid vaccine(s) have the right to object to your participation in the team(s) of experts which will evaluate (its) (their) vaccine(s). If such objection cannot be resolved in consultation with the manufacturer(s), WHO shall be entitled to terminate this Agreement or cancel part of the activities to be undertaken by you hereunder. The travel and per diem allowances payable to you under this Agreement will in such event be adjusted accordingly.

I hereby agree to the conditions and provisions contained in this document.

Signed: _____

Name (typewritten): _____

Institute: _____

Place: _____

Date: _____

Annex 2

Model certificate for the release of vaccines acquired by United Nations Agencies

(Revised 1988)

(to be completed by the national control authority of the country where the vaccines have been manufactured, and to be sent by the vaccine manufacturer to UNICEF)

The following lots of .....[1] vaccine produced by .....,[2] in .....,[3] whose numbers appear on the labels of the final containers, meet all national requirements,[4] Part A[5] of Requirements for Biological Substances No. .....[6] (Requirements for .....,[1] published in 19..... [if applicable, revised 19....., addendum 19.....]) and Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories, published in 1959; revised 19.....).[7]

Lot No.	*Expiry date*	*Lot No.*	*Expiry date*
.....	.....	.....	.....
.....	.....	.....	.....
.....	.....	.....	.....

As a minimum, this certificate is based on examination of the manufacturing protocol.

The Director of the National Control Laboratory (or Authority as appropriate)[8]

Name (typed).....

Signature.....

Date.....

[1] Indicate type of vaccine (measles, oral poliomyelitis, tetanus, diphtheria–tetanus, diphtheria–pertussis–tetanus, BCG).

[2] Name of manufacturer.

[3] Country.

[4] If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the national control authority.

[5] With the exception of the provisions on shipping, which the national control authority may not be in a position to control.

[6] Indicate the reference number of the relevant Requirements for Biological Substances published by WHO.

[7] These requirements were revised in 1965; a further revision is in preparation for consideration by the WHO Expert Committee on Biological Standardization in 1989.

[8] Or his or her representative.

F. UNICEF Application of Vaccine Vial Monitors

Since 1996, all oral polio vaccine purchased by UNICEF carries a Vaccine Vial Monitor. This is a time-temperature sensitive dot that provides an indication on the accumulative heat to which the vial has been exposed. It warns the end-user when exposure to heat is likely to have degraded the vaccine beyond an acceptable level.

G. UNICEF Pricing[39]

UNICEF reports continuing reductions in the price of vaccine purchased for EPI up to 1998. The average cost of airfreight has also been significantly reduced following negotiations with suppliers and a review of packaging and shipping methods. Freight usually does not exceed 10 percent of vaccine value. Vaccine type, packaging, destination, the urgency of the shipment and source of supply may cause some variation.

The manufacturers' quoted prices are generally valid for one year for all standard EPI vaccines. Prices for non-EPI vaccines are negotiated on a case-by-case basis, but a minimum of six months price validity is sought.

[39] Information provided by UNICEF (April 1998).

PART VI - GENERAL INFORMATION

A. Price and Cost Data 681
1. Vaccine prices 681
2. Vaccine cost components 682
B. Vaccine Sources and Supply Survey 692
1. WHO assessed sources 692
2. Other sources 692
3. Sample merger and acquisition history 692
4. Advertising 693
C. Payment Options 705
1. Cash in advance 705
2. Down payment 705
3. Open account or "on account" 705
4. Payment against documents 706
5. Letter of Credit 707
D. International Commerce 712
1. ICC (International Chamber of Commerce) 712
2. INCOTERMS (International Commerce Terms) 712
E. International Shipping Documents 721
1. Normally required documents 721
2. Common import documents 721
3. Additional documents 721
F. Transportation and Storage 750
1. Vaccine stability 750
2. Packaging and shipping vaccine 750
3. Vaccine storage—time/temperature 751
G. Vaccine Temperature Monitoring 782
H. Vaccine Vial Monitors 799

I. Vaccine-Related Products 806
1. Selection of injection equipment 806
2. Syringes and needles 806
3. Syringe incinerator box 806
4. Vaccine cold boxes/carriers 806

PART VI - GENERAL INFORMATION

A. Price and Cost Data

1. *Vaccine prices*

The vaccine price material included at the end of this section is presented in graphic form for comparison purposes. It represents the price history of EPI vaccines for the period from September 1992 through April 1998, calculated through periodic surveys of manufacturers.

Supplement VI.A.1., page 683

For current specific, accurate pricing, the prospective Buyer must request a price quotation or proposal from one or more vaccine producers or Suppliers. A draft fax titled "Request for Quotation" that may be used for such inquiry appears in Part II.D.13. of this manual.

Sample II.D.13.a., page 79

The price a manufacturer will offer for a specific vaccine often varies from customer to customer and from contract to contract according to the circumstances of the sale. Because of the nature of the vaccine market, the price of a dose of vaccine from the same supplier may vary from country to country dependent upon the marketing policy of the manufacturer. Two of the main considerations are the quantity to be supplied and public- versus private-sector status. Prices are usually lowest when a large quantity will be purchased for a public-sector immunization programme, and highest when a small quantity is purchased for use in private-sector settings. Other elements affecting price include:

- the number of shipments anticipated
- when the shipments are needed
- shipping distance
- currency of payment
- payment terms
- special labelling, packaging and handling requirements
- risk—for example, risk of nonpayment, risk of currency devaluation

In addition, a manufacturer may raise or lower the price of his vaccine on short notice according to his current inventory position. If a large quantity of unsold product is on hand, the manufacturer is usually willing to sell at a lower price; if there is a shortage, especially a world-wide shortage, often prices increase. Unexpected increases in the manufacturer's expenses can affect pricing also. Manufacturers sometimes offer very low

"introductory" prices to secure entry into a new market or win a new customer. In these cases, purchasers can usually expect higher prices for subsequent contracts.

2. *Vaccine cost components*

The price quoted by a supplier does not accurately represent the entire cost of a vaccine to the purchaser. In order to make an accurate comparison, the additional costs associated with each offer must be considered:

- Shipping
- Insurance
- Inspection
- Testing[40]
- Customs Duties
- Clearance Fees
- Other, such as:
  - financing costs
  - currency conversion costs
  - Letter of Credit costs
  - lost interest costs
  - documentary costs

Cost calculations can be facilitated by using a Budget Estimate Worksheet such as the one included at the end of this section (page 691).

Form II.C.3., page 52
Sample II.C.3., page 53
Form VI.A.2, page 691

(Text resumes on page 692)

[40] Testing costs are only incurred in exceptional circumstances. See Part IV - Quality Assurance, page 575.

Budget Estimate Worksheet

Date _____ Approved Budget _____

Supply Year _____

Estimated Cost of Commodities Required

Commodity _____

Quantity _____ x Cost / unit _____ = _____

Shipping Cost	_____
Insurance Cost	_____
Inspection Cost	_____
Testing Costs	_____
Customs Duties	_____
Clearance Fees	_____
Other	_____
Total Required	_____

Convertible Currency Required _____ (amount and currency)

Convertible Hard Currency Available _ yes _ no

B. Vaccine Sources and Supply Survey

1. WHO assessed sources

Supplement VI.B.1., page 694

WHO has assessed a number of vaccine manufacturers and found them acceptable, in principle, for the supply of specific vaccines to UNICEF and other UN agencies. This list changes from time to time so it is necessary to use the latest version, which can be obtained from the VSQ unit of WHO-GPV.

Manufacturers appearing on the WHO list have specifically applied to WHO for assessment. WHO can only advise UNICEF and other UN agencies whether vaccines effectively meet WHO recommended requirements if the vaccines have been assessed through the WHO procedure. Other vaccines that have not gone through this process may be as safe and effective as those that have actually been assessed.

2. Other sources

Supplement VI.B.2.a., page 699

Contact information for U.S. and other manufacturers of a wide range of vaccine and biologicals also appears at the end of this section. This is not intended to be a comprehensive list, nor infer an endorsement of the manufacturers that have been included. It was derived mainly from WHO documents and U.S. reference texts for a rapid survey that was conducted by a Ministry of Health in search of potential suppliers of specific vaccines and biologicals in addition to EPI vaccines. It is offered here as an example.

Supplement VI.B.2.b., page 701

Additional biologials manufacturers who belong to the International Federation of Pharmaceutical Manufacturers Association (IFPMA) are listed at the end of this section.

Supplement VI.B.2.c., page 703

Not all vaccines required by a country programme are available from the same producer. Thus, Buyers who are charged with obtaining a range of EPI and other vaccines and related products usually must deal with multiple sources. In order to quickly identify potential suppliers of specific vaccines other than those mentioned in Supplement VI.B.1, the procurement entity may wish to engage in a survey similar to the one mentioned above.

3. Sample merger and acquisition history

While every effort has been made to provide correct contact information, the 1990s have seen a number of mergers and acquisitions among the larger vaccine manufacturers so this information tends to become obsolete very quickly. For example, in the United States, Wyeth-Ayerst acquired Lederle

Laboratories. Chiron, a U.S. corporation, formed a joint partnership with Ceiba Geigy of Switzerland, and purchased Sclavo in Italy. Chiron also acquired Behringwerke Ag from Hoechst. A diagram illustrating some recent merger and acquisition history appears in Supplement VI.B.3.

Supplement VI.B.3., page 704

4. Advertising

In a procurement system where open tendering (international competitive bidding) is required, sources for vaccine may be located by advertising in local and international newspapers and journals for applications from vaccine manufacturers to pre-qualify for the supply of specific vaccines to the country's immunization programme.

Part II.B.7, page 39

(Text resumes on page 705)

Address List of Suppliers Approved in Principle for the Supply of Vaccines to United Nations Agencies

ADDRESS	VACCINES
Chiron-Behring GmbH & Co. Postfach 1630 D-5006 Marburg Germany Contact: Mr. K. Friederich Head, International Sales, Vaccines Telephone: +49-6421-39 40 99 Fax: +49-6421-39 40 95 Telex: 041-48 23 20 01	BCG DTP DT TT OPV
Chiron Vaccines (Italy) Via Fiorentina 1 53100 Siena Italy Contact: Mr. Bruno Alfonsi R.O.W. Marketing and Sales Manager Telephone: +39-577-24 32 14 Fax: +39-577-24 30 76 Telex: 043-5713 29	TT DT OPV Measles
Cheil Jedang Corporation 10th Floor, Cheil Bldg. 500 5Ka, Namdaemoon-Ro Choong-Gu, Seoul Republic of Korea Contact: Mr. T.H.Choi, General Manager Telephone: +82-2-31 19 560 / 585 / 569 Fax: +82-2-311 94 90	Hepatitis B (Plasma Derived)
CSL Limited 45 Poplar Road Parkville Victoria 3052 Australia Contact: Mr. S. McLeish, General Manager Telephone: +61-3-93 89 19 11 Fax: +61-3-93 89 18 87 European Office 20, rue Saint Victor CH-1227 Carouge Geneva Switzerland Contact: Dr. J. Marshall	DTP DT TT

ADDRESS	VACCINES
Eisai / Biken. Department of Export & Asian Operations 6 10 Loishikawa 4 Bunkyoku Tokyo 112-88 Japan Contact: Mr. Yasuhiro Hara, Senior Manager Telephone: +81-3-38 17 39 17 Fax: +81-3-38 11 35 74 Telex: 072-28282	Measles
Human Serum Production & Medicine Manufacturing Co. H-2100 Gödöllö Tancisics Mihaly ut 82 Hungary Contact: Dr. A. Bukovszky, Head, Export / Import Office Telephone: +36-133 0564 or +36-282 0733 Fax: +36-282 0177 Telex: 22-4014 (For Sole agent see Intervax Biologicals Ltd, Canada)	DT TT
Institute of Immunology Rockerfellerova 2 Zagreb Croatia Contact: Dr. Vlako Silobrcic, Director Telephone: +385-1-27 29 79 or +385-1-27 72 78 Fax: +385-1-27 72 78 Telex: 21864 (For Sole Agent see Intervax Biologicals Ltd, Canada)	Measles
Institut Pasteur de Dakar 36 Avenue Pasteur Boite Postale 220 Dakar Senegal Contact: Dr. Jean Moreau Telephone: +221-22 51 81 Fax: +221-23 87 72 Telex: 61241 Pasteur SG March 1998 - under reassessment by WHO. Please contact WHO / VSQ for information.	Yellow Fever

ADDRESS	VACCINES
Intervax Biologicals Ltd The Scotia Bank Commenrcial Tower 802 625 Cochran Drive Markham, Ontario L3R 9R9 Canada Contact: Dr. S. Tabbara, President Telephone: +1-905-940 8385 Fax: +1-905-940-8387 Telex: 021-696 2999 Sole agents for: • Human Serume Production & Medicine Co., Hungary • Institute of Immunology, Croatia • National Centre of I&P Diseases Bulgaria	(Agent)
Japan BCG Laboratory Koishikawa IS Building 4-2-6 Koshinata, Bunkyo-ku Tokyo 112 Japan Contact: Mr. Hashimoto, Manager, Foreign Trade Dept. Telephone: +81-3-5800 5303 Fax: +81-3-5800 5352 Telex: 0720-232 2357	BCG
Korea Green Cross Corporation 1465-4, Seocho-Dong, Seocho-ku Seoul Korea Contact: Mr Jee. J. Han, Managing Director of KGCC. Telephone: +82-2-584 0131 Fax; +82-2-584 2828 / 584 9802	Hepatitis B (Plasma derived)
L. G. Chemical Ltd. Pharmaceutical Division 20, Yoido-dong Youngdongpo-gu Seoul 150-721 Korea Contact: Mr. S. J. Jeon, General Manager Telephone: +82-2-3773 0605 Fax. +82-2-3773 7391	Hepatitis B (Recombinant)

ADDRESS	VACCINES
Medeva Group Development Evans House, Regent Park Kingston Road Leatherhead Surrey KT22 7PQ United Kingdom Contact: Mr. P. Gallard, Business Unit Manager. International Markets Telephone: +44-1372-36 40 00 Fax: +44-1372-36 40 18 Telex: 051-921 133	BCG Measles Yellow Fever
National Drug Institute Agency 26 Yanko Sakazov Boulevard Sofia 1504 Bulgaria Contact: Pof. Stefan Dundarov, Director Telephone: +359-2-434 7290 or +359-2-44 22 60 Fax: +359-2-44 22 60 (For Sole Agent see Intervax Biologicals Ltd. Canada)	BCG
Pasteur Merieux Connaught (Canada) 1755 Steeles Avenue West North York Ontario, M2R 3T4 Canada Contact: Mr. Stan Gillis, Director Telephone: +1-416-667 2296 Fax: +1-416-667 2961	BCG DTP DT TT OPV Measles
Pasteur Mérieux Connaught (France) B.P. 7046 58 Avenue LeClerc F-69348 Lyon Cedéx 07 France Contact: Mr. Patrick Laturnus Manager International Tenders Telephone: +33-472 73 70 75 Fax: +33-472 73 78 30 Telex: 042-900 266 e-mail: laturnus@bdr.pasteur-merieux.fr	BCG DTP DT TT OPV Measles Yellow Fever
PT Bio Farma (Persero) Jl. Pasteur No. 28 Bandung 40161 Indonesia Contact: Mr. Thamrim Poeloegan, DVM. Telephone:+62-22-23 37 55 / 23 40 18 Fax: +62-22-204 1306 / 23 40 18	OPV Measles

ADDRESS	VACCINES
Serum Institute of India (Pty) Ltd 283 Mahatma Gandhi Road Pune 411042 India Contact: Mr. S.R. Mundra, Director Telephone: +91-212-64 69 63 / 64 01 97 / 64 20 53 Fax: +91-212-64 51 84 Telex: 145 7216 SEAL IN	DTP DT TT Measles
Smith Kline Beecham - Pharmaceuticals rue de l'Institut 89 B-1330 Rixensart Belgium Contact: Ms. Eunice Miranda Associate Director Commercial Affairs Telephone: +32-2-656 8754 Fax: +32-2-656 9034 Telex: 046-63251 e-mail: miranda@sbbio.be	OPV Measles Hepatitis B (Recombinant)
Statens Serum Institut Artillerivej 5 DK-2300 Copenhagen S Denmark Contact: Mr. Jens Hummeluhr, Division Director Sales and Distribution Telephone: +45-32 68 34 04 Fax: +45-32 68 38 88 Telex: 31316	BCG
Swiss Serum & Vaccine Institute Berne P.O. Box 2707 CH-3001 Berne Switzerland Contact: Mr. Dr. L. Gruenenfelder, Sales Director Telephone: +41-31-980 6111 Fax: +41-31-980 6775 Telex: 045-91 26 18	DT TT Measles

Vaccine and Biologicals Manufacturers Contact Information

Company Name	Facsimile Number
Bayer Pharmaceutical Division, Berkley, CA USA	+1-510-705 5000
Biken Institute Tokyo Japan	+81-6-222 0470
Chiron Behring GmbH Marburg, Germany	+49-6421-39 40 95
Chiron Sclavo SpA Siena, Italy	+39-577-24 30 76
Cheil Jedang Corp. Ltd Seoul, Korea	+82-2-311 9490
Commonwealth Serum Laboratories CSL, Ltd., Parkville Victoria Australia	+61-3-93 89 18 87
Connaught Laboratories Inc (Pasteur Merieux Connaught) Swiftwater, PA. USA	+1-717-839 7235
Esai Co. Ltd. Tokyo, Japan	+81-3-38 11 35 74
Human Institute of Immunology (Human Co. Ltd.) Budapest, Hungary	+36-282 0177
Institute Of Immunology Zagreb, Croatia	+385-1-27 72 78
Intervax Biologicals Ltd Ontario, Canada	+1-905-940 8387
Japan BCG Laboratory Tokyo, Japan	+81-3-58 00 53 52
Korean Green Cross Corp Seoul, Korea	+82-2-584 2828
LG Chemical Ltd. Seoul, Korea	+82-2-37 73 73 91
Medeva International Leatherhead, United Kingdom	+44-1372-36 40 18

Company Name	Facsimile Number
Merck Biologicals USA	+1-215-652 0210
National Centre for Infectious and Parasitic Diseases Sofia, Bulgaria	+359-2-44 22 60
Pasteur Merieux Connaught Ontario, Canada	+1-416-667 2961
Pasteur Merieux Serums and Vaccins Lyon, France	+33-472 73 78 30
PT Bio Farma (Persero) Bandung, Indonesia	+62-22-204 1306
SA Vaccine Producers (PTY) Sandringham, South Africa	+27-11-822 0812
Serum Institute of India Pune, India	+91-254-64 51 84
SBL Vaccin AB Stockholm, Sweden	+46-8-730 3248
Smith Kline Beecham Pharmaceuticals Rixensart, Belgium	+32-2-656 9034
Statens Serum Institute Copenhagen, Denmark	+45-32 68 38 88
Swiss Serum and Vaccine Institute Berne, Switzerland	+41-31-980 6775
Institute of Immunology and Virology, Torlak. Beograd, Yugoslavia	+381-11-46 95 65
Wyeth-Lederle Vaccines Inc. Marietta, USA	+1-717-436 4731

Biologicals Manufacturer Members of the International Federation of Pharmaceutical Manufacturers Association (IFPMA)

ALK-ABELLO A / S
Boege Afte 6-8
DK-2970 Hoersholm
Denmark

Ares Serono S.A.
15bls, cherrin des Mines
PO Box 54
1211 Geneva 20
Switzerland

Arznelmittelhersteller E.V. -VFA
Johanna-Kinkel-Strasse 2-4
53175 Bonn
Germany

Bayer Pharmaceutical
400 Morgan Lane
West Haven CT 06516
USA

Bayer Pharmaceutical
Biological Products Division
4th & Parker Streets
P.O. Box 1986
Berkeley, CA 94701
USA

Celltech Therapeutics Limited
216 Bath Road, Slough
Berkshire SL1 4EN
UK

Chiron Behring GmbH & Co.
P.O. Box 1630
D-35041 Marburg
Germany

Chiron Vaccines
36, qual Fulchiron
69005 Lyon
France

CSL Limited
45 Poplan Road
Parkville, Victoria 3052
Australia

Farma Biagini SpA
Loc. Al Contl
55020 Castervecchio Pascoli
Lucca, Italy

Glaxo Wellcome
Langley Court
Beckenham
Kent BR3 3BS
UK

Immuno AG
Arabellastr, 4 (BayWa-Haus)
81925 Munchen
Germany

Instituto Sierovaccineogeno Italiano
ISI Spa
55020 Castelvecchio Pascoli
Lucca

The Kitasato Institute
6-111 Aral, Kitamoto
Saitarna 364-0026
Japan

Korea Green Cross Corporation
1465-4 Seocho-do
Seocho-ku, Seoul
Korea

Medeva Pharma Ltd.
Evans House, Regent Park, Kingston Road
Leatherhead, Surrey KT 22 7PQ
UK

Merck & Co. Inc.
WP 16-100
West Point PA 19486
USA

Norartis Pharma AG
Biotechnology
K-681.1.44
CH-4002 Basel, Switzerland

North American Vaccine, Inc.
10150 Old Columbia Road
Columbia MD 21044
USA

Pasteur Merieux Connaught
Connaught Laboratories Ltd
1755 Steeles Avenue West
North York, Ontario M2R 3T4
Canada

Pasteur Merieux MSD
8, rue Jonas Salk
69367 Lyon Cedex 07
France

Pasteur Merrieux Connaught
58 Avenue Leclerc
69007 Lyon
France

Pharmacia & Upjohn
Undhagensgatan 133
112 87 Stockholm
Sweden

SmithKline Beecham Biologicals
89 Rue de l'Institut
1330 Rixensart
Belgium

SmithKline Beecham Pharmaceuticals
709 Swedeland Road
P.O. Box 1539, UE0224
King of Prussia, PA 19406-0939

Wyeth-Lederle Vaccines
211 Bailey Road
West Henrietta NY 14586-9728
USA

Vaccine Supply Survey

Please fax the data mentioned in the below table to:

Name of Manufacturer: _____ Date: _____

Address of Manufacturer: _____ Fax No:

Please specify items produced by your organization by means of "Y" (Yes) and "N" (No) in appropriate box.

Products	**Number of doses in vials**					
	1	2	5	10	20	other
Hepatitis B Vaccine - R-DNA						
Hepatitis B Vaccine - Plasma derived						
Polio Vaccine - OPV						
Polio Vaccine - IPV						
Mumps Vaccine (monovalent)						
Measles Vaccine (monovalent)						
Measles & Mumps Vaccine						
BGG Vaccine						
DTP Vaccine						
DT Vaccine						
Td Vaccine						
"d" Diphtheria Vaccine (monovalent)						
Anti-Rabies Vaccine						
Anthrax Vaccine						
Yellow Fever Vaccine						
Tularemia Vaccine						
Leptospira Vaccine						
Brucella Vaccine						
Diphtheria antitoxin						
Tetanus antitoxin						
Tuberculin 2 UT PPD						
Rabies immune globulin						
Cholera Vaccine						
Meningococcal Vaccine - Groups A, C						
Miningococcal Vaccine - Group B						
Typhoid Vaccine						
Botulinum antitoxin A, B, E						

C. Payment Options

The Seller generally dictates payment terms, but the Buyer is free to negotiate. The most common payment terms are:

- Cash in advance
- Down payment
- On account
- Payment against documents
- Letter of Credit

Of the common practices listed above, the Letter of Credit is the most complicated, but it has important advantages for both the Buyer and the Seller.

1. Cash in advance

The Buyer, after purchasing the commodity under the original contract, sends the Seller cash pre-payment for the entire shipment. The Seller, upon receipt of the cash advance, makes shipment to the Buyer and provides all the necessary shipping documents. This is the simplest payment option.

What are the advantages and disadvantages of cash in advance?
This method of payment involves direct Buyer / Seller contact without commercial bank involvement and is therefore inexpensive. However, the Buyer faces a very high degree of payment risk while retaining little recourse against the Seller for poor quality goods or incorrect or incomplete documentation, and there is a possibility that an unscrupulous Seller may never deliver the goods even though the Buyer has made full prepayment.

2. Down payment

The Buyer pays the Seller a portion of the cost of the goods "in advance", when the contract is signed or shortly thereafter.

What are the advantages and disadvantages of down payment?
The down payment method induces the Seller to begin performance without the Buyer paying all of the agreed price in advance. The disadvantage is that there is a possibility the Seller may never deliver the goods even though it has the Buyer's down payment. This option must be combined with one of the other options to cover the full cost of goods.

3. Open account or "on account"

This payment method is virtually the opposite of "cash in advance". In this option, the Seller essentially extends credit to the Buyer. Upon shipment, the Seller prepares the normal documents (such as bills of lading and original invoices) and presents these to the Buyer directly, thus avoiding the

involvement of a commercial bank. The Buyer then pays the Seller directly, usually via wire transfer, upon receipt of the documents.

What are the advantages and disadvantages of open account?
Under an open account payment method, title to the goods usually passes from the Seller to the Buyer prior to payment and subjects the Seller to risk of default by the Buyer. Furthermore, there may be a time delay in payment, depending on how quickly documents are exchanged between Seller and Buyer. While this payment term involves the fewest restrictions and the lowest cost for the Buyer, it also presents the Seller with the highest degree of payment risk and is employed only between a Buyer and a Seller who have a long-term relationship involving a great level of mutual trust.

4. Payment against documents

This method of payment is primarily used for ocean shipment. It is generally inapplicable for vaccine procurement as vaccines are shipped by air. Since vaccines are shipped by air, the vaccines normally would arrive well before the documents.

While this method is not applicable to air cargo shipments, it is included here for completeness.

How does payment against documents work?
Under the original contract, the Seller makes shipment and then sends the shipping documents to his bank for collection. The Seller's bank sends the shipping documents along with a collection letter to the Buyer's bank, who sends a collection notice to the Buyer. The Buyer either makes payment upon receiving the notice and prior to possessing the shipping documents, (a cash against documents arrangement), or the Seller accepts a time draft obligating the Buyer to pay at a future date (a documents against acceptance arrangement). Only after payment or acceptance does the Buyer receive the original shipping documents.

What are the advantages and disadvantages of payment against documents?
The major advantage of a "cash against documents" payment method for the Buyer is the low cost, versus opening a Letter of Credit. The advantage for the Seller is that he can receive full payment prior to releasing control of the documents, although this is offset by the risk that the Buyer will, for some reason, reject the documents (or they will not be in order). Since the cargo would already be loaded (to generate the documents), the Seller has little recourse against the Buyer in cases of non-payment. A payment against documents arrangement involves a high level of trust between the Seller and the Buyer and should be adopted only by parties well known to each other.

5. *Letter of Credit*

There are three major types of Letter of Credit: revocable Letter of Credit, irrevocable letter of credit and confirmed irrevocable Letter of Credit. This manual discusses only the latter two because revocable is rarely used.

a. Irrevocable Letter of Credit

The irrevocable commercial Letter of Credit is a banking instrument that guarantees payment to the Seller (Beneficiary) when he has complied with its terms. Usually, these terms will include shipment of the contracted goods, compliance with specific contract requirements and presentation of specified documentary evidence to the bank proving compliance. The bank deals in documents only, not intentions, and therefore allows no discrepancies without the expressed approval of the Buyer (Applicant).

b. Confirmed Irrevocable Letter of Credit

The Seller relies on the bank's guarantee and therefore wants to be completely assured of its reliability. When Letters of Credit are issued through small local banks, the confirmation of a major international bank is often required. In other cases, the Seller simply wants payment to be guaranteed by a bank located in his own country. Thus, a "Confirmed" Letter of Credit is a double assurance of payment: The Issuing Bank makes a legally binding promise to pay a Beneficiary and a second bank (the Confirming Bank) adds its own legally binding guarantee to pay if the Issuing Bank defaults.

In selecting a bank to issue a Letter of Credit, it is important to choose one with an official correspondent relationship with a major international bank so that appropriate confirmations are possible.

c. Separate Contracts Relating to a Letter of Credit

There are three separate contracts in operation under a Letter of Credit arrangement and sometimes four:

- The sales contract between the Buyer and the Seller.

- The reimbursing agreement between the Buyer (Applicant) and the Issuing Bank (the Bank that issues the Letter of Credit). This is normally a deposit or set-aside of the Buyer's (Applicant's) funds in his own bank against the time the Seller (Beneficiary) fully complies with the requirements of the Letter of Credit and thus gains access to the payment. The Buyer (Applicant) is said to have collateralised the Letter of Credit when he deposits or sets aside[41] funds in the Issuing Bank. These funds may not be used for

[41] Banking process where access to specified funds in an existing bank account is restricted.

other purposes, but the Buyer (Applicant) earns interest, or other benefit, on the deposit until the Letter of Credit is paid.

- The Letter of Credit between the Issuing Bank and the Beneficiary (Seller).

- If the Letter of Credit is "confirmed" by another bank, then such bank (the Confirming Bank) undertakes its own contractual arrangement with the Beneficiary (Seller), in addition to that of the Issuing Bank.

  An Advising Bank simply provides information and there is no contractual undertaking; however, in practice, the Advising Bank may also be the Confirming Bank.

Each contract is independent and controls its relationship with the other parties.

d. Issuing a Letter of Credit

Supplement VI.D.5.d., page 710

The Buyer applies to his bank to issue a Letter of Credit in favour of the Seller. Exact terms are spelled out, such as:

- how much is to be paid
- in what currency
- time limits for shipment, presentation of documents for payment
- what documents must be presented in order to allow the bank to pay

In most cases, the Purchaser will be required to deposit funds or assign already deposited funds equal to the expected payment. During the period between deposit and payment, the bank may pay the Buyer (Applicant) interest, or other benefit, for the use of the funds deposited with the bank.

The bank issues its document to the Seller (Beneficiary) with a copy to the Buyer (Applicant). If there are errors in the document prepared by the bank, a no-cost amendment can be requested. Amendments can also be requested by either the Buyer or the Seller to accommodate changing conditions, provided they are acceptable to both parties. For instance, a delivery date may need to be amended based on an agreement between Seller and the Buyer.

e. Settlement of a Letter of Credit

Supplement VI.D.5.e., page 711

In order to receive payment, the Beneficiary (Seller) must submit specified documents to the paying bank as proof of his performance. These documents are:

- Commercial Invoice
- Insurance Certificate
- Transport Documents (for example, the Bill of Lading or Air Waybill)
- Certificate of Origin
- Inspection Certificate
- Authorisation for Shipment
- Other Certificates and Certifications (for example, Certificate of Analysis)

The first three items are required. The last four items are optional and are often used as tools to enforce contract provisions.

f. Cost

Banks charge a percentage of the value of the goods for the original Letter of Credit. This charge is normally paid by the Applicant (Buyer). Banks levy additional fees for amendments, payments and draw-downs. The Letter of Credit should stipulate who is responsible for paying these additional fees, the Beneficiary or the Applicant. Fees for opening a Letter of Credit will amount to at least several hundred dollars. Every bank is different so the Applicant should get this information at the initial contact. A typical fee ranges from 0.5 percent to 1.0 percent of the face value of the Letter of Credit.

g. What are the advantages and disadvantages of Letters of Credit?

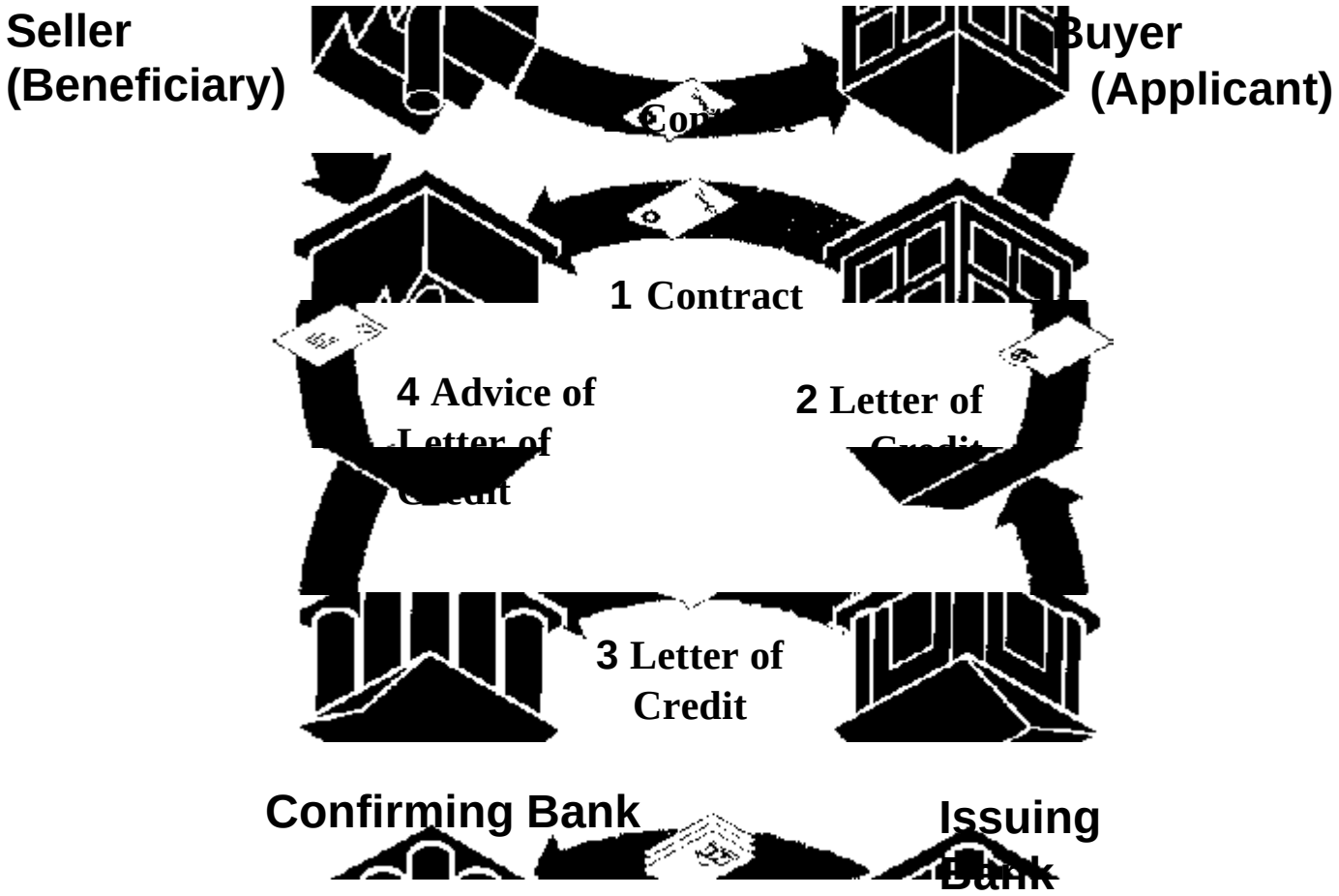
The Letter of Credit allows the Buyer to avoid payment in advance, accrue interest (or other consideration) on deposited funds until the goods are shipped and enforce quality assurance provisions in the contract by linking proof of compliance to payment. This proof may take the form of Quality Assurance Documents, Inspection or Testing[42] Certificates or an Authorisation for Shipment signed by the Buyer's representative, based on acceptable inspection or testing results.

The confirmed, irrevocable Letter of Credit presents the Seller with the least risk. Since the Buyer generally bears the cost of opening the Letter of Credit, it is the highest cost option for the Buyer. In addition, the existence of a Letter of Credit does not obligate the Seller to ship the goods purchased by the Buyer.

(Text resumes on page 712)

[42] Testing is only required in exceptional circumstances.

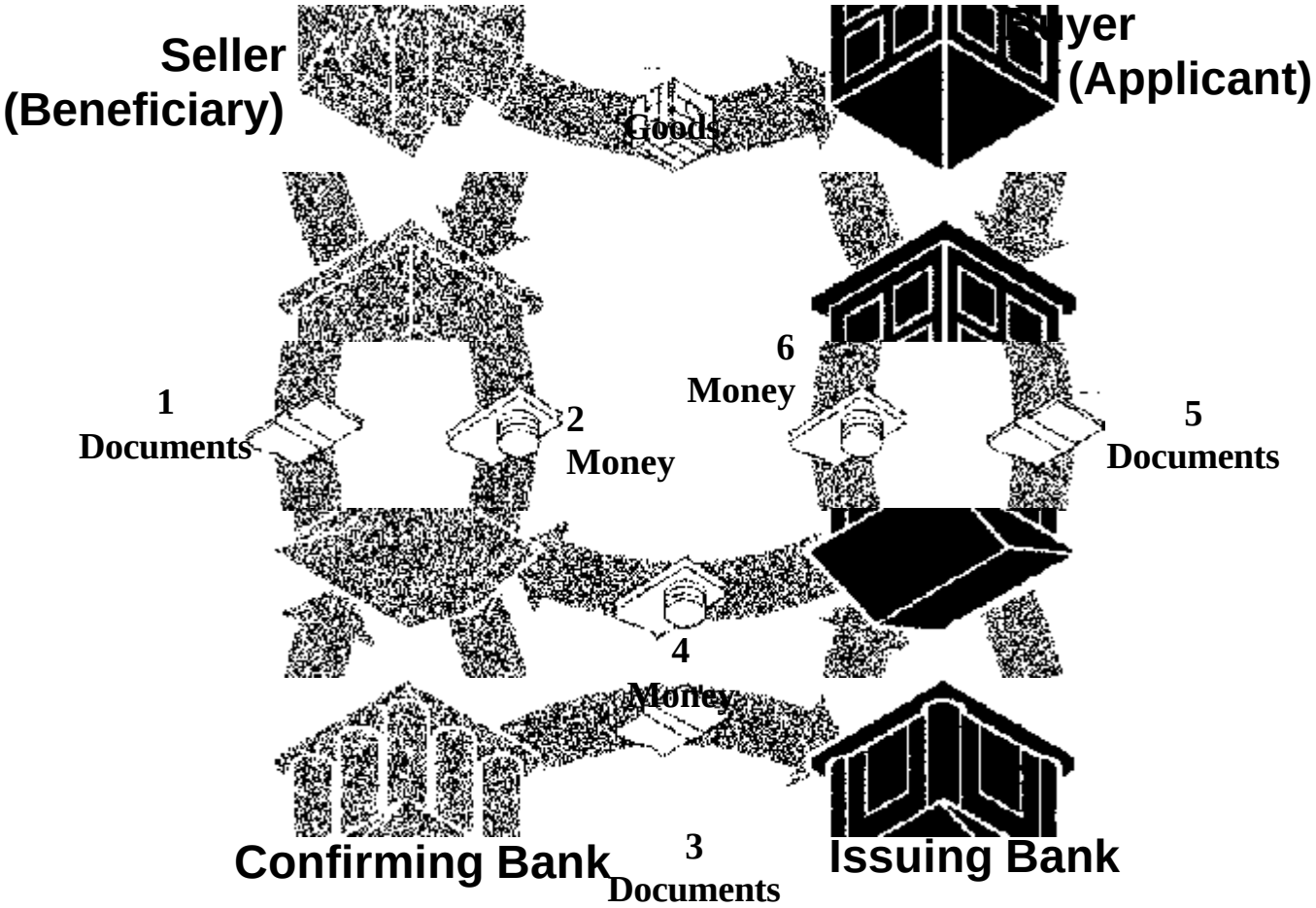
Issuing a Letter of Credit



1. **The Buyer and the Seller conclude a sales contract providing for payment by Letter of Credit.**
2. **The Buyer instructs his bank - the Issuing Bank - to issue a Letter of Credit in favour of the Seller (Beneficiary).**
3. **The Issuing Bank asks another bank, usually in the country of the Seller, to advise and perhaps also to add its confirmation to the Letter of Credit.**
4. **The Confirming Bank informs the Seller that the Letter of Credit has been issued.**

del Busto C., *ICC Guide to Documentary Credit Operations*. International Chamber of Commerce; June 1994.

Settlement of a Letter of Credit



1. **The seller sends the documents evidencing the shipment to the bank where the Letter of Credit is available (the Confirming Bank).**
2. **After checking that the documents meet the Letter of Credit requirements, the bank makes payment.**
3. **This bank then sends the documents to the Issuing Bank.**
4. **The Issuing Bank, after checking that the documents meet the Letter of Credit requirements, makes reimbursement in the pre-agreed manner (see UCP 500 Article 19).**
5. **The Issuing Bank then sends the Documents to the Buyer.**
6. **Issuing Bank is paid by Buyer in the pre-agreed manner.**

del Busto C., *ICC Guide to Documentary Credit Operations*. International Chamber of Commerce; June 1994.

D. International Commerce

1. ICC (International Chamber of Commerce)

The ICC is a non-governmental organisation serving and representing the world business community at national and international levels. It promotes world trade and investment based on free and fair competition, harmonises trade practices and formulates terminology and guidelines for importers and exporters, such as INCOTERMS (see 2. below). It also provides practical services to business and produces a wide range of useful publications including several of critical importance to vaccine purchasers.[43]

Supplement VI.D.1., page 713

2. INCOTERMS (International Commercial Terms)

INCOTERMS such as Ex Works, CIP and FOB define the responsibilities of Buyers and Sellers for delivery of goods under contract and stipulate how costs and risks are divided. These terms are regularly incorporated into sales contracts worldwide and are recognised by the United Nations. A chart summarising the most common terms is at the end of this section (Supplement VI.D.2.a.), along with an enlargement of the associated legend indicating air shipment, truck shipment, train shipment and sea shipment.

Supplement VI.D.2.a., page 719
Supplement VI.D.2.b., page 720

(Text resumes on page 721)

[43] *Guide to INCOTERMS 1990* and *ICC Guide to Documentary Credit Operations*. All ICC publications are available from ICC National Committees in some 60 countries or from ICC Publishing S.A., 38 Cours Albert 1er, 75008 Paris, France [Customer Service telephone (33 1) 49 53 29 23; fax (33 1) 49 5329 02] and ICC Publishing Inc., 156 Fifth Avenue, New York, NY 10010, U.S.A. [telephone (212) 206 1150, fax (212) 633 6025].

INCOTERMS

1. *Introduction*

The INCOTERMS are published by the International Chamber of Commerce [ICC] for the purpose of clearly defining the most common terms used in international trade. The shipping rules specify the point at which risks and responsibilities are transferred from the seller to the buyer.

The 13 INCOTERMS shipping terms are grouped into four basically different categories. The categories are described below. [Group C terms are the ones normally used when buying vaccines]:

Group E: Departure term.

Where the Seller makes the goods available to the Buyer at the Seller's own premises, (EXW).

Group F: Shipment terms - main carriage paid by Buyer.

Where the Seller is called on to deliver the goods to a carrier named by the Buyer, (FCA, FAS and FOB). These are shipment contracts with the shipment point named and carriage unpaid by the Seller.

Group C: Shipment terms - main carriage paid by Seller.

These terms are normally used when buying vaccines and are discussed in detail below.

Group D: Arrival terms.

Where the Seller has to bear all costs and risk needed to bring the goods to the country of destination, (DAF, DES, DEQ, DDU and DDP). These are arrival contracts.

2. *Details of Shipping Terms*

Group E: Departure term.

EXW - EX WORKS (... named place)

"Ex works" means that the Seller fulfills his obligation to deliver when he has made the goods available at his premises (i.e., works, factory, warehouse, etc.) to the Buyer. In particular, he is not responsible for loading the goods on the vehicle provided by the Buyer or for clearing the goods for export, unless otherwise agreed. The Buyer bears all costs and risks involved in taking the goods from the Seller's premises to the desired destination. This term thus represents the minimum obligation for the Seller.

This term should not be used when the Buyer cannot carry out directly or indirectly the export formalities. In such circumstances, the FCA term should be used.

Group F: Shipment terms - main carriage paid by Buyer.

FCA - FREE CARRIER (... named place)

"Free Carrier" means that the Seller fulfills his obligation to deliver when he has handed over the goods, cleared for export, into the charge of the carrier named by the Buyer at the named place or point. If no precise point is indicated by the Buyer, the Seller may choose within the place or range stipulated where the carrier shall take the goods into his charge. When, according to commercial practice, the Seller's assistance is required in making the contract with the carrier (such as in rail or air transport) the Seller may act at the Buyer's risk and expense.

This term may be used for any mode of transport, including multi-modal transport.

"Carrier" means any person who, in a contract of carriage, undertakes to perform or to procure the performance of carriage by rail, road, sea, air, inland waterway or by a combination of such modes. If the Buyer instructs the Seller to deliver the cargo to a person, e.g., a freight forwarder who is not a "carrier", the Seller is deemed to have fulfilled his obligation to deliver the goods when they are in the custody of that person. "Transport terminal" means a railway terminal, a freight station, a container terminal or yard, a multi-purpose cargo terminal or any similar receiving point.

"Container" includes any equipment used to unitize cargo, e.g., all types of containers and / or flats, whether ISO accepted or not, trailers, swap bodies, Ro-Ro equipment or igloos, and applies to all modes of transport.

FAS - FREE ALONGSIDE SHIP (... named port of shipment)

"Free Alongside Ship" means that the Seller fulfills his obligation to deliver when the goods have been placed alongside the vessel on the quay or in lighters at the named port of shipment.

This means that the Buyer has to bear all costs and risks of loss of or damage to the goods from that moment. The FAS term requires the Buyer to clear the goods for export. It should not be used when the Buyer cannot carry out directly or indirectly the export formalities.

This term can only be used for sea or inland waterway transport.

FOB - FREE ON BOARD (... named port of shipment)

"Free on Board" means that the Seller fulfills his obligation to deliver when the goods have passed over the ship's rail at the named port of shipment. This means that the Buyer has to bear all costs and risks of loss of or damage to the goods from that point.

The FOB term requires the Seller to clear the goods for export.

This term can only be used for sea or inland waterway transport. When the ship's rail serves no practical purpose, such as in the case of roll-on/ roll-off or container traffic, the FCA term is more appropriate to use.

Group C: Shipment terms - main carriage paid by Seller.

The Group C terms are normally used when purchasing vaccine. With these terms the Seller has to contract for carriage, but without assuming the risk of loss of or damage to the goods or additional costs due to events occurring after shipment and dispatch, (CFR, CIF, CPT and CIP).

These are shipment contracts with the destination point named and carriage paid by the Seller. There are two critical division points, one for the division of costs, the other for the division of risk. Costs being assumed by the Seller until the destination point; risk being transferred to the Buyer at the point of shipment.

CIF and CIP are the only INCOTERMS related directly to insurance coverage. In these the Seller arranges the contract of carriage and payment of freight and is regarded as being in a better position than the Buyer to arrange insurance.

CFR - COST AND FREIGHT (... named port of destination)

"Cost and Freight" means that the Seller must pay the costs and freight necessary to bring the goods to the named port of destination but the risk of loss of or damage to the goods, as well as any additional costs due to events occurring after the time the goods have been delivered on board the vessel, is transferred from the Seller to the Buyer when the goods pass the ship's rail in the port of shipment.

The CFR term requires the Seller to clear the goods for export.

This term can only be used for sea and inland waterway transport. When the ship's rail serves no practical purpose, such as in the case of roll-on/ roll-off or container traffic, the CPT term is more appropriate to use.

CIF - COST, INSURANCE AND FREIGHT (... named port of destination)

"Cost, Insurance and Freight" means that the Seller has the same obligations as under CFR but with the addition that he has to procure marine insurance against the Buyer's risk of loss of or damage to the goods during the carriage. The Seller contracts for insurance and pays the insurance premium.

The Buyer should note that under the CIF term the Seller is only required to obtain insurance on minimum coverage. The CIF term requires the Seller to clear the goods for export.

This term can only be used for sea and inland waterway transport. When the ship's rail serves no practical purposes such as in the case of roll-on/ roll-off or container traffic, the CIP term is more appropriate to use.

CPT - CARRIAGE PAID TO (... named place of destination)

"Carriage paid to... " means that the Seller pays the freight for the carriage of the goods to the named destination. The risk of loss of or damage to the goods, as well as any additional costs due to events occurring after the time the goods have been delivered to the carrier, is transferred from the Seller to the Buyer when the goods have been delivered into the custody of the carrier.

"Carrier" means any person who, in a contract of carriage, undertakes to perform or to procure the performance of' carriage, by rail, road, sea, air, inland waterway or by a combination of such modes.

If subsequent carriers are used for the carriage to the agreed destination, the risk passes when the goods have been delivered to the first carrier.

The CPT term requires the Seller to clear the goods for export.

This term may be used for any mode of transport including multi-modal transport.

CIP - CARRIAGE AND INSURANCE PAID TO (... named place of destination)

"Carriage and insurance paid to..." means that the Seller has the same obligations as under CPT but with the addition that the Seller has to procure cargo insurance against the Buyer's risk of loss of or damage to the goods during the carriage. The Seller contracts for insurance and pays the insurance premium.

The Buyer should note that under the CIP term the Seller is only required to obtain insurance on minimum coverage. The CIP term requires the seller to clear the goods for export.

This term may be used for any mode of transport including multi-modal transport.

Group D: Arrival terms.

DAF - DELIVERED AT FRONTIER (... named place)

"Delivered at Frontier" means that the Seller fulfills his obligation to deliver when the goods have been made available, cleared for export, at the named point and place at the frontier, but before the customs border of the adjoining country. The term "frontier" may be used for any frontier including that of the country of export. Therefore, it is of vital importance that the frontier in question be defined precisely by always naming the point and place in the term. The term is primarily intended to be used

when goods are to be carried by rail or road, but it may be used for any mode of transport.

DES - DELIVERED EX SHIP (... named port of destination)

"Ex Ship" means that the Seller fulfills his obligation to deliver when the goods have been made available to the Buyer on board the ship uncleared for import at the named port of destination. The Seller has to bear all the costs and risks involved in bringing the goods to the named port of destination. This term can only be used for sea or inland waterway transport.

DEQ - DELIVERED EX QUAY (DUTY PAID) (... named port of destination)

"Delivered Ex Quay (duty paid)" means that the Seller fulfills his obligation to deliver when he has made the goods available to the Buyer on the quay (wharf) at the named port of destination, cleared for importation. The Seller has to bear all risks and costs including duties, taxes and other charges of delivering the goods thereto.

This term should not be used if the Seller is unable directly or indirectly to obtain the import license.

If the parties wish the buyer to clear the goods for importation and pay the duty, the words "duty unpaid" should be used instead of "duty paid".

If the parties wish to exclude from the Seller's obligations some of the costs payable upon importation of the goods (such as value added tax (VAT)), this should be made clear by adding words to this effect: "Delivered ex quay, VAT unpaid (... named port of destination)",.

This term can only be used for sea or inland waterway transport.

DDU - DELIVERED DUTY UNPAID (... named place of destination)

"Delivered duty unpaid" means that the Seller fulfills his obligation to deliver when the goods have been made available at the named place in the country of importation. The Seller has to bear the costs and risks involved in bringing the goods thereto (excluding duties, taxes and other official charges payable upon importation) as well as the costs and risks of carrying out customs formalities. The Buyer has to pay any additional costs and to bear any risks caused by his failure to clear the goods for import in time.

If the parties wish the Seller to carry out customs formalities and bear the costs and risks resulting therefrom, this has to be made clear by adding words to this effect.

If the parties wish to include in the Seller's obligations some of the costs payable upon importation of the goods (such as value added tax (VAT)),

this should be made clear by adding words to this effect: Delivered duty unpaid, VAT paid, (... named place of destination).

This term may be used irrespective of the mode of transport.

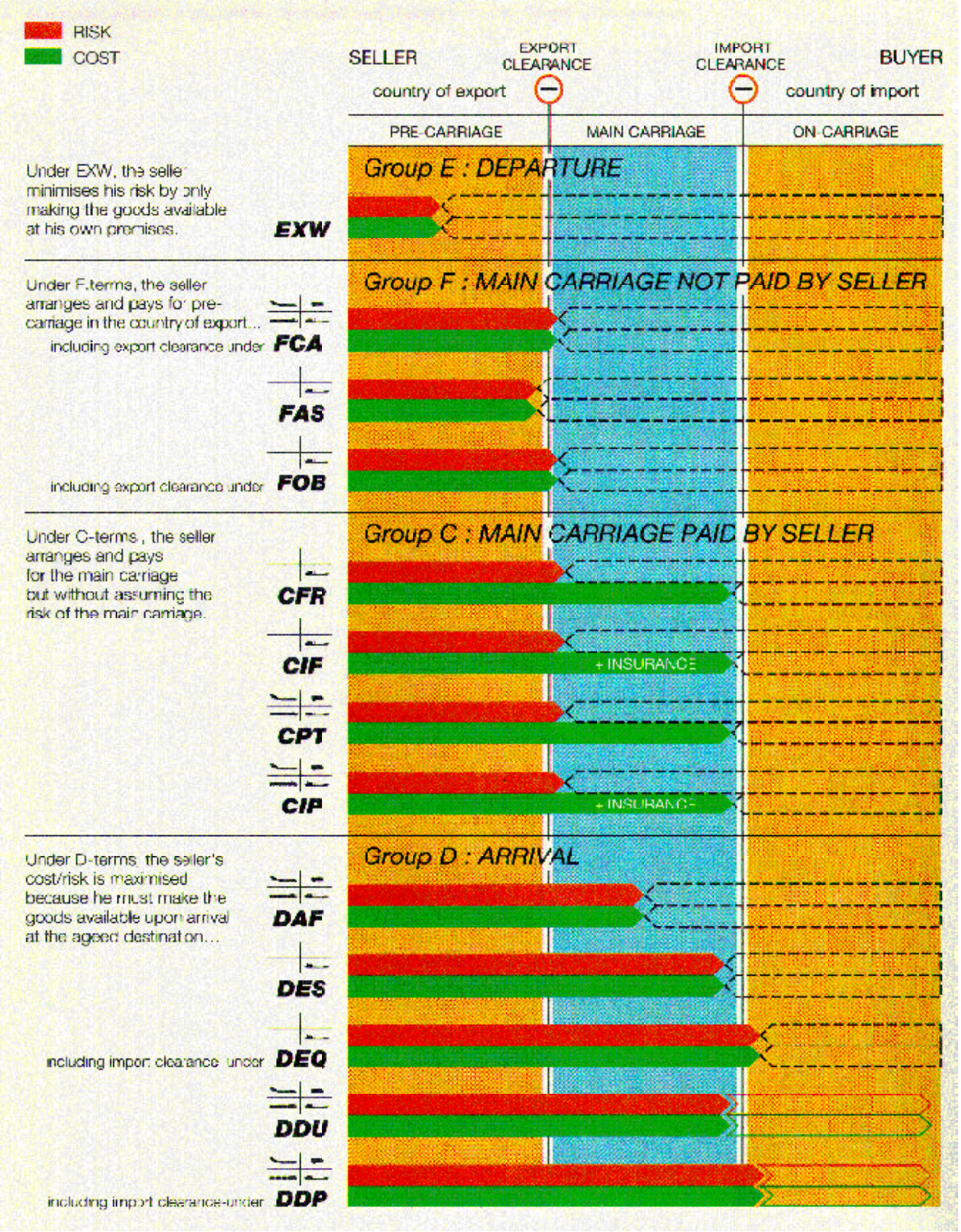
DDP - DELIVERED DUTY PAID (... named place of destination)

"Delivered duty paid" means that the Seller fulfills his obligation to deliver when the goods have been made available at the named place in the country of importation. The Seller has to bear the risks and costs, including duties, taxes and other charges of delivering the goods thereto, cleared for importation.

If the parties wish to exclude from the Seller's obligations some of the costs payable upon importation of the goods (such as value added tax (VAT)), this should be made clear by adding words to this effect: "Delivered duty paid, VAT unpaid (...named place of destination)".

This term may be used irrespective of the mode of transport.

Common Terms



E. International Shipping Documents

1. Normally required documents

The following documents are normally required by the country of export, the carrier of the goods and the importing country:

- Commercial Invoice
- Bill of Lading or Air Waybill
- Insurance Certificate
- Packing List

2. Common documents

The importing country might also require:

- Import License
- Certificate of Origin (sometimes)
- Certificate of Analysis (sometimes)

3. Additional documents

In addition, the purchaser or purchaser's National Control Authority may require:

- Certificate of Free Sale
- Certificate of Inspection
- Certificate of Registration
- Product License

Examples of all these documents are located at the end of this section, beginning on page 722.

COMMERCIAL INVOICE

1) **Shipper/Exporter:** CHEIL AMERICA 1234 Production Road Industry Town, State of New Jersey, USA	8) **Date and Number of Invoice:** CA-97-2007-HEP 9) **Number and Date of L/C:** P/O 97-0108
2) **For Account & Risk of Messrs.** Local Health Ministry 5678 Mission Compound, 1045 Lima PERU	10) **L/C Issuing Bank:** T/T payment 30 days from AWB date 11) **Remarks:** • Consignee same as notify • Freight prepaid and insured at 11% CIF value
3) **Notify Party:** Same as above General Director, Senor P. Gonsalves 011 525 0111	

4) **Port of Loading:** Seoul, Korea	12) **Final Destination:** Lima, Peru	13) **Delivery Terms:** CIF Lima Peru
6) **Carrier** LH8729	7) **Sailing/Shipping on or about:** September 12, 1997	14) **Payment To:** United Jersey Bank ABA No. 987654321 Acct. No. 1234567 Beneficiary: Cheil America or: bank draft payable to Cheil America 30 days from AWB date

15) **Marks and numbers:**	16) **Description of Goods:**	17) **Quantity**	18) **Unit price**	19) **Total**
Hepatitis B vaccine Heppaccine B	5 ml Adult Vial at 3mcg/ml per dose, with standard packaging and art work, in English language	6,000 doses	USD 7.50/ vial	USD 9,000.00

Country of Origin: Korea

20) Signed by: _____
"JH Ming, General Manager"

PASTEUR MÉRIEUX

——Sérums & Vaccins——

SIÉGE SOCIAL : 123, AVENUE INDUSTRIE - 56789 LYON - FRANCE

TEL:(33) 12 34 56 78 - TELEX : 900 100 MERIX LYON - FAX 98 76 54 32

Société anonyme au capital de 638 950 000 F - R.C.S. Lyon B 349 505 370 - Sinet 349 505 370 00016

No. identification T.V.A. FR 349 505 370

Lyon, le:	Invoice	Folio	No. Client	Reference de la Command/ Indent Reference
17/08/97	12345-ABC	1	00247	97-MED-0002

Address de Livration/Delivery Address	Address de facturation/ Invoicing address
UKRAINE HUMANITARIAN ASSISTANCE 12345 KIEV 10, GRUSHENKO ST. 7 MINISTRY OF HEALTH, UKRAINE, "FORWARDER: PREFERRED BROKERS"	NATIONAL HEALTH AGENCY 12345 KIEV 50, KOVTUN ST., 10 UKRAINE

Exdedition/ Dispatch	No. Delta/ Air waybill No.
AIR FREIGHT REMIS A SAGA ROUEN LE 39 AOUT 1997 VOL PS 702 DU 31 AOUT A 10H45 ARRIVE A KIEV A 15HOO	566-1000-3486

TELEPHONE
392 476 8855
TELEX/FAX
392 476 5588
NO TARRIF DOULANIER *CUSTOMS TARRIF*
NO. d'INSURANCE *(INSURANCE REF.)*

No. Colis *(No. of pieces)*	Poids Brut *(Gross Weight)*	Poids Net *Net Weight)*	Volume	Médécine *(Medicine)*
25	742.500 KG	650.000 KG	3.825	

NO. ARTICLE *ITEM NO.*	PRODUITS D'ORIGINE ET DE FRANÇAISE *PRODUCTS OF FRENCH ORIGIN AND MANUFACTURED IN FRANCE*	LOT NO. *BATCH NO.*	PEREMP *EXPIRY*	QUANTITÉ *QUANTITY*	PRIX UNITAIRE *UNIT PRICE*	NO. ARTICLE *(ITEM NO.)*
041011552	DT ADULT VACCINE, 10 VIALS/ 20 DOSE SHIPPED TODAY	K0619	12/31/97	2500	18.00	45000.00

Brut *Invoice Total*	Port *Freight*	Assurance *Insurance*	Douanes *Duties*	Consulaires *Consular fees*	Frais Divers *Misc. Charges*
45000.00	1645.80	31.50			

Dévise/*Currency*	Total Facture *Total Amount*
USD	46677.30

Mode de Facturation/*Invoicing Conditions*	Ville/*Port of Destination*
C.I.P.	Kiev

Mode de Paiement/ *Mode of Payment*	Conditions of Patment/*Terms of Payment*
BANK TRANSFER	30 DAYS/END MONTH FROM INVOICE DATE

Domiciliation Client/ *Client Bank*	Domiciliation Pasteur Mérieux/ *Payable on our bank*

023 | 8506 9902 Air Waybill, Example #1 023- 8506 9902

Shipper's Name and Address
CHEIL AMERICA
INDUSTRY ROAD
RIDGEFIELD NJ USA

Shipper's Account Number

Not Negotiable
Air Waybill

Issued by FedEx® Box 727 Memphis, TN, USA 38194-2112

023-8506 9902

Consignee's Name and Address
BIOVAC
HEALTH CARE ROAD
PO BOX ###
JOHANNESBURG SOUTH AFRICA
NOTIFY PARTY: SAME AS ABOVE

Consignee's Account Number

Copies 1, 2 and 3 of this Air Waybill are originals and have the same validity.

It is agreed that the goods described herein are accepted in apparent good order and condition (except as noted) for carriage SUBJECT TO THE CONDITIONS OF CONTRACT ON THE REVERSE HEREOF. ALL GOODS MAY BE CARRIED BY ANY OTHER MEANS INCLUDING ROAD OR ANY OTHER CARRIER UNLESS SPECIFIC CONTRARY INSTRUCTIONS ARE GIVEN HEREON BY THE SHIPPER, AND SHIPPER AGREES THAT THE SHIPMENT MAY BE CARRIED VIA INTERMEDIATE STOPPING PLACES WHICH THE CARRIER DEEMS APPROPRIATE. THE SHIPPER'S ATTENTION IS DRAWN TO THE NOTICE CONCERNING CARRIER'S LIMITATION OF LIABILITY. Shipper may increase such limitation of liability by declaring a higher value for carriage and paying a supplemental charge if required.

Issuing Carrier's Agent Name and City
DAEBO AIR CARGO CO., LTD.

Accounting Information
ATTN: MR. S. ANYANGWE
TEL (---) -- --- ----

Agent's IATA Code: ## # ####/####

Account No.

Airport of Departure (Addr. of First Carrier) and Requested Routing
SEOUL/KOREA

To	By First Carrier / Routing and Destination	to	by	to	by
KUL	MH067	JNB	MH		

Currency	CHGS Code	WT/VAL PPD	WT/VAL COLL	Other PPD	Other COLL	Declared Value for Carriage	Declared Value for Customs
USD		X		X		N.V.D.	

Airport of Destination
JNB/SOUTH AFRICA

Flight/Date | For Carrier Use Only | Flight/Date

Amount of Insurance: INSURANCE - If carrier offers insurance, and such insurance is requested in accordance with the conditions thereof, indicate amount to be insured in figures in box marked "Amount of Insurance".

TC

Handling Information
INVOICE, PACKING LIST ATTACHED
* DO NOT FREEZE
* STORE AT 2-8°C.

☐ International EXPRESSfreight ☐ Airport to Airport

SCI

No. of Pieces RCP	Gross Weight	kg / lb	Rate Class / Commodity Item No.	Chargeable Weight	Rate / Charge	Total	Nature and Quantity of Goods (incl. Dimensions of Volume)
22	367.0	K	Q	367.0	11.40		6,000 vials Hepatitis B vaccine; Hepaccine-B Inj. .05ml/vial INV NO: C8899 "FREIGHT PREPAID" PERISHABLE MATERIAL KEEP BETWEEN 2-8°C DO NOT FREEZE MEDICINES FOR HUMAN USE HIGHLY PERISHABLE MATERIALS NOT TO BE DELAYED CONNECTION BY BOOKED FLIGHT ~~PENDING RE SHIPMENT, STORE~~ AT 2-8°C DO NOT FREEZE

HEPATITIS B VACCINE
HEPACCINE B INJ.

TO: BIOVAC
JOHANNESBURG, SOUTH AFRICA

MANUFACTURED BY CHEIL FOODS & CHEMICALS

MADE IN KOREA
! UP

Prepaid | Weight Charge | Collect

Other Charges

Valuation Charge

Tax

Total Other Charges Due Agent

Total Other Charges Due Carrier

Shipper certifies that the particulars on the face hereof are correct and that **insofar as any part of the consignment contains dangerous goods, such part is properly described by name and is in proper condition for carriage by air according to the applicable Dangerous Goods Regulations.**

Signature of Shipper or his Agent

Total Prepaid | Total Collect

Currency Conversion Rates | CC Charges in Dest. Currency

October 1, 1997 SEL M.H. YEE
Executed on (date) at (place) Signature of Issuing Carrier or its Agent

For Carrier's Use Only at Destination | Charges at Destination | Total Collect Charges

023- 8506 9902
COPY 8 (FOR AGENT)

FedEx PART 147883 REV. 9/95 FORMAT #235 SR-Y 02/97

Air Waybill Example #2

566 CDG | 1000 3486 566-1000 3486

Nom et adresse de l'expéditeur / Shipper's Name and Address
PASTEUR MERIEUX
SERUMS & VACCINS
58 AVENUE LECLERC
69007 LYON CEDEX 07
FRANCE

Numéro de compte de l'expéditeur / Shipper's Account Number

LETTRE DE TRANSPORT AÉRIEN Non négociable / Not negotiable / AIR WAYBILL / Issued by
AIR UKRAINE INTERNATIONAL
KIEV AIRPORT
BORISPOL UKRAINE
CITA KBPAPPS
UKRAINE

Les exemplaires 1, 2 et 3 de cette lettre de transport aérien sont originaux et ont la même validi
Copies 1, 2 and 3 of this Air Waybill are originals and have the same validity.

Nom et adresse du destinataire / Consignee's Name and Address
UKRAINE HUMANITARIAN
ASSISTANCE 252021 KIEV 21,
GRUSHEVSKOGO ST.7 MINISTRY OF
HEALTH, UKRAINE, "UKRAVACCINA"
GAL DIRECTOR GEORGIJ BRUJ

Numéro de compte du destinataire / Consignee's Account Number

Il est convenu que les marchandises décrites dans le présent document sont acceptées pour le transport en bon état appar (sauf annotation contraire) et que le transport est SOUMIS AUX CONDITIONS DU CONTRAT QUI FIGURENT AU VERSO. L MARCHANDISES PEUVENT ÊTRE TRANSPORTÉES PAR TOUT AUTRE MOYEN Y COMPRIS PAR ROUTE OU PAR TOUT AUT TRANSPORTEUR A MOINS QUE DES INSTRUCTIONS CONTRAIRES PRECISES, A CE SUJET, NE SOIENT DONNEES PAR L'EXPEDITE L'ATTENTION DE L'EXPÉDITEUR EST ATTIRÉE SUR L'AVIS CONCERNANT LA LIMITATION DE RESPONSABILITE DU TRANSPORTE L'expéditeur peut augmenter cette limitation de responsabilité en déclarant une valeur pour le transport plus élevée et en payant frais supplémentaires s'il y a lieu.
It is agreed that the goods described herein are accepted in apparent good order condition (except as noted) for carriage SUBJECT TO T CONDITIONS OF CONTRACT ON THE REVERSE HEREOF. ALL GOODS MAY BE CARRIED BY ANY OTHER MEANS INCLUDING ROAD ANY OTHER CARRIER UNLESS SPECIFIC CONTRARY INSTRUCTIONS ARE GIVEN HEREON BY THE SHIPPER. THE SHIPPE ATTENTION IS DRAWN TO THE NOTICE CONCERNING CARRIER'S LIMIT OF LIABILITY. Shipper may increase such limitat of liability by declaring a higher value for carriage and paying a supplemental charge if required.

Nom et ville de l'agent du transporteur émetteur / Issuing Carrier's Agent, Name and City
SAGA AIR
AEROPORT ROUEN VALL.SEINE
76520 BOOS

Renseignements comptables / Accounting information
STATUT DOUANIER C BUREAU 039

Code IATA de l'agent / Agent's IATA Code
20 4 7061

Numéro de compte / Account Number

Aéroport de départ (Adresse du premier transporteur) et itinéraire demandé / Airport of Departure (Address of First Carrier) and Requested Routing
CDG ROISSY C.D.G.

à / to	Par premier transport / By First Carrier	Routage et Dest. / Rout. and Dest.	à / to	par / by	à / to	par / by
IEV	AIR UKRAINE					

Monnaie / Currency	Frais / CHGS code	Poids/Val. Wt/Val. Payé / PPD	Poids/Val. Wt/Val. Dû / COLL	Autres / Other Payé / PPD	Autres / Other Dû / COLL	Valeur déclarée pour le transport / Declared Value for Carriage	Valeur déclarée pour la douane / Declared Value for Customs
FRF	PX	X		X		N.V.D	N.C.V

Aéroport de destination / Airport of Destination
KIEV

Vol/Flight/Date 702 /3108

Réservé au transporteur / For Carrier Use only **Vol/Flight/Date**

Montant de l'assurance / Amount of Insurance
XXXXXX

ASSURANCE : Si le transporteur propose une assurance et que l'expéditeur en fait la demande conformeme aux présentes conditions, indiquer le montant à assurer en chiffres dans la case " Montant de l'assurance
INSURANCE : If carrier offers insurance, and such insurance is requested in accordance with the conditions there indicate amount to be insured in figures in box marked " Amount of insurance

Renseignements pour le traitement de l'expédition / Handling Information

25 PARCELS ADR/PLI SERVICE

Nombre de colis / Number of Pieces RCP	Poids brut / Gross Weight	kg / lb.	Classif. du tarif / Rate class	N° d'article de la marchandise / Commodity Item No	Poids de taxation / Chargeable Weight	Tarif/Montant / Rate/Charge	Total / Total	Nature et quantité des marchandises (y compris dimensions ou volume) / Nature and Quantity of Goods (incl. Dimensions or Volume)
25	742,5	K	Q		742,5	12,00	8910.00	MEDECINE HUMAINE VOLUME 3,825 M3
								PRODUITS MEDECINE HUMAINE. URGENT. A CONSERVER A +4°C. PERISSABLES PREVENIR LE DESTINATAIRE A L'ARRIVEE. COLDSTORAGE. TEL. 70.44.518.27.74 - FAX. 70.44.518.26.81 - FAX. 70.44.518.04.18 REF: 114880.01.00DS
25	742,5						8910,00	

Port payé / Prepaid 8910,00

Taxation au poids / Weight Charge

Port dû / Collect

Autres frais / Other Charges
CHC 58,00 AWA 36,70

Taxation à la valeur / Valuation Charge

Taxe / Tax

Total des autres frais dûs à l'agent / Total Other Charges Due Agent 36,70

Total des autres frais dûs au transporteur / Total Other Charges Due Carrier 58,00

L'expéditeur certifie que les indications portées sur le présent document sont exactes et que, dans la mesure ou une partie quelconque de l'expédition contient des marchandises dangereuses, cette partie d'expédition est correctement dénommée et bien préparée pour le transport par air conformément à la réglementation applicable.
Shipper certifies that the particulars on the face hereof are correct and that insofar as any part of the consignment contains dangerous goods, such part is properly described by name and is in proper condition for carriage by air according to the applicable Dangerous Goods Regulations.

SAGA AIR Dossier n° 9484/202123

Signature de l'expéditeur ou de son agent / Signature of Shipper or his Agent

Total port payé / Total Prepaid 9004,70

Total port dû / Total Collect

Taux conversion monnaie / Currency Conversion Rates

Port dû en monnaie du pays de destination / Collect Charges in Destination Currency

Fait le / Executed on 18/AUG/94 (Date) (Date) ROUEN à / at (Place)

SAGA AIR MORY
AIMM
AÉROPORT ROUEN VALLÉE DE SEINE
76520 BOOS
Tél. 35.09.12.86

Frais à l'arrivée / Charges at Destination

Total du Total Collect Charges

Réservé au transporteur à destination / For Carrier's Use only at Destination

ORIGINAL 3 (POUR L'EXPÉDITEUR) *ORIGINAL 3 (FOR SHIPPER)*

dep. 527 525 818 09.90 1/1-14

Extract-Translation
from the General Conditions of Marine Insurance on Goods
(GCMI 1988)
(The original wording in German or French shall be decisive in the case of dispute)

The term assured is hereinafter understood to include: the part contracting the insurance, the holder of title to claim as well as the persons for whose acts the aforementioned are responsible.

Scope of Insurance

Art. 2 Restricted Insurance

The insurance covers loss and damage only when they are the direct consequence of one of the following events (so-called specified accidents):
- shipwreck
- stranding
- springing of leaks necessitating putting into a port of refuge
- jettison and washing overboard of entire packages
- collision, overturning or breakdown of the conveyance
- derailment
- falling aircraft or spacecraft or parts thereof
- collapse of structures
- fire, explosion, lightning, earthquake, volcanic eruption, flooding, avalanche, land and snowslip, falling rock, flash flood, hurricane (windspeed in excess of 100km per hour)
- falling of goods during loading, transshipment or discharge.

Theft and disappearance of entire packages (goods and packing) or-for good in bulk-of whole consignments are also covered.

Art. 3 Extended Insurance
The insurance covers loss and damage; however, unless otherwise agreed, the following special risks are excluded:
- wetting by freshwater as well as damage caused by condensation of water vapors in ships holds or containers
- rust and other forms of oxidation
- breakage
- extraordinary leakage
- loss and damage caused by rats and mice or other vermin from an external source
- extraneous odour.

These special risks are nevertheless covered if the loss or damage has been caused by an accident specified in art. 2.

Art. 4 Insurance against all risks
The insurance covers loss and damage.

Art. T Inclusions common to all forms of cover
Also insured by all forms of cover are:
a) General average contributions chargeable to the insured goods in accordance with a legally valid statement, as well as goods sacrificed in general average, the foregoing subject to the exclusions of art. 6.
b) insofar as there is an insured loss or damage or this immediately threatens,
-the costs of the surveyor's intervention
-the costs incurred for the purpose of averting or minimizing the loss or damage
c) Should there be an insured occurrence in accordance with art. 2 the additional costs of transshipment, storage and forwarding, insofar as the assured considers this to be necessary according to the circumstances or the insurer orders it to be carried out.

Art.6 Exclusions common to all forms of cover
a) The insurer is not liable for the consequences of:
-seizure, confiscation or temporary seizure (quarantine) by government authority or power, subject to art. 6 d
-delay, howsoever caused, in transit or delivery
-deliberate actions of the assured; in the case of negligence of the assured, the insurer is entitled to reduce his payment in corresponding relation to the degree of negligence
-false declaration
-infringement of import, export or transit regulations as well as of currency and customs regulations
-infringement of carrier's regulations with the assured's knowledge
-insolvency or delay in paying on the port of the owner, charterer or operator of a conveyance or any other financial disputes with the named parties, insofar as the assured chose these parties himself or decisively affected the choice.

Furthermore, the insurer is not liable for loss or damage attributed to:
-humidity of the air
-influence of temperature
-the nature of the goods such as self-deterioration, heating, spontaneous combustion, shrinkage, wastage and ordinary leakage
-vermin originating in the goods insured
-the unsuitable condition of the goods for the insured voyage
-inadequate or insufficient packing
-inappropriate stacking tin the conveyance or container by the assured
-normal wear and tear
b) The following are also excluded:
-damage to the packing unless specifically insured
-liability to third parties for loss or damage caused by the insured goods
-damage caused by nuclear energy
-indirect damage such as:
-losses not directly sustained by the insured goods (e.g. loss of interest, difference of exchange, loss of market, loss of use or consequential loss)
-compensation for trouble taken in connection with loss or damage
-demurrage and supplements of freight of whatever nature as well as costs other than those covered in accordance with art.5b or c.
c) The insurer is released from all liability if, with the assured's knowledge,
-the voyage or conveyance do not correspond to the agreement
-the goods are carried by inappropriate vehicles or containers
-routes are used which are unsuitable or officially closed to traffic.
d) Unless otherwise agreed, the insurer is not liable for the following political or social risks:
-war
-warlike occurrences (e.g., occupation of foreign territory, border incidents)
-civil war, revolution, rebellion
-preparations for war or war measures
-explosion or other effects of mines, torpedoes, bombs or other engines of war
-confiscation, requisition, sequestration or detainment by a government, authority or power
-strike, lockout and disturbances of whatever kind
-violent or malicious acts.

Moreover, the insurer is not liable whenever the loss or damage, the cause of which cannot be ascertained, is likely to be the consequence of one of the foregoing events.

Art. 7 Special cases
Unless otherwise agreed, the following are einsured only in accordance with art. 2:
-unpacked goods
-returned goods
-reforwarded goods
-used goods
-goods dispatched in damaged condition

Extract-Translation
from the General Conditions of Marine Insurance on Goods
(GCMI 1988)
(The original wording in German or French shall be decisive in the case of dispute)

-goods stored on deck with the knowledge of the assured, unless in a seaworthy container in a specially equipped ship.

Duration of Insurance

Art. 10 Commencement and Termination
The insurance is operative from warehouse to warehouse. The insurance attaches as soon as the goods are loaded onto the means of transportation or into the container with which they undertake the journey. It terminates when, at the end of the insured voyage, the goods are unloaded from the means of transportation or out of the container.
If no vehicle or means of transportation are used at departure point or for deliver, the insured journey begins as soon as the goods to be dispatched leave the storage place at point of departure and ends as soon as they reach the consignee.

Art. 11 Delay
In the event of delay in the execution of the insured voyage, cover is limited to 30 days any one delay. Nevertheless, should the delay be due to circumstances beyond the assured's control, cover remains in force for a further period of 30 days.
At intermediate places delay is understood to be the period of time between the arrival of the conveyance and the departure of the on-carrying conveyance; the day of arrival and the day of departure are taken into account in assessing the delay. Cover during delay may be extended by special agreement.

Definition of Values

Art. 15 Underinsurance
Whenever the sum insured is less than the replacement value, the insurer is liable only for such proportion of loss or damage, general average contributions or expenses as the sum insured bears to the replacement value.

Obligations in Case of Loss or Damage and Claims

Art. 20 Notification of loss or damage and salvage
The assured must notify the insurer forthwith of any loss or damage which comes to his knowledge. Furthermore, in the event of loss or damage, it is the duty of the assured to take forthwith all measures for the preservation and salvage of the goods and for minimizing the loss or damage. The insurer also may take such measures. In the event of non-compliance with these obligations, the indemnification can be reduced commensurate with the assured's degree of fault.

Art 21 Preservation of rights of recovery
All rights of recovery against third parties who may be held responsible for the loss or damage shall be preserved. In particular the following steps shall be taken:

a) Outwardly apparent loss or damage must be notified to the carrier in writing before delivery of the goods is taken.
b) Whenever loss or damage is presumed, valid reserves must be lodged within the period prescribed by law or contract.
c) The carrier must be summoned to a joint survey of the loss or damage.

The assured is responsible for all acts or omission which prejudice the rights of recovery.

Art 22 Survey
In case of loss or damage in Switzerland the insurer, and abroad his surveyor, must be called in forthwith for survey purposes and to take whatever steps may be necessary. Should the loss or damage not be outwardly apparent, survey must be requested within one week of the delivery of the goods to the consignee. If the insurer has not appointed any surveyor, application shall be made to the Lloyd's Agents or if there is no such agent to ____ competent surveyor. If the loss or damage occurred during carriage by mail ____ the transport administration shall be required to issue ___ report. The surveyor's fees and expenses are to be paid by the applicant and are refundable by the insurer if, and to the extent that, the claim is recoverable under the policy. The insurer is released from all liability to pay the claim if the loss or damage has not been ascertained in the he prescribed manner.

Art. 26 Claims
The claimant must justify his title to claim by means of the policy or insurance certificate. He must, moreover prove that in the course of the insured voyage the goods sustained loss or damage for which the insurer is liable. To this end he shall submit the claim statement together with all necessary documents (invoices, waybills, survey reports, official reports, expert's reports, etc.)

Legal Questions

Art. 28 Enforcement of rights of recovery
Last paragraph: The assured may not accept any compensation offered by third parties without the insurer's consent.

Art. 29 Forfeiture
Legal claims against the insurer are forfeited unless suit is brought within two years of the event giving rise to the claim. Claims in respect of general average contributions become void unless suit is brought within one year of the completion of the statement.

Art. 30 Effect of measures taken by the Insurer and the surveyor
The measures ordered by the insurer or the surveyor for the purpose of surveying, minimizing or averting loss or damage or of preserving or enforcing any rights of recovery do not constitute an admission of liability.

L'U.A.P. INCENDIE ACCIDENTS S.A.
Entreprise régie par le Code des Assurances
Capital Social 500.00 de Frs RC Paris B77349 192
Siége Social: 9 Plsc Vendome
75052 Paris Cedex 01

CERTIFICATE D'ASSURANCE

No.

Sauf indication contraire, le présent certificate est établi en seul exemplaire originale.

Description des marchandises:No. 25 colis no. 114880.01 Medicin Humaine	Poids: 742,500 KGS BRUT	No.
	Marques:	
Navire de mer et/ou autre moyen de transport: PAR AVION	Voyage assuré - Lieux de transit ou de transbordement éventueis PARIS KIEV	
En case d'avaries à destination, pour les contratation s'addresser à: STE MARINEX I.T.S. PROSPERKT VERNADSKOGO 41 5 FLOOR ROOM 500 118971 MOSCOU (RUSSIE)	Pour le paiement des dommages susceptibles d'être mis à la charge de l'assureur, addresser le dossier complet à: STE MARINEX I.T.S. PROSPERKT VERNADSKOGO 41 5 FLOOR ROOM 500 118971 MOSCOU (RUSSIE)	
Valeur assurée (chiffres): USD 46.667,30	Lettres: QUARANTE SIX MILLE SIX CENT SOIXANTE DIX SEPT USD 30 USD.	

Conditionsl générales:	Ordre ou avis d'aliment	No.	Date
CODE DES ASSURANCES			

RÉSUMÉ DES PRINCIPAL CNODITIONSL D'ASSURANCE:

TOUS RISQUES, VOL.

Signature de L'Assureer:

18 Aout 1997

Toutes indemnities pour pertes ou avaries seront payées, dans les conditions prévues á L'articles 27 de la Police Française d'Assurance Maritime sur Facultés (imprimé de 30.6.1983, modifié le 16 février 1990) entre les mains du porteur de l'original du certificate d'assurance et des piéces justificatives de la réclamation. En application de l'articles 13 de la police, l'assureur pourra opposer aux bénéficiares de l'assurance la compensation de la prime afférente á l'assurance dont ils revendiquent le bénéfice.

PACKING LIST

Shipper/Exporter Eugene Tech International Incorporated Business Building 123 Industrial Road Production City, New Jersey, USA	No. & Date of Invoice PA9701 August 29, 1997
For Account and Risks Messrs.: American Embassy - NAMRID Attn: Supply Department Reference: Purchase Order 97-VAC-0008 JORGE CGAVAX INTERNATIONAL AIRPORT, LIMA PERU	Remarks : • Freight prepaid • Store between 2-8 °C • DO NOT FREEZE • PERISHABLE - REFRIGERATE ON ARRIVAL. • CONSIGNEE: AMERICAN EMBASSY NAMRID RE: PURCHASE ORDER 97VAC0008 JORGE CHAVEZ INT'L AIRPORT LIMA, PERU
Port of Loading: SEOUL/KOREA	Final Destination: LIMA/PERU
Carrier	Sailing on or about:

Marks and Numbers	Description of Goods	Quantity	Net Weight	Gross Weight
Hepatitis B Vaccine Hepaccine-B Inj. 5ml vial As Consignee Manufactured by: Cheil Foods & Chemicals INC. Made in Korea C/No : 1 up	Hepatitis B Vaccines as follows: 5 adult at 3 mcg/ml per dose with standard packaging and artwork.	6,000 doses (1,200 vials)		

Mr. Kim, General Manager
EUGENE TECH INTERNATIONAL, INC.

PACKING LIST

Shipper/Exporter Eugene Tech International Incorporated Business Building 123 Industrial Road Production City, New Jersey, USA		No. & Date of Invoice PA9701 August 29, 1997		
For Account and Risks Messrs.: American Embassy – NAMRID Attn: Supply Department Reference: Purchase Order 97-VAC-0008 JORGE CGAVAX INTERNATIONAL AIRPORT, LIMA PERU		Remarks: • Freight prepaid • Store between 2-8 °C • DO NOT FREEZE • PERISHABLE – REFRIGERATE ON ARRIVAL. • CONSIGNEE: AMERICAN EMBASSY NAMRID RE: PURCHASE ORDER 97VAC0008 JORGE CHAVEZ INT'L AIRPORT LIMA, PERU		
Port of Loading: SEOUL/KOREA	Final Destination: LIMA/PERU			
Carrier	Sailing on or about:			
Marks and Numbers	Description of Goods	Quantity	Net Weight	Gross Weight
Hepatitis B Vaccine Hepaccine-B Inj. 5ml vial As Consignee Manufactured by: Cheil Foods & Chemicals INC. Made in Korea C/No : 1 up	Hepatitis B Vaccines as follows: 5 adult at 3 mcg/ml per dose with standard packaging and artwork.	6,000 doses (1,200 vials)		

Mr. Kim, General Manager

EUGENE TECH INTERNATIONAL, INC.

PACKING LIST

VDR 08/08/97

CONSIGNER

PASTEUR MERIEUX SERUMS & VACCINS
123 AVNUE BIEN PLACE
12345 LYON
FRANCE

CONSIGNEE

UKRAINT HUMANITARIAN AISSISTANCE
123456 KIEV 10, GRUSHENKO ST.
MINISTRY OF HEALTH
FORWARDER: PREFERRED BROKERS

ORDER NO
114-253-01-00

NR PARCEL	WEIGHT GROSS BY PARCEL	WEIGHT NET BY PARCEL	VOLUME BY PARCEL	BATCH NO.	DATE SHELF LIFE	QUANTITY BY PARCEL
1 – 25	29,700	26,000	0.153			
	DT ADULT VACCINE 10 VLS 20 DS			K09453	12/31/00	100

NUMBER OF PARCELS:		25
TOTAL GROSS WEIGHT:	KG	742,500
TOTAL NET WEIGHT:	KG	650,000
TOTAL VOLUME:	M3	3,825

VIA AIR FREIGHT

SH
SR

SECRETARIAT OF HEALTH
SUB SECRETARIAT OF SANITARY PRODUCTS
DEPARTMENT OF HEALTH PRODUCTS CONTROL

DH
RC

Complete in triplicate, submit white and yellow copies

APPLICATION FOR SANITARY AUTHORIZATION FOR IMPORT OR EXPORT	*For office use only*
Company Name: "Local Health Agency"	
Company Address: "1234 Main City Road" "Mexico City, DF, MEXICO"	No. of Expediter: Air Freight Express Co.
Company Telephone: "25-123-4567"	Federal registration number: TEC.84037-GS4
Application for Sanitary Authorizations is for: Importation ☒ Temporary Importation ☐ Export ☐	Sanitary License Number: 10080007795
Port of Entry: At Laredo TX USA via Nuevo Laredo, MEXICO Boarder	Date: 05 (Mo.) 22 (Day) 98 (Year)
Name and address of product manufacturer: (for imports) "Worldwide Vaccines" "6789 North Factory Road" "Industrial City, State of New Jersey, U.S.A."	Exporter Registration number (for export applications only): _____
Name and address of destination (only for exports):	We certify that the information above is true and correct: [illegible] Authorized officer of applicant

Approval

Exceptions: Note: This authorization is for the sole use of products to be distributed under programs sponsored by the Ministry of Health. Commercial distribution of products imported under this license are prohibited. Authorization valid for 180 days after issuance. Multiple imports allowed during validity period. Authorization not tranferrable to other parties. Shipments subject to inspection upon arrival.	Permit Number: MEX06.27.4593 Date: 05-31-98

[illegible]
Chief, Department of Sanitary Controls of Imports and Exports

[illegible]
Sub Director, Department of Sanitary Controls of Imports and Export

[illegible]
Director General, Department of Health Product Controls

COPY

CERTIFICATE OF ORIGIN

issued by

THE KOREA CHAMBER OF COMMERCE & INDUSTRY

Seoul Republic of Korea

1. Seller:
CHEIL FOODS AND CHEMICALS INC.
10TH FLOOR
CHEIL BUILDING 500
FACTORY PROVINCE, SEOUL, KOREA

2. Consignee
AMERICAN EMBASSY
ATTENTION: SUPPLY DEPARTMENT

REFERENCE: PURCHASE ORDER 97-VAC-0008
JORGE CHAVAX INT'L AIRPORT
LIMA PERU

4. Buyer *(if other than Consignee)*
EUGENE TECH INT'L INC.
BUSINESS BUILDING
123 INDUSTRIAL ROAD
PRODUCTION CITY, NJ USA 02006

3. Particulars of Tranport *(where required)*

carrier: LH
From: Seoul/Korea
To: Lima/Peru
Sailing on or about Aug. 30, 1997

5. Country of Origin:
The Republic of Korea

6. Invoice Number and Date:
PA97007 & Aug 29 1997

7. Shipping Marks	8. Number and Kind of Packages; Description of Goods:	9. Quantity:	10. Gross Weight or Measurement
Hepatitis B vaccine Hepaccine-B inj. 5ml/vial As Consignee Manufactured by Cheil Foods & Chemicals, Inc. Made in Korea	Hepatitis B vaccines as follows: 5 adult doses at 3 mcg/1ml per dose with standard packaging and artwork	6,000 doses	52 KG

10. Other Information

The Korean Chamber of Commerce & Industry hereby certifies on the basis of relevant invoice adn other documents, that the above mentioned goods ariginate in the country shown in column 5.

CERTIFIED BY
1 SEP. 1997
THE KOREA CHAMBER OF COMMERCE & INDUSTRY
EXECUTIVE OFFICER. KANG SENGUIL
The Republic of Korea

THE KOREA CHAMBER OF COMMERCE AND INDUSTRY

Swiss Serum and Vaccine Institute Berne

CERTIFICATE OF ANALYSIS

PRODUCT	DiTe Anatoxal Berna for adults
LOT-NO	137 100 101
EXPIRY DATE	29,08,1997
PRESENTATION	Vials 50 x 10 ml

TEST	METHODE	SPECIFICATION	RESULTS
DiTe Anatoxal Berna for adults lot No. 137 100 101			
Identity	flocculation test	identified as DiTe Anatoxal	identified as DiTe Anatoxal Date of Test: 02.09.1994
DiTe Anatoxal adults Filling Lot lot No. 13710 01			
Sterility	according to Ph. Eur. direct inoculation	sterile	sterile Date of start: 10.08.1994 Date of end: 26.08.1994 Date of test: 26.08.1994
Innocuity	inoculation of mice	no evidence of any abnormal sign	no evidence of abnormal sign Date of test: 26.08.1994
Visual Inspection	visual inspection	free of foreign particles	free of foreign particles Date of test: 08.08.1994

P.O. Box, CH-3001 Berne - Telephone +41 31 980 6 111 - Telex 912618 - Telefax +41 31 980 6 785

Swiss Serum and Vaccine Institute Berne

CERTIFICATE OF ANALYSIS

PRODUCT	DiTe Anatoxal Berna for adults
LOT-NO	137 100 101
EXPIRY DATE	29,08,1997
PRESENTATION	Vials 50 x 10 ml

TEST	METHODE	SPECIFICATION	RESULTS

DiTe Anatoxal adults Final Bulk lot No. 13710

Potency Diptheria according to Ph. Eur. — LLC=> 2 UI/0.5ml

16.63.IU/0.5ml

Toxoid lot no used:	304
Weight of guinea-pigs:	250-350g
No of animals per dose toxoid:	8
Date of immunization:	14.07.1994
Vol of dilutions administered:	1.0ml
Date of challenge or bleed:	11.08.1994
Challenge dose:	100 LD50
Date of end of test:	17.08.1994

Challenge test: Dilutions No. of survivors ED50

Ref toxoid	1.26 IU	4.5 mcl	0/8
(280 IU/ml)	2.52 IU	9.0 mcl	5/8 2.26 IU
	5.4 IU	18.0 mcl	8/8

Test toxoid:	45 mcl	2/8
	90 mcl	5.8 68.34 mcl
	180 mcl	8/8

Potency of test toxoid 16.63 per single humen dose 95% confidence limits of potency 10.76 - 25.77 IU

Date of Test: 02.09.1994

Potency Tetanus according to Ph. Eur. — LLC =>40 IU/0.5ml

100.28 IU/0.5ml

Toxoid lot no used:	154
Weight of mice:	14-16g
No of mice per dose/vac:	16
Date of immunization:	15.07.1994
Challenge dose:	50 LD50
Date of challenge:	10.08.1994
Date of end of test:	16.08.1994

P.O. Box, CH-3001 Berne - Telephone +41 31 980 6 111 - Telex 912618 - Telefax +41 31 980 6 785

Swiss Serum and Vaccine Institute Berne

CERTIFICATE OF ANALYSIS

PRODUCT	DiTe Anatoxal Berna for adults
LOT-NO	137 100 101
EXPIRY DATE	29,08,1997
PRESENTATION	Vials 50 x 10 ml

TEST	METHODE	SPECIFICATION	RESULTS
			Challenge test: Dilutions No of survivors ED50 Ref vaccine: 0.224IU 0.64mcl 0/16 (350 IU/ml) 0.56 IU 1.6 mcl 8/16 0.60 IU 1.40 IU 4.0 mcl 15/16 Test toxoid 1.6 mcl 2/16 4.0 mcl 8/16 2.99 mcl 10.0 mcl 15/16 Potency of test toxoid 100.28 IU per single human dose 95% confidence limits of potency 67.82 - 148.27 IU Date of test: 02.09.1994
Sterility	according to PH. Eur. direct inoculation	sterile	sterile Date of start: 04.07.1994 Date of end: 20.07.1994 Date of test: 20.07.1994
Innocuity	according to PH. Eur.	no evidence of any abnormal sign	no evidence of abnormal sign Test in mice / Test in guinea-pigs No of animals: 5 / 2 Route of injection: ip / ip volume of injection: 1.0ml / 2.0ml observation period: 7 days / 7 days Date of test: 04.08.1994

P.O. Box, CH-3001 Berne - Telephone +41 31 980 6 111 - Telex 912618 - Telefax +41 31 980 6 785

Swiss Serum and Vaccine Institute Berne

CERTIFICATE OF ANALYSIS

PRODUCT	DiTe Anatoxal Berna for adults
LOT-NO	137 100 101
EXPIRY DATE	29,08,1997
PRESENTATION	Vials 50 x 10 ml

TEST	METHODE	SPECIFICATION	RESULTS
Limit of Binding Diptheria	US minimal requirements	<2 BU/ml	0.45 BU/ml Date of Test: 16.08.1994
Limit of Binding Tetanus	US minimum requirements	25-38 BU/ml	31 BU/ml Date of Test: 11.07.1994
Specific toxicity	according to WHO	passed	passed (07.07 - 18.08.1994) Date of Test: 18.08.1994
Aluminiumphosphate	complexometric	<7.68 mg/ml (= 1.7mg Al/ml)	4.3 mg/ml Date of Test: 11.07.1994
Formaldehyde	colorimetric	<200 mcg/ml	0.2 mcg/ml Date of Test: 11.07.1994
NaCl	mercurimetric	0.85-0.95 % w/v	0.87% Date of Test: 11.07.1994
pH	potentiometric according to Ph. Eur.	6.0 - 7.0 at 20°C	6.8 Date of Test: 11.07.1994

P.O. Box, CH-3001 Berne - Telephone +41 31 980 6 111 - Telex 912618 - Telefax +41 31 980 6 785

Swiss Serum and Vaccine Institute Berne

CERTIFICATE OF ANALYSIS

PRODUCT	DiTe Anatoxal Berna for adults
LOT-NO	137 100 101
EXPIRY DATE	29,08,1997
PRESENTATION	Vials 50 x 10 ml

Page 5

TEST	METHODE	SPECIFICATION	RESULTS
Thimerosal	AAS	max 110 mcg/ml	105.1 mcg/ml
			Date of Test: 12.07.1994

We certify that:

the above mentioned batch was prepared and tested according to our valid standard operating procedures and has been found in conformity with our requirements.

Swiss Serum and Vaccine Institute Berne
Quality Control Department

(S. Varallyay, MD)

Furthermore, we certify that this batch was released for sale by our National Control Authority: the FEDERAL OFFICE OF PUBLIC HEALTH.

Swiss Serum and Vaccine Institute Berne
Head of Quality Assurance
P. Paroz, Ph.D.

Berne, 02.09.1994

P.O. Box, CH-3001 Berne - Telephone +41 31 980 6 111 - Telex 912618 - Telefax +41 31 980 6 785

CERTIFICAT DE LIBRE VENTE

Free Sale Certificate
Certificado de libre venta

Il est certifis que les laboratoires

It is hereby certified that laboratories
Se certifica que los laborotorios

PASTEUR MERIEUX
Sérums & Vaccins
--, Avenue XXXXXX
Lyon, FRANCE

sont autoriés à fabriquer er à vendre des spécialités pharmaceutiques.
are authorized to manufacture and to sell pharmaceutical specialties.
estan autorizados para la fabricación y la venta de especialidades farmaceúticas.

Ils exploitment notament les medicaments ci-apres dssignés.
They also manufacture and sell the following drugs.
Expotan y commercializan particularmente los medicamentos mencionados abajo.

Ces médicaments on fait l'objet d'une autorization de Ministare chargé de la santé.
The Ministry of Health has delivered an authorization for the marketing of these pharmaceutical specialities.
Estos medicamentos han recibido una autorización del Ministro de la Salud.

Ladite autorisation n'est assortie d'aucune autre restriction ou precaution d'emploi que celles figurant, le cas échéant, sur cette autorisation.
The said authorization is subject to no restriction with respect to its use unless specifically stated hereunder.
Dicha autorización no tiene otra restrición o precaución de empleo que las que figuran en esta autorización, si se presenta el caso.

Td adsorbé - vaccine diphtérique et tétanique de rappel

Fait à Paris, le 9 JUIN 1995

Le Ministre chargé de la santé

Pour le Ministre et par délégation
Florence de SAINT-MARTIN
Pharmacien Inspecteur Principal de la Santé

Bundesamt für Gesundheitswesen
Office fédéral de la santé publique
Ufficio federale della sanità pubblica

CERTIFICATE

THE FEDERAL OFFICE OF PUBLIC HEALTH (Switzerland) certifies that the immunobiological product:

DiTe Anatoxal adults Berna

batch No. 137 090 102 fabrication No. 13 709 has been found in conformity with the Swiss Regulations and released for sale in Switzerland under our marketing authorization number 3672.

FEDERAL OFFICE OF PUBLIC HEALTH
Section for Immunobiological Products

F. Reigel, Ph.D., Head of Section

FEDERAL OFFICE OF PUBLIC HEALTH

Berne, September 6, 1996

SGS

Certificate No. S-50.004/90

SGS KOREA CO., LTD.

SSANG LIM BLDG.
G.P.O. BOX: 8293
SEOUL

CABLE SUPERVISE
TELEX K24882 SGS SEL K
PHONE 265 8671-5, 278-6121
FAX 274-8142

Certificate No. 6901/S-50.004/90
of Inspection

SEATTLE FIRST NATIONAL BANK, SEATTLE, LETTER OF CREDIT NUMBER 76011SI011690 dated 16 JANUARY 1990

MERCHANDISE REF.: PO 90-0007

SHIPPERS : PATH, Seattle, USA

MAKERS :

BENEFICIARY:

CONSIGNEE : PATH Indonesia, Tifa Building Suite 1103 JL Kuningan Barat 26, Jakarta 12710, Indonesia

ACCOUNTEE : PIACT/PATH, 4 Nickerson Street Seattle, WA 98109

From/To : Kimpo Airport, Korea/Jakarta Airport, Indonesia

* * * * * * *

THIS IS TO CERTIFY THAT we attended at the Maker's premises in Yongin, Korea on 24th January 1990 in order to carry out the pre-shipment inspection in accordance with the instructions received from Accountee and report as follows:

CARGO : **HEPAVAX-B WITHOUT SYRINGE**
30,000 Doses 0.5ml, 5ug/V

MARKING : **(On Carton No. 1-50)**
PATH INDONESIA
L/C NO. : 76011SI011690
INVOICE NO. : C 900102
INVOICE DATE: JAN. 10, '90
LOT NO. : H066303
EXPIRY DATE : MAY 19, '91
PERISHABLE MATERIAL,
KEEP REFRIGERATED (2-8°C)
KEEP FROM FREEZING
GROSS WEIGHT: 25 KG
BOX #: 1-50
AIRPORT OF DESTINATION: JAKARTA, INDONESIA
TELEPHONE CONSIGNEE UPON ARRIVAL
- TELEPHONE: 5200737

- continued -

Packing: Each vial packed in individual paper box.
10 paper boxes in one PE bag and such 10 PE bags (100 vials) stuffed in styrofoam box with 4 packs of ice cooler (500 ml/pack).

One freezing indicator was affixed on the inside wall of each styrofoam box (100 vial), just on the upper part of the vial packages.

Then such styrofoam box is packed into an inner carton of card-board and such 6 cartons in one master carton. Total of 50 master cartons.

The packing was in apparent good order and condition at time of our attendance.

Labeling: Label on each vial was as follows:

- continued -

Package insert: Package insert in individual box was as follows:

Hepatitis B Vaccine

Hepavax® -B

Description
Hepavax-B is a non-infectious, inactivated subunit viral vaccine derived from hepatitis B surface antigen (HBsAg). It is prepared exclusively using HBsAg purified from plasma obtained from health HBsAg carriers by three purification/inactivation steps; pepsin digestion heating at 60°C for 10 hours and formatin treatment Hepavax-B is a suspension for intramuscular injection.

Composition
0.5ml contains
Hepatitis B surface antigen protein, inactivated.....5μg
Adjuvant, Aluminium hydroxide gel.....0.25mg (as Al)
Preservative, Thimerosal.....0.01 w/v%

Indication
For the prevention of hepatitis B in neonates

Dosage and Administration
Inject intramuscularly
- Primary immunization
  Two doses of 0.5ml (5μl) at interval of a month and one dose of 0.5ml (5μl) on the sixth month after the first injection.
- Booster immunization
  An appropriate dose according to ages every five years.
  Younger children (<10 years old): 0.5ml (10 μg)
  Older children and adults (>10 years old) 1 mg (20μg)
  In the case of booster immunization, Hepavax-B in children and adults shall be available.

Considerations
the doses given at longer or shorter intervals (about one week) in the second or third injection provide equally protective effects.
Inject a booster dose when anti-HBs liter produced by primary immunization is insufficient.
The injection in the anterolateral thigh is recommended for infants or small children.
Hepavax-B produces neither therapeutic nor adverse effects in HBV carriers.
Hepavax-B may be administered with hepatitis B immune globulin at different sites simultaneously, which does not interfere with production of anti-HBs.
For neonates born to HBsAg positive mothers, inject 1 ml of HBIG (2001.U) intramuscularly with o.5ml (5μg) of Hepavax-B at a different site simultaneously within 48 hours after birth and complete vaccination according to the recommended schedule.

Contraindication
Hypersensitivity to any component of the vaccine.

Precaution
Caution and appropriate care should be exercised when Hepavax-B is administered to individuals with severely compromised cardiopulmonary status or to others in whom a lebrile or systemic reaction could pose a significant risk.
Local reactions including erythema, swelling and warmth, or systemic complaints including fever, malaise, fatigue, headache, dizziness, myalgia, and nausea may occur, but disappear in several days.
Because of the long incubation period of hepatitis B, it is possible for unrecognized infection to be present at the time Hepavax-B is given.
Hepavax-B may not present hepatitis B in such individuals.

Storage
Store unopened and opened vials at 10°C or less. Do not freeze, since freezing may induce destruction of potency. Further stability tests show that Hepavax-B at higher temperatures will be stored without loss of its potency.
Maximum validity: 26 months

How Supplied
0.5ml/vial x 1, 5, 10, 50

EXAMINE
DOCUMENTATION: Examined the concerned documents of Commercial Invoice and Packing List and found them to be in accordance with the Shipping Instructions.

(Refer to the attached copy of Invoice and P/L.)

- continued -

SGS

Certificate No. S-50.004/90

SGS KOREA CO., LTD.

Quality: The certificate of analysis and quality control records provided by manufacturer were carefully examined and found to be in conformance with vaccine specifications with following analysis results:

Commodity: Hepavax-B (Hepatitis B Vaccine)
Lot No.: H066303 (5ug, 0.5ml/vial)
Date of Mfg.: 1989. 3. 20
Analysis: As follows

Test items	Specifications	Results
Appearance	Opalescent	Pass
pH	Value on determination	6.28
Thimerosal content	NMT 0.012 W/V %	0.0101 %
Formaldehyde content	NMT 0.01 W/V %	0.00026 %
Aluminum content	NMT 1.25 mg/ml	0.461 mg/ml
Filling volume	NLT 0.68 ml	0.68 ml
Foreign material	None	None
HBsAg Content	9-11 ug/ml	10.99 ug/ml
Sterility	No growth in FTM media (31, 36 °C)	Conformed
	No growth in SDB media (25 °C)	Conformed
Abnormal toxicity	Freedom from abnormal toxicity	Conformed
Potency	NLT Reference	Pass

The issuance of this certificate does not exonerate buyers or sellers from exercising all their rights and discharging all their liabilities under the Contract of Sale. Stipulations to the contrary are not binding on us. The Company's responsibility under this certificate is limited to gross negligence proven by Principals and will in no case be more than ten times fees or commission.

* * * * * * *

Seoul, 30t January 1990
SGS KOREA CO., LTD.

Y. S. Chun/Redwood

MINISTRY OF SOCIAL AFFAIRS AND EMPLOYMENT
PHARMACY AND DRUG DIRECTION
1 place de Fontenoy
75700 PARIS

FRENCH REPUBLIC

THE MINISTER FOR SOCIAL AFFAIRS AND EMPLOYMENT

TAKING INTO ACCOUNT Volume V of the Public Health Code, and in particular its Article L603.

TAKING INTO ACCOUNT the request presented by the Head of the Laboratories INSTITUTE MERIEUX, -- rue Bourgeiat. 69002 LYON, in regard to the pharmaceutical product Td adsorbed – diphtheria and tetanus booster vaccine.

Considering that the manufacture of this drug possesses adequate industrial installations and utilizes manufacturing and quality control procedures offering the necessary guarantees of safety.

Considering that justificatory documents of good practice in regard to manufacture and quality control, as recommended by the World Health Organization, have been supplied,

Considering that the laboratory in which this drug is manufactured is subjected to inspections, at appropriate intervals.

AUTHORISATION NUMBER
A.E. Nc 2398

RULES AS FOLLOWS:

ARTICLE 1: An authorization as provided for in Article L603 of the Public Health Code is granted to the applicant for the drug called:

Td adsorbed – Diphtheria and tetanus booster adsorbed vaccine

which has the following formula:

Each vaccinating dose contains:

- Purified diphtheria toxoid ..... 1 booster vaccinating dose
- Purified tetanus toxoid ..... 1 booster vaccinating dose
- Aluminum hydroxide expressed as Al. Maximum of.....1.25 mg
- Sodium mercurothiciate (preservative) maximum..... 0.05 mg
- 0.9% sodium chloride solution, to ..... 0.5 ml

The booster vaccine dose of purified diphtheria toxoid is a reduced dose of the normal vaccinating dose, namely a maximum of 2 flocculating units (Lf)

The booster vaccinating dose of purified tetanus toxoid corresponds to at least 40 international units when the protective activity of the vaccine is measured against the WHO international standard or against a sub-standard calibrated with reference to the international standard,

- And which is presented in the following forms:
- 5 ml (10 vaccinating doses) in vial (glass)
- 5 ml (10 vaccinating doses) in vial (glass), box of 10
- 10 ml (10 vaccinating doses) in vial (glass)
- 10 ml (10 vaccinating doses) in vial (glass), box of 10

ARTICLE 2: The Director of Pharmacy and Drug is responsible for notifying the persons concerned regarding this decision.

Drawn up in Paris on 23 October, 1995

For the Minister for Social Affairs
and Employment and by Delegation,
The Deputy Director for Drugs Economic Policy

Certified:
Florence SAINT MARTIN
Health Inspector Pharmacist
(Signed)

Aline GODARD

MINISTERE DES AFFAIRES SOCIALES ET DE L'EMPLOI

REPUBLIC FRANCAISE

DIRECTION DE LA PHARMACIE ET DUE MEDICAMENT

1, Place de Fontenoy
75700 PARIS

LE MINISTRE DES AFFAIRES SOCIALES ET DE L'EMPLOI

Vu le levre V du Doce de la Snate Publique, notament son article L603

Vu la demande presente par le responsible des Laroatoires:

INSTITUTE MERIEUX, -- rue Bourgelat 69002 LYON

en ce concerne la spécialité Td adsorbé - vaccine diphtéerique et tétanique de rappel.

Considerant que le fabricant de ce medicament possede des installations industrielles adequates et met oeuvre des procedes de fabrication et de controle offrant les garanties de securite necessaires,

Considerant que les justifications de bonnes pratiques en matiere de fabrication et de controle de la qualite, recommandees par l'Organisation Mondiale de la Sante, ont ete fournies,

Considerant que le laboratoire dans lequei ce medicament est fabrique est soumis, a intervailes appropries, a des inspections.

NUMERO D'AUTORISATION
A.E. N°

2398

Article 1 - Une autorisation prevue a l'article L603 du Code de la Santé est accordee au demandeur pour le medicament denomme:

repondant a la formue suivante:

Chaque dose vaccinate renferme:

- Anatoxine tetanique purifée	1 dose vaccinate de rappei
- Anatoxine tétanique purifée	1 dose vaccinante de rappei
- Hydroxyde d'aluminium exprimй en Al au maximum	1.25 mg
-Solution de chlorure de sodium a 0.9% pour cent	a.s.p. 0.5 ml

-La does vaccinante de rappel d'antatixine tétanique purifée correspond à au mains 40 unités internationales quand le pouvoir protecteur correspondet presente: du vaccine est mesuré en parallèle avec l'étalon international de HOMS or avec un sous étalon calibré par rapport a 'l'étalon international.

- 5 ml (10 doses vaccinantes) en flacon (verre)
- 5 ml (10 doses vaccinantes) en flacon (verre), boite de 10
- 10 ml (20 doses vaccinantes) en flacon (verre)
- 10 ml (20 doses vaccinantes) en flacon (verre), boite de 10

Article 2. 0 Le Directeur de la Paramacie et du Medicament est charge de notifier cetter décision aux interesses.

Fait a Paris le 23 OCT 1996

Pour le Ministere des Affaires Sociales et de L'Emploi et par delegation Le Soux-Directeur le la Politique Economique Reglement

MINISTERE DES AFFAIRES SOCIAL DIRECTION DE LA PHARMACIE ET DU MEDICAMENT DE L'EMPLOI

Pour Ampliation

Pharmacien Inspecteur de la Santé

Aline GODARD

MINISTRY OF HEALTH AND SOCIAL AFFAIRS
REPUBLIC OF KOREA
SEOUL 110, KOREA

REFERENCE:

Cable Address: MOHSASBOK
Telex: MOHSA S K2330

PRODUCT LICENSE

****

The Ministry of Health and Social Affairs, Republic of Korea licensed on June 20, 1983 that the following Pharmaceutical Speciality;

Name of Product:	: HEPAVAX-B (Trade Name) Hepatitis B Vaccine (General Name)	
Composition	: Each 1 vial (1 ml) Contains:	
	Hepatitis B surface antigen, inactivated	20 ug
	Aluminium Hydroxide Gel	0.5 mg(as Al)
	Thimerosal	0.01 W/V%
	Isotonic Sodium Chloride Injection	q.s.
Indication	: Prophylaxis of Hepatitis B	
Storage and Expiry Date	: Should be stored at 2-8°C, Hermetic Container It: expiration date is not later than __ years after approval of the national control tests.	
Pack Size	: 1 ml/V, 1 ml/V x 5, 5 ml/V, 10 ml/V	

is produced by the firm and sold in domestic and overseas markets open to the public under the following registration numbers:

Registered No. of Manufacturer	: 102
Registered No. of the Product	: 133
Classification No.	: 631

F. Transportation and Storage

Vaccines are perishable, biological products that must be kept within a specific temperature range from manufacture until use. Not all vaccines require the same temperature range. Freezing destroys some but extends the shelf life of others. Thus, packing, transportation and storage arrangements for vaccine need to provide protection from temperatures outside of specified ranges.

1. Vaccine stability

Supplement VI.F.1., page 752

The BASICS / WHO document Safe Handling, Cold Chain and Immunizations: A Manual for the Newly Independent States provides guidance on vaccine stability. A relevant excerpt appears at the end of this section.

2. Packaging and shipping vaccine

a. Because of their perishable nature, vaccines are usually shipped by air. Air freight is priced according to weight with additional charges when volume exceeds a ratio of 6 cubic meters for one ton of weight. Vaccine packaging can therefore have a substantial impact on costs, particularly if small, elaborate packages are used. During 1997, UNICEF and WHO reviewed actual vaccine packing practices in order to begin eliminating nonessential packing.

Supplement VI.F.2.a., page 754

The World Health Organization is in the processes of revising its Guidelines on the International Packaging and Shipping of Vaccines. The current draft (revision 6) appears as Supplement VI.F.2.a.

Supplement VI.F.2.b., page 760

b. WHO provides guidance for estimating storage capacity requirements in its 1986 document WHO Guide to Estimating Capacity of Equipment Required for Storage and Transporting EPI Vaccines. A one-page 1997 summary is attached to the end of Supplement VI.F.2.b.

A standardised vaccine arrival report has been included as Annex One of the WHO Guidelines on the International Packaging and Shipping of Vaccines. This is a simple, standardised management and technical tool to stimulate analysis, systematically document feedback on the conditions of international vaccine shipments and take corrective action. Many countries are now using this form to report problems at the time of arrival of international vaccine shipments in order to prevent recurrences. The form is a mechanism to provide feedback on how well the supply system functioned in bringing the vaccine into the country and is used to improve vaccine delivery at the highest level of the cold chain, which is the most important and easily fixed link from the manufacturer to the national store.

The vaccine arrival report also is needed by donors who want reassurance that their financial inputs are being appropriately and efficiently used and by MOHs who ultimately are responsible for protecting their populations and have the greatest interest in ensuring trouble-free shipments.

The lack of a systematic feedback mechanism from the field to vaccine suppliers results in delivery problems going unrecognised, uncorrected and even repeated. Inadequate compliance on the part of vaccine manufacturers with international standards and procedures related to vaccine shipment occurs. Some of the documented problems include vaccines arriving:

- without sufficient advance notification
- without sufficient ice packs
- without cold chain monitors
- with short expiry dates
- without regard for requested black-out dates, such as weekends and holidays
- trans-shipped via cities lacking cold rooms
- without documentation and labelling needed for easy customs clearance
- without regard for requested vial sizes
- consigned to the wrong party, etc.

The MOH and vaccine suppliers in each country specify procedures for receiving international vaccines and identify a person acting as a focal point with high-level support who has responsibility for each step in the process of ordering, receiving and documenting vaccine shipments. Upon the arrival of each international shipment of vaccine supplied, the focal point completes the vaccine arrival report and officially submits it to the supplier or, in the case of vaccine supplied through UNICEF, to UNICEF and WHO for their information.

3. Vaccine storage - time/temperature

a. The World Health Organisation has published recommended temperature and storage times at different stages of the cold chain on its World Wide Web site.

Supplement VI.F.3.a., page 772

b. In 1991, the U.S. Centers for Disease Control published Recommendations for Handling and Storage of Selected Biologicals.

Supplement VI.F.3.b., page 774

(Text resumes on page 782)

Vaccine Stability

All vaccines are sensitive biological substances that progressively lose their potency (i.e., their ability to give protection against disease). This loss of potency is much faster when the vaccine is exposed to temperatures outside the recommended storage range. Once vaccine potency has been lost, returning the vaccine to correct storage conditions cannot restore it. Any loss of potency is permanent and irreversible. Thus, storage of vaccines at the correct recommended temperature conditions is vitally important in order that full vaccine potency is retained up to the moment of administration. Although all vaccines are heat-sensitive, some are far more sensitive than others are. Those listed in Section 1.2 can be arranged in order of decreasing sensitivity to heat as follows:

Most sensitive
- Live oral polio vaccine (OPV)
- Measles (Lyophilized)*
- Pertussis and Mumps (Lyophilized)
- Hepatitis B
- Adsorbed Diphtheria-Pertussis-Tetanus vaccine (DPT)
- Adsorbed Diphtheria-tetanus vaccine (DT, Td)
- BCG (Lyophilized)*
- Tetanus Toxoid (TT)

Least sensitive

**Note: These vaccines become much more heat sensitive after they have been reconstituted with diluent.*

Some vaccines are also highly sensitive to being cold. Such vaccines will lose their potency entirely if frozen, although others can sustain freezing without any damage whatsoever. (Refer to Table 1). It is therefore vitally important to know the CORRECT storage conditions for each vaccine, and to ensure that each is kept always at the recommended conditions.

Table 1: Sensitivity of Vaccines to Freezing

Vaccines Damaged by Freezing	Vaccines Unaffected by Freezing
DPT	BCG*
DT	OPV
Td	Measles*
TT	Mumps
Hepatitis B	

Note: Vaccines freeze at temperatures just below zero.

- *BCG and Measles vaccines must not be frozen after reconstitution
- diluent for any vaccine must never be frozen

In addition to being temperature-sensitive, several vaccines are also highly sensitive to strong light, and thus need to be kept in the dark as far as possible. BCG and measles are those most affected. These vaccines must never be exposed to sunlight, and are given some protection by being supplied in vials of dark brown glass to reduce the penetration of light. This alone will not prevent light damage, however, and great care must be taken to protect them during use. As with loss of potency due to heat, any loss of potency due to light is also permanent and irreversible.

Note that all losses of potency are CUMULATIVE; the is, each time a vaccine is exposed to incorrect temperature or strong light its potency will decrease. Since the vaccine may have already been exposed previously, any new exposure, however small, will increase the damage to the vaccine. Ultimately, due to cumulative damage, the vaccine may be completely destroyed, with all its potency lost.

Note also that even when stored at the correct temperature vaccines do not retain potency forever. Therefore, the **expiry date** marked on a vial or packet of vaccine must be strictly observed even when correct storage temperatures have always been maintained.

EPI/CCIS/81.4 Rev.6
Original: English

GUIDELINES ON THE INTERNATIONAL PACKAGING AND SHIPPING OF VACCINES

(Draft Revision** November, **1998: Last revised July 1992)

The following guidelines are jointly endorsed by UNICEF and WHO. They relate specifically to the international shipment of vaccine to countries implementing the Expanded Programme on Immunization. The guidelines may be cited in part or in full in invitations to bid for vaccine supply

1. Insulated packaging standards

Class A: The vaccine shall be packed to ensure that the warmest storage temperature of the vaccine does not rise above +8°C in continuous outside ambient temperatures of +43°C, for a period of at least 48 hours. One WHO approved cold chain monitor card shall be packed in each shipping carton of vaccine

Class B: The vaccine shall be packed to assure that:

- at the time of packing, the storage temperature is as recommended by the manufacturer,
- the warmest storage temperature of the vaccine does not rise above +30°C in continuous outside ambient temperatures of +43°C for a period of at least 48 hours and that
- the coolest storage temperature of the vaccine does not fall below freezing in continuous external temperatures of –5C for a period of at least 48 hours.
- Freeze dried vaccine diluents should be packed within the vaccine in the same primary or secondary packaging[1].
- one WHO approved cold chain monitor card shall be packed in each shipping carton of vaccine

[1] Laboratory testing shows that liquid vaccines have sufficient thermal capacity to achieve this standard of cooling without additional icepacks if the number of doses within the package is sufficient. This finding may also apply to diluent packed with freeze dried vaccine.

Class C: The vaccine need not be packed in insulated cartons with icepacks for international air transport. Suitable chemical temperature threshold indicators shall be packed with each 3,000 doses of vaccine.

Table 1: Classification of current vaccines

Class	Vaccine	Class	Vaccine
A	Oral poliomyelitis	B	BCG
B	DPT	B	Measles freeze dried
B	Yellow fever freeze dried	C	Hepatitis B
C	Tetanus Toxoid	C	Td
C	DT	C	HIB

2. Storage volume standards

Vaccine manufacturers shall state the storage volumes occupied per infant dose of vaccine in trade literature and in tender documents submitted to the purchaser. Maximum recommended volumes per infant dose are as follows:

Table 2: Maximum packed volume[2] per vaccine dose

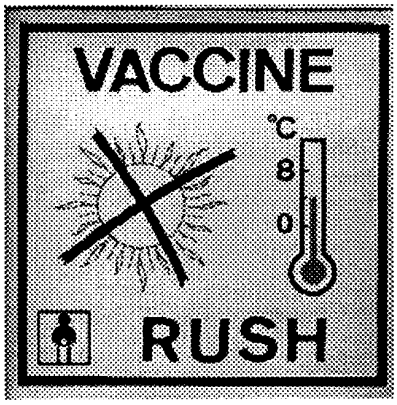
Vaccine	Doses per vial	Cm3/dose	Vaccine	Doses per vial	Cm3/dose
BCG freeze dried	20 or 10	1.0	Measles freeze dried[1]	10	3.0
Hepatitis B	2	6.0	Hepatitis B	10	3.0
Oral polio	10	2.5	Oral polio	20	1.5
TT, Td, Dt, DT	20	3.0	TT, Td, Dt, DT	20	2.5
Yellow fever	10	3.0			

[2] The "packed volume" includes the vaccine vial, the packet containing the vaccine vial and any intermediate packing.

3. Labelling and packaging standards

The external surface of vaccine packaging shall be white. A label of the design, see sample below, shall be affixed to each outside face of every vaccine package. Although the label shown is in English, other languages can be used as follows: ***Vaccin Urgent - Vacuna Urgente - Imptstoff Eilt .***

Labels on each vaccine vial (or ampoule) shall be fixed with water-resistant adhesive. The expiry date shall be printed on each vial or vial label in indelible ink.



4. Standard shipping procedures

Vaccines shall travel by a direct route whenever possible. If trans-shipment is unavoidable, the journey shall be planned, whenever possible, through airports with a temperate climate and cold store facilities. Shipments shall be scheduled to arrive on *Monday, Tuesday, Wednesday or Thursday only.*

Vaccine consignments shall be booked well ahead of the date of departure. At least *one week* before the date of despatch, telexes or *cables* shall be sent to the consignee *and* to the local WHO or UNICEF office, *stating the following:*

- Number of vials and doses per vial
- Type of vaccine
- Number of cartons
- Gross weight (in kgs)
- Value of shipment
- Flight number, date and expected time of arrival (ETA) at final destination
- Airwaybill (AWB) number
- Instructions re collection: "Please arrange immediate collection or telex immediately if vaccine does not arrive."

The following information shall be stated *on the airwaybill:*

- Consignee's name, address and *tele-phone number.*
- Type of vaccine and quantity
- Instructions to: "Telephone consignee upon arrival (repeat telephone number)"

- Handling information: "Medicines - Vaccine - For human use - Highly perishable - Not to be delayed - Connection by booked flight - Pending reshipment or collection, store at 0°C to +8°C (35°F to 50°F)".

Two copies of the invoice or pro-forma invoice with packing details shall be attached to the airwaybill for customs clearance at destination. At the time of shipping, the following shall be ***sent to the consignee by mail:***

- Copy of the airwaybill
- Copy of the invoice with packing details
- Copy of the standard vaccine arrival report (See Annex 1)

All invoices shall quote the vaccine batch numbers and the dates of expiry of the lots included in the consignment.

ANNEX 1
VACCINE ARRIVAL REPORT

FLIGHT DETAILS
NO.....

REPORT

DATE.....

...

AIRPORT INFORMATION		DATE AND TIME		
AIR WAY BILL NO.		Arrival	Departure	Flight No.
ORIGIN				
STOPOVER				
FINAL				
SUPPLIER	UNICEF/JICA/WHO/.....			

VACCINES ORDER REFERENCE.....

VACCINE	MANUFACTURER	NUMBER OF DOSES	DOSES PER VIAL	LOT NUMBER	EXPIRY DATE

DILUENT

VACCINE	MANUFACTURER	NUMBER OF DOSES	DOSES PER VIAL	LOT NUMBER	EXPIRY DATE

ARE VACCINE VIAL MONITORS ATTACHED	YES	NO

IS AIRWAY BILL ATTACHED ?	YES	NO
IS PACKING LIST ATTACHED ?	YES	NO

SHIPPING PROCEDURES

WAS ADVANCE FAX RECEIVED ? (DATE)	
WAS IT SENT TO THE CORRECT ADDRESS ?	
WHO WAS IT SENT BY ?	
WERE THERE DIFFERENCES BETWEEN THE FAXED INFORMATION AND THE ACTUAL ARRIVAL ? WHAT ?	

COLD CHAIN MONITOR

5

VACCINE						
INDEX	A B C D		A B C D		A B C D	A B C D
DATE OF PACKING		DATE		SIGNATURE		
NAME						
DATE OF ARRIVAL						

DPT AND TT SHIPPING INDICATORS

WERE TT SHIPPING INDICATORS INCLUDED ?	YES	NO
WERE ANY INDICATORS BLACK ?	YES	NO

VACCINE TRANSPORT BOXES

TOTAL NO. OF BOXES	

6

IS THE CARGO PROPERLY LABELLED ?	YES	NO
IS THE TELEPHONE NUMBER OF THE CONSIGNEE ON THE CARGO ?	YES	NO
DOES THE LABEL STATE "STORE VACCINES AT +0C TO +8C"	YES	NO
DOES THE LABEL STATE "DO NOT FREEZE" (IF DPT OR TT OR Td VACCINES.)	YES	NO
WERE THE PACKAGES LABELLED "VACCINE RUSH"	YES	NO
WERE THE PACKAGES LABELLED "CONTAINS VACCINE"	YES	NO

WHAT WAS THE STATE OF PACKAGES ON ARRIVAL ? ANY OTHER COMMENTS

WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTE

WHO/EPI/CCIS/86.3
ORIGINAL: ENGLISH

Expanded Programme on Immunization

A GUIDE TO ESTIMATING CAPACITY OF EQUIPMENT REQUIRED FOR STORAGE AND TRANSPORTING EPI VACCINES[1]

Table of Contents

Page

Introduction 1

1. First estimates 1

2. Detailed analysis 3

   2.1 Population growth 3
   2.2 Net vaccine requirements 3
   2.3 Cold chain equipment options 5
   2.4 Equipment selection 6
   2.5 Vaccine transport requirements 8

3. Summary of cold chain requirements for sample country .. 11

Introduction

When assessing the Cold Chain requirements for a new or Expanded Programme on Immunization, it is often helpful to consider the needs in two distinct steps. First, based on national, regional and district populations, make a broad estimate of the size and type of cold chain. This will give a general idea of costs and equipment. Follow this with a more detailed analysis of needs, identifying specific equipment requirements and drawing up lists of equipment plus a firm budget for the cold chain system.

This paper is intended to be used as a guide for assessing country cold chain needs, following these two steps.

1. FIRST ESTIMATES

Table 1 gives approximate capacities required for a cold chain, assuming routine immunization based on a number of assumptions, using a normal fixed centre strategy combined with out-reach services. This may be used as a first estimate for cold chain system planning, and will give a general indication of cold chain needs at each level.

[1]This document revises and replaces the original document on this subject, EPI/CCIS/80/10.

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted, quoted or translated without the agreement of the World Health Organization. Authors alone are responsible for views expressed in signed articles.

Ce document ne constitue pas une publication. Il ne doit faire l'objet d'aucun compte rendu ou résumé ni d'aucune citation ou traduction sans l'autorisation de l'Organisation mondiale de la Santé. Les opinions exprimées dans les articles signés n'engagent que leurs auteurs.

Table 1: Approximate Cold Chain System Requirements

Level	Temperature of storage Deg.C	Population served	Type of equipment needed	Gross storage volume needed*
NATIONAL OR REGIONAL STORES (4 months stock)	0 to +8	Up to 8 million	Refrigerators	300 litres/ million
	0 to +8	Over 8 million	Cold room	0.75 m3/ million
	-15 to -20	Up to 30 million	Freezers	164 litres/ million
	-15 to -20	Over 30 million	Cold room	0.41 m3/ million
TRANSPORT TO REGION (Quarterly deliveries to all regions simultaneously)	0 to +8	No limits	Cold boxes	30 litres/ million
TRANSPORT TO LOCAL STORES (Monthly deliveries to all stores simultaneously)	0 to +8	No limits	Cold boxes	12 litres/ hundred thousand
LOCAL STORES INC. HEALTH CENTRES (6 weeks stock)		Up to 15 million	Refrigerator with icepack freezing	1.50 litres/ ten thousand

* Based on the assumptions outlined in Section 2 below. Note that the choices indicated above are only a guide to selection; they do not include local factors which are important in all cases. The choices do not, for example, take account of possible savings through selecting cold rooms built on site because of lower local costs. They also do not reflect the extra difficulty of installing cold rooms, compared to refrigerators or freezers. In all cases where the choice is not clear, the final choice must rest on local factors, such as the relative ease of importing, transporting, installing, operating and maintaining the chosen equipment.

This approximation is not a substitute for detailed country planning of cold chain equipment needs from the health unit to the central level. Before selecting a system or placing orders, the step-by-step analyses outlined in Section 2 must be followed, with actual conditions in the country substituted for the various assumptions used in the examples below. The methods of calculation, however, remain essentially the same.

2. DETAILED ANALYSIS

2.1 Population Growth

When planning for cold chain needs,a five or ten year planning period should always be considered, and the growth of population over that period estimated. The cold chain chosen must be large enough for programme needs at the end of the planning period.

For example, if a country has a population of 8.5 million today and the population growth rate is 2.8% per annum, by next year the population will have increased to:

$$8{,}500{,}000 \times 1.028 = 8{,}738{,}000$$

And, by the end of a five-year planning period, the population will have further increased to:

$$8{,}738{,}000 \times 1.028 = 8{,}983{,}000 \quad \text{(2nd year)}$$
$$8{,}983{,}000 \times 1.028 = 9{,}234{,}000 \quad \text{(3rd year)}$$
$$9{,}234{,}000 \times 1.028 = 9{,}493{,}000 \quad \text{(4th year)}$$
$$9{,}493{,}000 \times 1.028 = \underline{9{,}759{,}000} \quad \text{(5th year)}$$

Or, alternatively,

$$8{,}500{,}000 \times (1.028)^5 = \underline{9{,}759{,}000}$$

Therefore, in this case we would need to plan the cold chain for a population of about 9,760,000 persons.

2.2 Net Vaccine Requirements

For each 1 million people in the country, let us assume the following:

- the target of infants to be immunized is 35,000 each year;
- 26,000 children entering school will require DT and BCG boosters;
- 35,000 pregnant women require 2 injections of tetanus toxoid.

We will work on the assumption that 100% of these target numbers of immunizations may be reached. Thus, our targets for the year are:-

- 35,000 infants (3.5% of population)
- 26,000 children entering school (5-6yr age group)
- 35,000 pregnant women (3.5% of population)
- 100% immunization coverage.

Vaccine volumes per dose vary widely from manufacturer to manufacturer. In Table 2 below the volume per dose, including packaging, for each vaccine is the maximum recommended by WHO/EPI. This volume excludes diluent for freeze-dried vaccine.

Table 2. Net Vaccine Requirements per Million Population

Target group	Vaccines (doses/ vial)	Target Imm. this year	No. of doses	cm^3 per dose incl. packing	wastage rate.		convert cm^3 to litres	Net vacc. requ ltrs.	Storage temp. °C
Infants	BCG(20)	35,000	x1	x1.0	x2.0	÷	1000	70	+ 4
	DPT(20)	35,000	x3	x2.5	x1.3	÷	1000	341	+ 4
	Meas(10)	35,000	x1	x3.0	x1.3	÷	1000	137	-20
	Polio(20)	35,000	x4	x1.5	x1.3	÷	1000	273	-20
			Sub-total for Infants				=	411	+ 4
							=	410	-20
School Children	DT(20)	26,000	x1	x2.5	x1.3	÷	1000	85	+ 4
	BCG(20)	26,000	x1	x1.0	x2.0	÷	1000	52	+ 4
			Sub-total for School Children				=	137	+ 4
Pregnant Women	Tetanus Tox. (20)	35,000	x2	x2.5	x1.3	÷	1000	228	+ 4
			Sub-total for Pregnant Women				=	228	+ 4

Total vaccine volume per million pop. at + 4°C = 776 litres
Total vaccine volume per million pop. at -20°C = 410 litres

We may now find the total net vaccine requirements for the whole population. For our sample country, we have estimated that the population by the end of the five-year planning period will be 9.76 million. Thus, the total net vaccine storage needs for the country by that time will be:

at + 4°C: 776 litres x 9.76 = 7574 litres
at -20°C: 410 litres x 9.76 = 4002 litres

Assuming this amount is delivered to the country in 4 consignments at 3-monthly intervals, we therefore need to store the following quantities of vaccine at the national level, including a 25% reserve stock:-

at +4°C: $\frac{7574}{4}$ + 25% reserve = 2367 litres of vaccine including reserve

at -20°C: $\frac{4002}{4}$ + 25% reserve = 1251 litres of vaccine including reserve

2.3 Cold Chain Equipment Options

In addition to the space occupied by the vaccines, we must allow extra space for air movement, loading and unloading clearances and, in the case of cold rooms, walk-around space. This allowance is called a "grossing factor".

There are also practical considerations which affect the choice between different types of equipment. Figure 3 shows the normal limits for the various types of cold chain equipment.

Table 3. Application Ranges for Cold Chain Equipment

Type of Cold Chain Equipment	Total Vaccine Requirement (Inc.reserve) in Litres	Maximum Practical Number of Units per Installation	Grossing Factor	Range of gross Capacities in litres of Available Equipment*
Front loading Refrigerators at +4°C	up to 1400 litres	up to 10	2.0	100 - 280
Top loading refrigerators at +4°C	up to 2000 litres	up to 10	1.2	15 - 250
Front loading Freezers at -20°C	up to 700 litres	up to 10	2.0	140
Top loading Freezers at -20°C	up to 4000 litres	up to 10	1.2	15 - 525
Cold rooms at +4°C	above 2000 litres	no limit	3.0	3000 and above
Cold rooms at -20°C	above 4000 litres	no limit	3.0	3000 and above
Cold boxes	no limit	no limit	1.1**	0.3 - 72***

* Approved equipment as reported in WHO Product Information Sheets SUPDIR 55 Amt5 (1985)
** "Spacing Factor" for Cold Boxes to allow for loading and unloading
*** Net capacity figures in the case of Cold Boxes, with allowances made for the necessary number of ice-packs

For large cold rooms, remember that it may be advantageous to divide the space into two or more separate rooms, or perhaps to have individual rooms for each vaccine, in order to facilitate storekeeping and stock control.

2.4 Equipment Selection

(a) The Central Store

The choice of equipment for the central store may now be made from Table 3. For storage of vaccine at +4°C, we have a requirement for 2367 litres of vaccine including reserve. Thus, we choose a cold room at +4°C, and this will need a gross capacity of:

2367 x 3.0 (grossing factor) = 7101 litres or 7.1m^3 gross storage capacity

At -20°C we require 1251 litres of vaccine including reserve, so the choice is for top loading freezers, which will need a gross capacity of:

1251 x 1.2 (grossing factor) = 1501 litres gross storage capacity

(Note that we have not included any space for icepacks at the moment. The calculation for this will be done later.)

(b) Regional Storage

Assume that the country has 10 regions, and that all regions have equal populations. Thus, each region has:-

$$\frac{9.76}{10} \text{ million} = 0.976 \text{ million}$$

From Table 2, we will therefore need an annual amount of vaccine storage space for each region as follows:

at + 4°C: 776 x 0.976 = 757 litres, and
at -20°C: 410 x 0.976 = 400 litres

In many countries, vaccine is delivered to regional stores in 4 consignments at 3-monthly intervals and thus, including a 25% reserve stock, we have to store:-

at + 4°C: $\frac{757}{4}$ = 189 litres of vaccine,
+ 25% reserve = 237 litres of vaccine including reserve, and

at -20°C: $\frac{400}{4}$ = 100 litres of vaccine,
+ 25% reserve = 125 litres of vaccine including reserve.

Referring again to Table 3, if we choose top-loading refrigerators at +4°C, we need a gross capacity of:

237 x 1.2 (grossing factor) = 284 litres, gross storage capacity,

and for frozen vaccine, a top loading freezer at -20°C, having a gross capacity of:

125 x 1.2 (grossing factor) = 150 litres, gross storage capacity.

(c) District Storage

If we further assume each region to have 5 districts of equal size, each district will have a population of approximately:

$$\frac{0.976}{5} \text{ million} = 195{,}000 \text{ persons}$$

At the district level, all vaccine will be stored at +4°C so, from Table 2, the annual amount of vaccine storage required per district is:

$$\text{at } +4^{\circ}\text{C: } (776 + 410) \times \frac{195{,}000}{1{,}000{,}000} = 231 \text{ litres}$$

Vaccine will normally be delivered every month, so the quantity to store, including 25% reserve stock, is:

$$\text{at } +4^{\circ}\text{C: } \frac{231}{12} = 19 \text{ litres of vaccine,}$$

$$+ 25\% \text{ reserve} = 24 \text{ litres of vaccine including reserve}$$

From Table 3 we choose a top loading refrigerator at +4°C, which will need a gross capacity of:

24 x 1.2 (grossing factor) = 29 litres, gross storage capacity

(d) Health Centre Storage

If each district is assumed to have 5 health centres of equal size, each will serve a population of:

$$\frac{195{,}000}{5} = 39{,}000 \text{ persons}$$

At this level also, all vaccine is stored at +4°C, so the annual amount to be stored at +4°C per health unit is:

$$(776 + 410) \times \frac{39{,}000}{1{,}000{,}000} = 46 \text{ litres of vaccine}$$

Assuming a monthly delivery of vaccine, and 25% reserve stock, the quantity to store becomes:

$$\text{at } +4^{\circ}\text{C: } \frac{46}{12} = 3.83 + 25\% \text{ reserve} = 4.8 \text{ litres, vaccine inc. reserve}$$

The best choice of equipment would be a top-loading refrigerator having a gross capacity of:

4.8 x 1.2 (grossing factor) = 5.8 litres, gross storage capacity

2.5 Vaccine Transport Requirements

The choice of cold box to be used will depend on the quantity of vaccine to be transported, and the time taken for the longest journey. Table 4 give a guide to the approved cold boxes available, in terms of their carrying capacity and cold life.

Table 4. Vaccine Carrying Capacity and Cold Life of Cold Boxes

Net vaccine storage capacity in litres	Cold Life in Hours at +43°C Ambient Temperature					
	<24	24-48	49-72	73-96	97-168	>168
more than 50	-	E/32	-	-	-	-
30.1 to 50.0	-	E4/33	E4/31	E4/08 E4/39 E4/50	E4/13 E4/38	-
20.1 to 30.0	E4/06	-	E4/09 E4/29	-	E4/37	E4/05 E4/30
10.1 to 20.0	-	-	E4/28	-	-	-
5.1 to 10.0	-	-	E4/10 E4/51	E4/16 E4/26 E4/36 E4/45 E4/49	E4/22	-
2.5 to 5.0	E4/21	E4/40	E4/20	-	-	-
Less that 2.5	E4/44 E4/46	E4/18 E4/19 E4/35 E4/41	E4/34	-	-	-

Note: Code numbers refer to approved equipment, as reported in the WHO Product Information Sheets, SUPDIR 55 Amt 5 (1985).

(a) Transport from Central Stores to Regional Stores

Vaccine deliveries to the 10 regions of the country are assumed to be made every 3 months. Since all vaccines transported by cold box will be carried at +4°C, the net vaccine volume at this temperature becomes:

(from para. 2.4 (b) "The Regional Store" above):
189 + 100 = 289 litres of vaccine per region

Allowing for loading and unloading space, the gross cold box capacity required becomes:

289 x 1.1 (grossing factor) = 318 litres carrying capacity per region

Assuming a cold life of at least 100 hours is considered to be necessary, together with the largest available cold box, Table 4 indicates that type E4/13 or E4/38 would be suitable. If type E4/13 were selected, with a capacity of 31 litres of vaccine and requiring 11.4 litres of ice packs (19 icepacks at 0.6 litres each), each regional delivery would need:

$$\frac{318}{31} = 11 \text{ cold boxes}$$

and 11 x 11.4 = 125 litres of ice-making capacity

At the Central Store, we therefore need a special icepack fast freezer (e.g type E3/26) or a separate chest freezer for freezing this volume of icepacks.

Remember that various types of vaccine delivery strategies may be employed. A single set of cold boxes may be used to deliver to one region at a time, thus necessitating 10 journeys every three months; alternatively, deliveries may be made to several points during a single journey. The strategy chosen will affect the number of cold boxes needed, and also the amount of ice needed at each level.

(b) Transport from Regional Store to District Store

Transport to the district is made every month and each district will need, allowing for loading and unloading space:

(from para. 2.4 (c) "District Storage" above):
19 litres of vaccine x 1.1 = 21 litres carrying capacity

If type E4/05 is used at district level, each district needs 1 box at 22 litres, and 14.4 litres of ice production would have to be provided at the regional store for producing the necessary ice packs. This can, as before, be met by a special icepack fast freezer or a separate chest freezer:

(c) Transport from District to Health Centre

The type of cold box selected above would be too large for deliveries to health centres individually, but since each centre requires 3.83 litres of vaccine per month, (from 2.4 (d) above) and each district has 5 centres:

5 x 3.83 = 19 litres of vaccine have to be delivered per month

One possible solution might therefore be to deliver all vaccines from the same 22 litre capacity cold box on a single delivery journey around the district. A freezer for producing the necessary 14.4 litres of ice-packs would also be required at the district level.

(d) Transport from Health Centres to Outreach Clinics

For those health centres which operate outreach services, the approximate attendance at each outreach site will enable the neccessary capacity of vaccine carriers to be calculated.

Suppose we know the attendance at a certain outreach clinic is usually about 50 mothers. We assume that each mother will bring at least one child to the clinic and, depending on the time of day when the session will be held, she may also bring older, school-age children. Some children who attend will probably be eligible for more immunizations than others, but an estimate of vaccine requirements can be made as follows:-

30 doses BCG = $\frac{30}{20}$ = 2 x 20-dose vials

40 doses DPT = $\frac{40}{20}$ = 2 x 20-dose vials

30 doses measles = $\frac{30}{10}$ = 3 x 10-dose vials

40 doses polio = $\frac{40}{20}$ = 2 x 20-dose vials

30 doses TT = $\frac{30}{20}$ = 2 x 20-dose vials

Obviously, we must allow for at least one vial of every vaccine we expect to use during the session, but it is usually a good policy to carry one or more extra vials in addition, in case attendance is greater than expected or a vial is damaged etc.

Thus, the vaccine carrier needed for this session should have a capacity of:-

VACCINE	NUMBER OF VIALS	NUMBER OF DOSES	VOLUME PER DOSE CM^3	NET VOLUME CM^3
BCG	2+1	60	1.0	60
DPT	2+1	60	2.5	150
Measles	3+1	40	3.0	120
Polio	2+1	60	1.5	90
TT	2+1	60	2.5	150
Approximate total volume :				570 (= 0.6 litres approx.)

Outreach clinics usually vary in size from session to session so, in order to be safe, it might be worthwhile to allow for more volume than the calculated amount. It would also be a good policy to standardise equipment as far as possible, so that choosing a vaccine carrier large enough for the largest outreach attendance in the country would ensure sufficient capacity for all other sessions.

Thus, in our example, we might decide to standardize on a 1 litre vaccine carrier, and decide to supply this model to all health centres in the country where outreach clinics are held. Remember that for each vaccine carrier used, ice-packs must be available, and that each health centre operating outreach services must therefore have a refrigerator capable of freezing the necessary number of ice-packs, usually four per carrier. (Approximately 1.2 litres or kgs. of ice)

3. SUMMARY OF COLD CHAIN REQUIREMENTS FOR SAMPLE COUNTRY

Table 5. National Cold Chain System Requirements

Level	Population Served	Type of Equipment Selected	Gross Cold Chain Capacity Calculated (litres)		
			Vaccine +4°C	Vaccine -20°C	Icepack Freezing
National Store	9.76 million	Cold Room	7101	-	-
		Top Loading Freezers	-	1501	125
Transport to Regions	976,000 each	Cold Boxes	318	-	-
Regional Store	976,000 each	Top Loading Refrigerator	284	-	-
		Top Loading Freezer	-	150	14.4
Transport to Districts	195,000 each	Cold Box	21	-	-
District Store	195,000 each	Top Loading Refrigerator	29	-	14.4
Transport to Health Centre	39,000 each	Cold Box	22 (shared for 5 health units)	-	-
Health Centre	39,000 each	Top Loading Refrigerator	5.8	-	1.2
		Vaccine Carrier	1 (one for each outreach session)	-	-

= = =

1.2 Vaccine storage volumes

The following table provides data for estimating the packed volume of vaccine required per fully immunized child. Adjusting any of the figures which have been assumed in this table will require that the table be re-calculated according to the prescription at the head of each column.

Vaccine	No. doses per vial	No. doses per F.I.	Packed volume per dose (cm^3)	Vaccine wastage (%)**	Wastage factor	Vaccine storage volume (cm^3)***
	A	B	C	D	E	F
Polio	20	4	1.5	40	1.6	9.6
Yellow Fever	10	1	3.0*	40	1.6	4.8
Measles	10	1	3.0*	40	1.6	4.8
BCG	20	1	1.5*	50	2.0	3.0
DPT	20	3	2.5	40	1.6	12.0
TT	20	2	2.5	40	1.6	8.0
Hepatitis B	10	3	3.0	40	1.6	12.0
Total net storage volume per fully immunized child (F.I.) cm^3						**54.2**

* Volume without diluent

** E = 100 / (100-D)

*** F = B x C x E

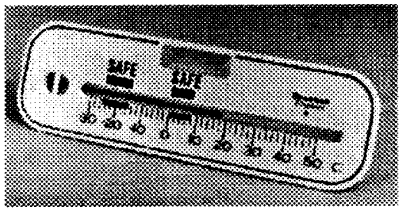
Although the total net storage capacity is estimated above as 54.2 cm^3, the following factors should be kept in mind:

- At higher levels of the cold chain, the vaccines are stored at different temperatures (see table of page 3).
- The wastage rates (D) are only general estimates: they must be checked with local records (vials used versus doses administered) to make correct national estimates. WHO/EPI has revised its policy on the use of Opened Vials of liquid vaccines in subsequent immunization sessions (WHO/EPI/LHIS/95.1). The implementation of this policy and the availability of Vaccine Vial Monitors may result in substantial reduction of the vaccine wastage rates for DTP, OPV, TT , DT and hepatitis B vaccines. Countries should monitor the vaccine wastage rates and adjust accordingly the quantities needed at each level.
- The packed volumes (C) are WHO maximum standards (EPI/CCIS/81.4 Rev.5). Locally made vaccines may occupy more space and this should be checked locally.
- Diluent for freeze dried vaccines doubles the storage space necessary at health centre level (diluent does not need to be cooled except at the level at which it is to be used).

Home
News
Polio Eradication
Diseases + Vaccines
Vaccines are safe
About us
Documents
Site Map

Temperature Monitoring

Quality of Immunization Services (QIS)



[Home] [Up]

Vaccine	Central storage with electricity: up to 6 months	Regional storage with electricity: up to 3 months	District & Health Centre storage with or without electricity: up to 1 month
Oral polio Yellow fever	-15°C to -25°C		
Measles BCG*	-15°C to -25°C or 0°C to +8°C		
IPV DTP DT TT Hepatitis B Hib	0°C to +8°C		

- Storage times given in the above table are recommended maximum figures. Remember to check the expiry dates of the vaccines.
- Do NOT freeze BCG if the diluent is included in the packaging.
- At any stage of the cold chain, vaccine is transported at a temperature of between 0 and +8°C. Polio vaccine may be thawed and frozen again without danger to the vaccine.
- IPV, DPT, DT, Hepatitis-B and TT vaccines are seriously damaged by being frozen at temperatures below 0°C. (Hepatitis B vaccine, for instance freezes around -0.5°C)
- Once potency has been lost through exposure to heat or cold, it *cannot be regained* by returning the vaccine to the correct storage temperature.
- If potency is lost through heat exposure, vaccines do not change their appearance. So a complete laboratory test is the only means of assessing whether a vaccine in a vial has lost its potency.

Reference to documents on this subject, are listed below.

- *Protocol for a cold chain survey using cold chain monitors & computer software.*

WHO/EPI/LHIS/94.09.

Detailed guidelines on how to plan, set up and conduct a survey based on readings from cold chain monitors, from the time they are initially packed with the vaccines to the time the last vial in the consignment is used. The results of this survey are analysed with the aid of two computer programmes which identify problem areas and enable management to focus on specific improvements. The recommended software is also available from GPV/HQ and is described in Section 5.2 of the GPV Catalogue 1998: Computer Applications - Software:EPIC (and E-Mate), Cat. ID# 354.

- *Vaccine cold chain monitor*. WHO/EPI/CCIS/85.01

  The vaccine cold chain monitor is packed with shipments of DPT, BCG, measles and polio vaccine, supplied for WHO and UNICEF. This document describes how to use the monitor and is written for manufacturers and/or suppliers who pack the vaccine, as well as for health workers who receive and handle them. It is complemented by the detailed training guide, Module 27 of the "Cold Chain and Logistics for Primary Health Care Training Series. How to use the monitor and the Cold Chain Monitor poster".

VACCINE MANAGEMENT

Recommendations for Handling and Storage of Selected Biologicals

March 1991

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
CENTERS FOR DISEASE CONTROL
CENTER FOR PREVENTION SERVICES
DIVISION OF IMMUNIZATION
ATLANTA, GEORGIA 30333

Recommendations for Handling and Storage of Selected Biologicals

(These recommendations are not a substitute for the package insert included with each different biologic.)

DTP: Diphtheria Toxoid, Tetanus Toxoid, Pertussis Vaccine

Shipping Requirements
Should be shipped in insulated container. Maintain temperature at 2°-8°C (35°-46°F). **Do not freeze** or store vaccine in direct contact with refrigerant.

Condition on Arrival*
Should not have been **frozen.** Refrigerate on arrival.

Storage Requirements
Refrigerate immediately on arrival. Store at 2°-8°C (35°-46°F). **Do not freeze.**

Shelf Life
Up to 18 months. Check date on vial or container.

Instructions for Reconstitution or Use
Shake vial vigorously before withdrawing each dose.

Shelf Life after Reconstitution, Thawing, or Opening
Until outdated, if not contaminated.

Special Instructions
Rotate stock so that the shortest dated material is used first.

Td—Adult: Tetanus-Diphtheria Toxoids
DT—Pediatric: Diphtheria-Tetanus Toxoids

Shipping Requirements
Should be shipped in insulated container. Maintain temperature at 2°-8°C (35°-46°F). **Do not freeze** or store vaccine in direct contact with refrigerant.

Condition on Arrival*
Should not have been **frozen.** Refrigerate on arrival.

Storage Requirements
Refrigerate immediately on arrival. Store at 2°-8°C (35°-46°F). **Do not freeze.**

Shelf Life
Up to 2 years. Check date on container or vial.

Instructions for Reconstitution or Use
Shake vial vigorously before withdrawing each dose.

Shelf Life after Reconstitution, Thawing, or Opening
Until outdated, if not contaminated.

Special Instructions
Rotate stock so that the shortest dated material is used first.

*If you have questions about the condition of the material at the time of delivery, you should: 1) **Immediately** place material in recommended storage; and 2) Notify the Quality Control office of the vaccine manufacturer; or 3) Notify the Division of Immunization, CDC, Atlanta, Georgia.

Recommendations for Handling and Storage of Selected Biologicals

(These recommendations are not a substitute for the package insert included with each different biologic.)

OPV: Poliovirus Vaccine — Live Oral Trivalent

Shipping Requirements
Pack with dry ice. Should be delivered within 3 days.

Condition on Arrival*
Should be frozen. If thawed but still cold—below 8°C (46°F)—refreeze immediately. Place in freezer on arrival. If thawed and warm—above 8°C (46°F)—or vaccine is cloudy, see footnote (*) below.

Storage Requirements
Maintain continuously in the frozen state—14°C (7°F) or lower. The vaccine may be refrozen. A maximum of 10 freeze-thaw cycles are permissible provided the total cumulative duration of thaw does not exceed 24 hours, and provided the temperature does not exceed 8°C (46°F) during the period of thaw.

Shelf Life
Up to 1 year. Check date on package or dispette.

Instructions for Reconstitution or Use
Thaw before using—may be rubbed between hands for rapid thawing.

Shelf Life after Reconstitution, Thawing, or Opening
Vaccine in liquid state in unopened dispettes may be used for up to 30 days provided it has been stored at 2°-8°C (35°-46°F). A maximum of 10 freeze-thaw cycles are permissible provided the total cumulative duration of thaw does not exceed 24 hours and provided the temperature does not exceed 8°C (46°F) during the period of thaw.

Special Instructions
Rotate stock so that the shortest dated vaccine is used first.

Color Change: This vaccine contains phenol red as a pH indicator. The usual color of the vaccine is pink, although some containers of vaccine shipped or stored in dry ice may exhibit a yellow coloration due to the very low temperature or possible absorption of carbon dioxide. The color of the vaccine prior to use (red-pink-yellow) has no effect on the virus or efficacy of the vaccine.

*If you have questions about the condition of the material at the time of delivery, you should: 1) **Immediately** place material in recommended storage; and 2) Notify the Quality Control office of the vaccine manufacturer; or 3) Notify the Division of Immunization, CDC, Atlanta, Georgia.
NOTE: All materials used for administering live virus vaccines should be burned, boiled or autoclaved prior to disposal.

Recommendations for Handling and Storage of Selected Biologicals

(These recommendations are not a substitute for the package insert included with each different biologic.)

IPV: Poliovirus Vaccine—Inactivated

Shipping Requirements
Should be shipped in insulated container with refrigerant.

Condition on Arrival*
Should not have been **frozen**. Refrigerate on arrival.

Storage Requirements
Refrigerate immediately on arrival. Store at 2°-8°C (35°-46°F). **Do not freeze**.

Shelf Life
Up to 18 months. Check date on package.

Instructions for Reconstitution or Use
Ampoule — 1 dose: Tap the ampoule to ensure that the solution is in the lower portion rather than in the neck of the ampoule. With sterile needle and syringe, withdraw the contents of the ampoule into syringe, holding the ampoule in such a way that the point of the needle is kept immersed throughout the withdrawal.

Vial — 10 dose: Withdraw 1.0 cc of vaccine into separate sterile needle and syringe for each immunization.

Shelf Life after Reconstitution, Thawing, or Opening
Ampoule: Discard if not used immediately.

Vial: Until outdated if not contaminated.

Special Instructions
Rotate stock so that shortest dated vaccine is used first. The vaccine should be perfectly clear and pink or red in color. Any vaccine showing particulate matter, turbidity, or change of color should be discarded.

*If you have questions about the condition of the material at the time of delivery, you should: 1) **Immediately** place material in recommended storage, and 2) Notify the Quality Control office of the vaccine manufacturer; or 3) Notify the Division of Immunization, CDC, Atlanta, Georgia.

Recommendations for Handling and Storage of Selected Biologicals

(These recommendations are not a substitute for the package insert included with each different biologic.)

Measles Virus Vaccine, Mumps Virus Vaccine, Rubella Virus Vaccine Measles/Mumps/Rubella-MMR Vaccine, Measles/Rubella-MR Vaccine

Shipping Requirements

Vaccine — Use insulated container. Must be shipped with refrigerant. Maintain at 10°C (50°F) or less. If shipped with dry ice, diluent must be shipped separately.

Diluent — May be shipped with vaccine but **do not freeze**.

Condition on Arrival*

Should be below 10°C (50°F). If above this temperature, see instructions (*) below. **Do not use warm vaccine**. Refrigerate on arrival.

Storage Requirements

Vaccine may be stored separately from diluent. Store as follows: Vaccine—refrigerate immediately on arrival. Store at 2°-8°C (35°-46°F). **Protect from light at all times**, since such exposure may inactivate the virus.

Diluent may be stored at 15°-30°C (59°-86°F) room temperature. **Do not freeze**.

Special Note: Freeze dried (lyophilized) vaccines may be maintained at freezer temperatures.

Shelf Life

Vaccine — Up to 2 years. Check date on container or vial.

Diluent — Check date on container or vial.

Instructions for Reconstitution or Use

Reconstitute just before using. Use **only** the diluent **supplied** to reconstitute the vaccine.

Single Dose Vials — Inject diluent into the vial of lyophilized vaccine and agitate to ensure thorough mixing. Withdraw entire contents into syringe and inject total volume of vaccine subcutaneously.

Multidose Vials — Withdraw **all** diluent from vial into syringe. Inject into vial of lyophilized vaccine and agitate to ensure thorough mixing.

10-Dose Vials — Withdraw 0.5cc of reconstituted vaccine into separate sterile needle and syringe for each immunization. Licensed for jet injector use.

50-Dose Vials — Use on jet injector only, with dosage set at 0.5cc.

Shelf Life after Reconstitution, Thawing, or Opening

After reconstitution, use immediately or store in a dark place at 2°-8°C (35°-46°F). **Discard if not used within 8 hours**.

Special Instructions

Rotate stock so that shortest dated vaccine is used first.

10-Dose Vials — May be used for both jet injector and needle and syringe methods of immunization.

50-Dose Vials — For jet injector use. Should not be utilized via needle and syringe method of immunization.

*If you have questions about the condition of the material at the time of delivery, you should: 1) **Immediately** place material in recommended storage; and 2) Notify the Quality Control office of the vaccine manufacturer; or 3) Notify the Division of Immunization, CDC, Atlanta, Georgia.

NOTE: All materials used for administering live virus vaccines should be burned, boiled or autoclaved prior to disposal.

Recommendations for Handling and Storage of Selected Biologicals

(These recommendations are not a substitute for the package insert included with each different biologic.)

HBCV: Haemophilus Conjugate Vaccine

Shipping Requirements
Should be shipped in insulated container to help prevent **freezing**.

Condition on Arrival*
Should not have been **frozen**. Refrigerate on arrival.

Storage Requirements
Refrigerate immediately on arrival. Store at 2°-8°C (35°-46°F). **Do not freeze**—this reduces potency.

Shelf Life
Up to 2 years. Check date on container or vial.

Instructions for Reconstitution or Use
Reconstitute before use. If the product requires reconstitution, record date of reconstitution on vial. Use only diluent supplied.

Shelf Life after Reconstitution, Thawing, or Opening
Multidose vials — Stable for 30 days after reconstitution if stored at 2°-8°C (35°-46°F) when not in use.

Single dose vials — Discard unused reconstituted vials after 8 hours.

Special Instructions
Rotate stock so that shortest dated vaccine is used first.

Influenza Vaccine

Shipping Requirements
Should be delivered in the shortest possible time. Should not be exposed to excessive temperatures.

Condition on Arrival*
Should not have been **frozen**. Refrigerate on arrival.

Storage Requirements
Refrigerate immediately on arrival. Store at 2°-8°C (35°-46°F). **Do not freeze**.

Shelf Life
Formulated for use within current influenza season.

Instructions for Reconstitution or Use
Shake vial vigorously before withdrawing each dose.

Shelf Life after Reconstitution, Thawing or Opening
Until outdated, if not contaminated.

Special Instructions
Rotate stock so that shortest dated vaccine is used first.

*If you have questions about the condition of the material at the time of delivery, you should: 1) **Immediately** place material in recommended storage, and 2) Notify the Quality Control office of the vaccine manufacturer; or 3) Notify the Division of Immunization, CDC, Atlanta, Georgia.

Recommendations for Handling and Storage of Selected Biologicals

(These recommendations are not a substitute for the package insert included with each different biologic.)

HB Vaccine: Hepatitis B

Shipping Requirements
Use insulated container. Must be shipped with refrigerant.

Condition on Arrival*
Should not have been **frozen**. Refrigerate on arrival.

Storage Requirements
Refrigerate immediately on arrival. Store at 2°-8°C (35°-46°F). **Do not freeze**.

Shelf Life
Up to 3 years. Check date on package or vial.

Instructions for Reconstitution or Use
Shake vial vigorously before withdrawing each dose.

Shelf Life after Reconstitution, Thawing, or Opening
Until outdated, if not contaminated.

Special Instructions
Rotate stock so that shortest dated vaccine is used first.

HBIG: Hepatitis B Immune Globulin

Shipping Requirements
Should be shipped in insulated container. **Do not freeze**.

Condition on Arrival*
Should not have been **frozen**. Refrigerate on arrival.

Storage Requirements
Refrigerate immediately on arrival. Store at 2°-8°C (35°-46°F). **Do not freeze**.

Shelf Life
Up to 1 year. Check date on container or vial.

Instructions for Reconstitution or Use
Shake vial vigorously before withdrawing each dose.

Shelf Life after Reconstitution, Thawing, or Opening
Until outdated, if not contaminated.

Special Instructions
Rotate stock so that shortest dated material is used first.

*If you have questions about the condition of the material at the time of delivery, you should: 1) **Immediately** place material in recommended storage; and 2) Notify the Quality Control office of the vaccine manufacturer; or 3) Notify the Division of Immunization, CDC, Atlanta, Georgia.

Recommendations for Handling and Storage of Selected Biologicals

(These recommendations are not a substitute for the package insert included with each different biologic.)

Pneumococcal Polysaccharide Vaccine—Polyvalent

Shipping Requirements
Should be shipped in insulated container with refrigerant. **Do not freeze.**

Condition on Arrival*
Should not have been frozen. Refrigerate on arrival.

Storage Requirements
Refrigerate immediately on arrival. Store at 2°-8°C (35°-46°F). **Do not freeze.**

Shelf Life
Up to 2 years. Check date on container or vial.

Instructions for Reconstitution or Use
Vials — Shake vial vigorously before withdrawing each dose.

Prefilled Syringes — Follow manufacturer's directions.

Shelf Life after Reconstitution, Thawing, or Opening
Until outdated, if not contaminated.

Special Instructions
Rotate stock so that shortest dated vaccine is used first. **Do not inject intravenously.** Intradermal administration may cause severe local reactions and should be avoided.

*If you have questions about the condition of the material at the time of delivery, you should: 1) **Immediately** place material in recommended storag and 2) Notify the Quality Control office of the vaccine manufacturer; or 3) Notify the Division of Immunization, CDC, Atlanta, Georgia.

☆U.S. GOVERNMENT PRINTING OFFICE : 1987-730-009/45671

G. Vaccine Temperature Monitoring

1. WHO recommends that manufacturers include cold chain monitor cards with each shipment of vaccine at a rate of one monitor per 3000 doses of DTP or Measles.

Supplement VI.G.1.a., page 783

The Cold Chain Monitor card, which now arrives with every consignment of vaccine from UNICEF and WHO, indicates when the temperature limits of the cold chain have been violated, but not the effect on the vaccine itself. Accidental heat exposures may not individually render the vaccine useless but cumulatively can be significant. As a result, EPI programmes have had to adopt very conservative guidelines about vaccine handling and the disposal of vaccines that have received relatively minor heat exposures. This is changing due to the development and use of Vaccine Vial Monitors (see discussion on page 799).

Supplement VI.G.1.b., page 784

Part VI.H., page 799

2. Several other specific-use monitors are available, including one for shipping DT and TT vaccines.

Supplement VI.G.2., page 794

3. Where it is anticipated that DTP, DT, TT, hepatitis B or Hib vaccine may be exposed to temperatures below freezing point, freeze watch indicators may be used. An indicator should be placed in each shipment box and if the freeze watch indicator has burst, the vaccine in that box should not be used. Care should be taken to ensure the indicator is placed in the box in such a way that it will not be crushed by any weight placed on the box.

Supplement VI.G.3., page 795

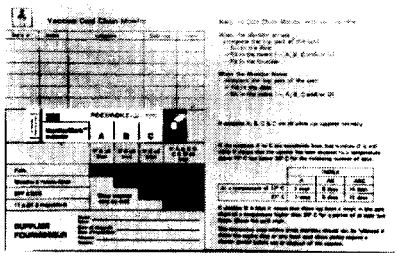
4. An electronic temperature recorder specifically designed for shipping applications is another option for protecting expensive vaccine shipments. This device not only indicates a violation of cold chain parameters, but pinpoints responsibility and the extent of the violation by recording time and temperature at pre-set intervals of as little as one minute. Recording capsules are retrieved from the shipping carton(s) at the receiving warehouse and downloaded on a lap-top computer containing software for generating and printing time/temperature charts. These devices can also be employed for warehouse temperature monitoring. Made in both the United States and United Kingdom, the single-use recording modules are available for US$50 to US$75 and multiple-use modules are available from US$140 to US$275. Corresponding software is available at about US$175. Depending on the overall volume of the shipment, from one to four devices plus one set of software are required for effective monitoring.

Supplement VI.G.4., page 796

(Text resumes on page 799)

130

CODE
PIS
E6/16



Vaccine cold chain monitor card

COMPANY NAME AND ADDRESS

Berlinger & Co. AG
Postfach 67
9608 Ganterschwil
Switzerland
Telephone: *41 (71) 982 8811;*
Fax: *41 (71) 982 8839*

SPECIFICATIONS

Temperature thresholds	+10 and +34°C
External dimensions	120x150 mm.
External materials	Card
Weight	1.7 kgs.
Number per package	250

COMMENTS

Test Report: Wolff Lab (1988)
Meets WHO/UNICEF Standard E6/IN2
Function: This indicator changes irreversibly from white to blue if exposed to temperatures higher than +10 and +34°C.
Use: Packaged with vaccine (1/3,000 doses) to monitor conditions in transit and in storage.
French and English stocked. Other language versions available on special order*.

1997 PRICES (EX WORKS)

Shipping weight/vol (250 pcs) *1.7 kgs/0.0055 m3*
Minimum order: *500 (2 cartons)*

Language	Price/unit
English card	CHF 4.00 US$ 2.92)
French card	CHF 4.00 US$ 2.92)

* *Other languages direct order from Berlinger at EXW price of CHF 4.00 each.*

WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTE

DISTR.: GENERAL(E)

EPI/CCIS/85.1 Rev.5*

ORIGINAL: ENGLISH

Expanded Programme on Immunization

VACCINE COLD CHAIN MONITOR

Table of Contents

Page

1. INTRODUCTION 1

2. THE NEW COLD CHAIN MONITOR..... 1

2.1 How temperatures are monitored 2
2.2 How to interpret temperature readings 3

3. INFORMATION FOR HEALTH WORKERS:

3.1 How to use the monitor in routine onforwarding of shipments 4
3.2 What action if there is a break in the cold chain: 4
- With the vaccine 4
- With the cold chain 4

4. INFORMATION FOR VACCINE MANUFACTURERS AND DISTRIBUTORS:

4.1 What to do when packing monitors cards with vaccine despatches 5
4.2 How to store monitors..... 5

5. AVAILABILITY, PRICE AND PURCHASING DETAILS 6

ANNEX 1: List of Countries and Languages 7
ANNEX 2: Vaccine Cold Chain Monitor Poster 11

1. INTRODUCTION

This document describes the new and simplified version of the Vaccine Cold Chain Monitor which is being packed by vaccine manufacturers with vaccines supplied by WHO and UNICEF.

To retain potency, vaccines must be kept at a temperature of 8°C or less, at all points of the cold chain - that is, from the time the vaccines are manufactured, to their shipment to central warehouses and their distribution to health centres at regional, district and village levels.

Each cold chain monitor has a heat-sensitive indicator in the form of a strip with 4 windows stuck to it. This indicator operates at two different temperatures (10°C and temperatures above 34°C) and serves as an effective check of the cold chain during shipment by giving health workers some guidance on whether or not to use the vaccines they receive.

2. THE NEW VACCINE COLD CHAIN MONITOR

The new monitor functions as the previous one did. However, it has been modified so that it has only 4 windows for registering temperature changes and, as from July 1987, the card is entirely heat stable until it has been activated. The instructions for interpreting the readings are printed on the monitor.

* Revised July 1988.

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2.1 HOW TEMPERATURES ARE MONITORED

Unlike previous versions of the monitor card, the version available as from July 1987 is entirely heat stable until it is activated by pulling out the small tab on the left hand side of the strip (see explanation on activation given on page ..).

Once the monitor has been activated, temperatures above 10°C are registered in windows A, B and C; temperatures above 34°C are registered in window D.

When the indicator is exposed to temperatures above +10°C, it functions as follows in windows A, B and C:

- A blue colour begins to appear in the first window, marked "A". If the temperature then drops below +10°C the blue colour stops spreading.
- Each time the strip is exposed to temperatures over +10°C the blue colour will spread further across the monitor from A to C. The colour change is irreversible: colour can stop spreading, but it will NEVER RETURN down the scale.

The higher the temperature the faster the blue colour will spread.

When the indicator is exposed to temperatures above +34°C, it functions as follows in window D:

- Window D turns blue within 2 hours.
- Once it has changed colour to blue it will never change back again to white.

2.2 HOW TO INTERPRET THE TEMPERATURE READINGS (Indexes)

A simplified interpretation guide [(3) in Figure 1] explains how to interpret the colour changes (referred to as "indexes") on the indicators in relation to the vaccines with which the monitor card is packed:

- Windows A, B, C and D are all white, no blue is showing:
  This means the cold chain is good; temperatures have been kept under 10°C and the vaccines are good:

  Polio: Vaccine may be used;
  Measles: Vaccine may be used;
  DPT & BCG: Vaccines may be used;
  TT & DT: Vaccines may be used.

- Window A is all blue, the other windows are white:

  Polio: Use vaccine within 3 months;
  Measles: Vaccine may be used;
  DPT & BCG: Vaccines may be used;
  TT & DT: Vaccines may be used.

- Windows A and B are all blue:

  Polio: Test vaccine before use;
  Measles: Use vaccine within three months;
  DPT & BCG: Vaccines may be used;
  TT & DT: Vaccines may be used.

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  Polio: Vaccine may be used;
  Measles: Vaccine may be used;
  DPT & BCG: Vaccines may be used;
  TT & DT: Vaccines may be used.

- Window A is all blue, the other windows are white:

  Polio: Use vaccine within 3 months;
  Measles: Vaccine may be used;
  DPT & BCG: Vaccines may be used;
  TT & DT: Vaccines may be used.

- Windows A and B are all blue:

  Polio: Test vaccine before use;
  Measles: Use vaccine within three months;
  DPT & BCG: Vaccines may be used;
  TT & DT: Vaccines may be used.

- Windows A, B and C are all blue:

  Polio: Test vaccine before use;
  Measles: Test vaccine before use;
  DPT & BCG: Use vaccines within 3 months;
  TT & DT: Vaccines may be used.

- Windows A, B, C and D are all blue:

  Polio: Test vaccine before use;
  Measles: Test vaccine before use;
  DPT & BCG: Test vaccines before use;
  TT & DT: Test vaccines before use.

3. INFORMATION FOR HEALTH WORKERS

3.1 HOW TO USE THE MONITOR IN ROUTINE ONFORWARDING OF VACCINE SHIPMENTS

The storekeeper is responsible for entering information on the record form on the top half of the monitor at each level of the cold chain.

When the monitor arrives with a vaccine shipment, the storekeeper fills in:

- the date the shipment arrived;
- index* registered on the indicators;
- location of the store.

When the monitor leaves the store with the vaccine, the storekeeper fills in:

- the date the shipment leaves;
- Index (A, B, C or D), as registered on the monitor*

3.2 What action to take if there is a break in the cold chain:

- With the vaccines: Consult the interpretation guide on the front of the monitor.

- With the cold chain: By reading the record form on the front top part of the monitor, the weak parts of the cold chain can be identified. Every break in a cold chain should be investigated and some action taken to prevent a repeat breakdown. Priority should be given to the higher levels of the cold chain - the central store, regional stores and their transport facilities. These stores are easier to visit and supervise and the volume of vaccine stored is bigger.

---

* To enter the index or colour change in the "Index Column" of the record form, fill in as follows:

If there is no blue colour showing in any windows, fill in a dash (-).
If window A is entirely blue, write "A" in the index column.
If windows A and B are entirely blue, write "AB" in the index column".
If windows A, B and C are entirely blue, write "ABC" in the index column.
If windows A, B, C and D are blue, write "ABCD" in the index column".

Note: Only record the index for that window when that window is entirely blue. For instance if window A is blue and window B is just beginning to turn blue, the index to enter is "A".

4. INFORMATION FOR VACCINE MANUFACTURERS

4.1 What to do when packing the monitor cards with vaccine despatches

(i) Select the Cold Chain Monitor with the correct language: English, French, Spanish, Portuguese, Arabic or Russian. The list in the index gives the language (within the choice available) appropriate for most countries of the world.

(ii) Fill in the bottom part of the Monitor labelled "Supplier" with the name of the vaccine supplier, date of despatch and the type of vaccine being despatched.

(iii) Select sufficient monitors so that you have:

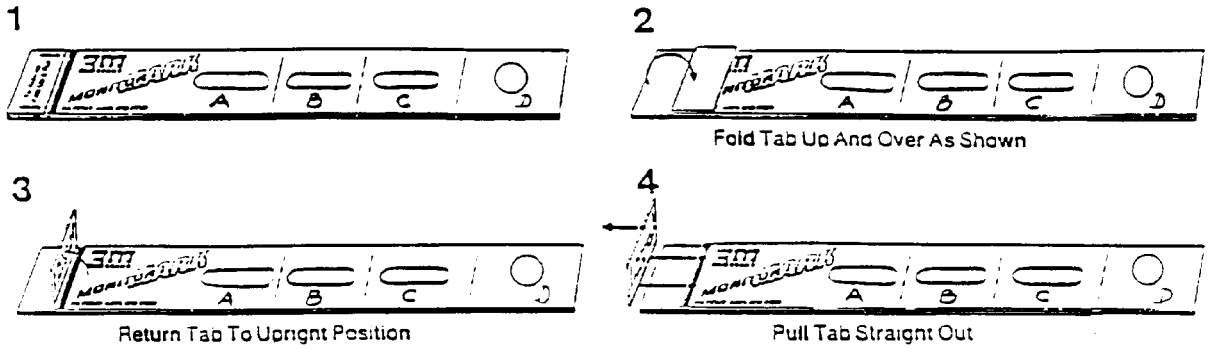
One Monitor per 3 000 doses of DPT, polio or measles vaccine

(iv) Activate the monitors you will be using. The activation is done in two steps:

(a) Pre-cool the monitors to a temperature less than 10°C for at least 30 minutes.

NOTE: Recent laboratory tests have shown that if the Monitor Marks are activated at a normal room temperature, some blue colour will begin to show on the Monitor - even if the Monitor is immediately cooled to below 10°C.

(b) Pull off the activation tabs. See below.



(v) Pack the Monitors with the vaccine.

4.2 How to store monitors

With the version of the cold chain monitor available as from July 1987 no special precautions need to be taken during storage or transport as this monitor is entirely heat stable until it has been activated.

5. AVAILABILITY, PRICE AND PURCHASING DETAILS

The Vaccine Cold Chain Monitors are available in five languages, each language printed on different coloured cards:

Arabic	- on a green card
English	- on a yellow card
French	- on an orange card
Portuguese	- on a grey card
Spanish	- on a white card

The current price of the Vaccine Cold Chain Monitors is Swiss Francs 380 per 100 cards, including packaging but excluding shipping costs. This price is subject to change without notice.

Orders for the Vaccine Cold Chain Monitors should be addressed to:

Berlinger and Company AG
9608 Gansterschwil
Switzerland

Telephone No. (073) 33 22 33
Telegrams Berlinger Ganterschwil
Telex: 883280 BEGA

Minimum order: 500 monitors

ANNEX 1

LIST OF COUNTRIES & LANGUAGES

The Cold Chain Monitors exist in Arabic, English, French, Portuguese and Spanish. The list below indicates the appropriate language (within the choice of five languages available to be selected for each country. If two languages are listed, the first is the preferred one

Afghanistan, English
Albania, French
Algeria, Arabic, French
Andora, Spanish
Angola, Portuguese
Antigua and Barbuda, English
Argentina, Spanish
Australia, English
Austria, English

Bahamas, English
Bahrain, Arabic, English
Bangladesh, English
Barbados, English
Belgium, French
Belize, English
Benin, French
Bermuda, English
Bhutan, English
Bolivia, Spanish
Botswana, English
Brazil, Portuguese
Brunei, English
Bulgaria, French
Burkina Faso, French
Burma, English
Burundi, French
Bylorussian SSR, English

Cameroon, French
Canada, English, French
Cape Verde, Portuguese
Cayman, English
Central African Republic, French
Chad, French
Chile, Spanish
China, English
Colombia, Spanish
Comoros, French
Congo, French
Cook Islands, English
Costa Rica, Spanish
Cuba, Spanish
Cyprus, English
Czechoslovakia, English, French

Democratic Kampuchea, French
Democratic Yemen, Arabic, English
Denmark, English
Djibouti, Arabic, French
Dominica, English
Dominican Republic, Spanish

Annex 1

Ecuador, Spanish
Egypt, Arabic, English
El Salvador, Spanish
Equatorial Guinea, Spanish
Ethiopia, English

Fiji, English
Finland, English
France, French

Gabon, French
Gambia, English
German Democratic Republic, English
Germany (Federal Republic of) English
Ghana, English
Gibraltar, English
Greece, English, French
Grenada, English
Groenland, English
Guadeloupe, French
Guatemala, Spanish
Guam, English
Guinea, French
Guinea-Bissau, Portuguese
Guyana, English

Haiti, French
Holy See, French
Honduras, Spanish
Hong-Kong, English
Hungary, English

Iceland, English
India, English
Indonesia, English
Iran, English, French
Iraq, Arabic, English
Ireland, English
Israel, English
Italy, French
Ivory Coast, French

Jamaica, English
Japan, English
Jordan, Arabic, English

Kenya, English
Kiribati, English
Korea (People's Democratic Republic of), English
Korea (Republic of), English
Kuwait, Arabic, English

Lao People's Democratic Republic, French
Lebanon, Arabic, English
Lesotho, English
Liberia, English
Libyan Arab Jamahiriya, Arabic, English
Liechtenstein, French
Luxembourg, French

Annex 1

Macao, Portuguese, English
Madagascar, French
Malawi, English
Malaysia, English
Maldives, English
Mali, French
Malta, English
Martinique, French
Mauritania, French, Arabic
Mauritius, English, French
Mexico, Spanish
Monaco, French
Mongolia, English
Monstserrat, English
Morocco, Arabic, French
Mozambique, Portuguese

Namibia, English
Nauru, English
Nepal, English
Netherlands, English
New Caledonia, English, French
New Zealand, English
Nicaragua, Spanish
Niger, French
Nigeria, English
Norway, English

Oman, Arabic, English

Pakistan, English
Panama, Spanish
Papua New Guinea, English
Paraguay, Spanish
Peru, Spanish
Philippines, English
Poland, French
Polynesia (French), French
Portugal, Portuguese

Qatar, Arabic, English

Réunion, French
Romania, French
Rwanda, French

Saint Christopher & Nevis, English
Saint-Helena, French
Saint Lucia, English
Saint Vincent & the Grenadines, English
Salomon Islands, English
Samoa, English
San Marino, French
Sao Tome & Principe, French
Saudi Arabia, Arabic, English
Senegal, French
Seychelles, English

Annex 1

Sierra Leone, English
Singapore, English
Somalia, English
South African, English
Spain, Spanish
Sri Lanka, English
Sudan, Arabic, English
Suriname, English
Swaziland, English
Sweden, English
Switzerland, French
Syrian Arab Republic, Arabic, English

Tanzania (United Republic of), English
Thailand, English
Togo, French
Tonga, English
Trinidad and Tobago, English
Tunisia, Arabic, French
Turkey, English, French

Uganda, English
Ukrainian SSR, English
United Arab Emirates, Arabic, English
United Kingdom, English
United States of America, English
Uruguay, Spanish
USSR, English

Vanuatu, English
Venezuela, Spanish
Viet Nam, French, English

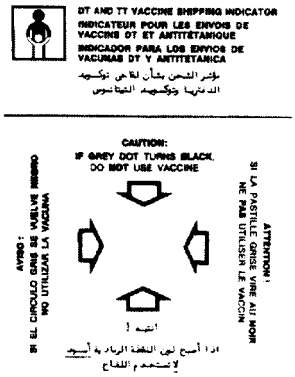
Yemen, Arabic, English
Yugoslavia, English

Zaire, French
Zambia, English
Zimbabwe, English

UNWRA, Arabic, French

129

CODE
PIS
E6/15



DT & TT Shipping indicator

COMPANY NAME AND ADDRESS

Berlinger & Co. AG
Postfach 67
9608 Ganterschwil
Switzerland
Telephone: *41 (71) 982 8811;*
Fax: *41 (71) 982 8839*

SPECIFICATIONS

Temperature threshold	+48°C
External dimensions	120x90 mm.
External materials	Card
Weight (per package)	1.25 kgs.
Number per package	600

COMMENTS

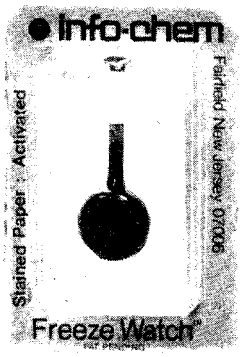
Test Report: Wolff Lab (1988)
Meets WHO/UNICEF Standard E6/IN1
Function: The temperature sensitive dot turns from silver grey to black instantaneously at temperatures above +48°C. This change is irreversible.
Use: The indicator is attached to each 3,000 doses of DT, DPT and TT vaccine in order to warn of high temperature exposure. Such temperatuers are reached in poorly ventilated spaces exposed to the sun, e.g. in vehicles parked in the sun.

1997 PRICES (EX WORKS)

Shipping weight/vol (600) *1.25 kgs/0.0036 m3*
Minimum order: *600 (orders in multiples of 600)*

Quantity	Price/unit			
up to 2,000	1/CHF	0.88	(US$	0.64)
2001-15,000	1/CHF	0.69	(US$	0.50)
15,001 +	1/CHF	0.50	(US$	0.36)

CODE
PIS
E6/45



Freeze watch indicator (0°C): Model recorder number: 9805

COMPANY NAME AND ADDRESS

Berlinger & Co. AG
Postfach 67
9608 Ganterschwil
Switzerland
Telephone: *41 (71) 982 8811;*
Fax: *41 (71) 982 8839*

SPECIFICATIONS

Temperature threshold	0°C
External dimensions	60x40x10 mm.
External materials	PVC Plastic
Weight	0.016 kgs.
Number per package	400

COMMENTS

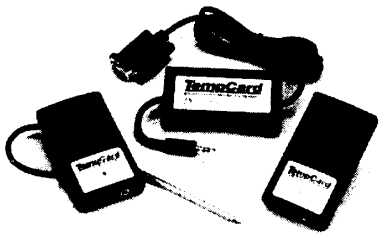
Test Summary: CCIS.83.8
Meets WHO/UNICEF Standard E6/IN3
An irreversible temperature indicator which shows if a product, such as vaccine, has been exposed to freezing temperatures. It consists of a white backing card with a small vial of red liquid, all contained in a plastic casing. If the indicator is exposed to temperatures below 0°C for more than one hour, the vial bursts and releases the red liquid, staining the white backing card. The indicator is used to warn of freezing and is packed with DPT, TT and DT vaccines (freezing point of -6.5°C) as well as with Hepatitis B (-0.5°C). It replaces E6/14, that bursts at -4.5°C. This change is due to the introduction of Hepatitis B, with a freezing point of -0.5°C, in the standard EPI schedule.

1997 PRICES (Ex works, USA)

Shipping weight/vol (400 pcs) *6.5 kgs/ 0.033 m3*
Minimum order: *400*

Quantity	Price/unit			
400-999	1/CHF	2.80	(US$	2.04)
1000-1,599	1/CHF	2.65	(US$	1.93)
1,600-3,199	1/CHF	2.50	(US$	1.82)
3,200	1/CHF	2.45	(US$	1.79)

CODE
PIS
E6/42



Temperature data logger -- TempTale

COMPANY NAME AND ADDRESS

TSS
Box 135
182 12 Danderyd, Sweden
Telephone: *+46 (8) 622 5141/45*
Fax: *+46 (8) 622 65 31*
E-Mail: *michael.thelven@mailbox.swipnet.se*

SPECIFICATIONS

Temperature range	-30 to +85°C
Accuracy	± 0.5°C
Measuring interval	from 0.5 secs-2.13 hrs
Memory size	4K, 6K, 8K and 16K
Size (lxwxh)	88 x 50 x 15 mm
External materials	Plastic
Weight	0.085 kg.
Power source	Lithium battery
Battery life available	365 days

COMMENTS

Test Report: Certified by TUV, April 1995
No WHO/UNICEF Standard exists.
Use: TempCard is a portable, self-contained, user-programmable, temperature monitor for use with goods sensitive to temperature and/or humidity. As a "reusable cold chain monitor", it gives a complete history and is suitable for use in vaccine refrigerators, vaccine shipments and cold chain studies. Recorded data can be downloaded and stored via computer COM port connection to software in Dos or Windows format. Ambient or probe models available.

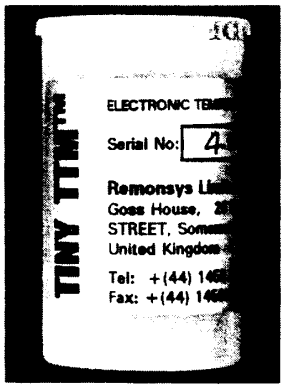
1997 PRICES (FOB)

Shipping volume *0.1088 m3*
Minimum order: *1*

Reusable* data logger	**1-10**	US$ 225
	11-200	US$ 195
Software and cable		US$ 245
Complete demo package		US$ 100

***A single use model also available from $30 and up.**

CODE
PIS
E6/43



Temperature data logger: Model Tiny TTM Type G

COMPANY NAME AND ADDRESS

Remonsys Ltd.
Goss House, 26 High Street
Street, Somerset BA16 OEB United Kingdom
Telephone: *+44 (1458) 840 088*
Fax: *+44 (1458) 841 188*
E-Mail: *lewis@autolog.u-net.com*
Internet *site: http://www.u-net.com/~autolog/remindex.html*

SPECIFICATIONS

Temperature range	-40°C to +75°C
Accuracy	± 0.2°C
Measuring interval	0.5 secs to 4.8 hrs
Memory size	1.8K
Size (dxl)	30 x 50 mm.
External materials	plastic case
Weight	0.05 kg.
Number per package	5
Power source	battery 1,5 V (1/2AA)
Battery life available	2 years

COMMENTS

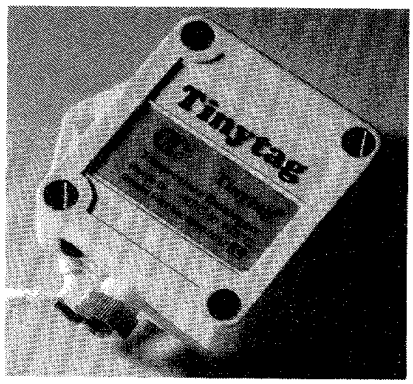
Test Report: Univalle, 1992
No WHO/UNICEF Standard exists.
Use: Functions as a "reusable cold chain monitor" for use in vaccine refrigerators, shipments and cold chain studies. The TTM is battery operated and small enough to fit inside a 35 mm. plastic film container. It stores data which can be downloaded by special cable to the serial port of a PC computer with DOS/MS Windows supported software.

1997 PRICES (FOB)

Shipping volume (5 pcs) *555 ccm*

Item	Min. order.	Price/unit	
Tiny TTM Type G	5	1/GB£ 85.00	(US$ 144.07)
I/Fcable & software	1	1/GB£ 80.00	(US$ 135.59)
Interface cable	1	1/GB£ 20.00	(US$ 33.90)

CODE
PIS
E6/44



Temperature data logger: Model Tiny TTM G IP68

COMPANY NAME AND ADDRESS

Remonsys Ltd.
Goss House, 26 High Street
Street, Somerset BA16 OEB United Kingdom
Telephone: *+44 (1458) 840 088*
Fax: *+44 (1458) 841 188*
E-Mail: *lewis@autolog.u-net.com*
Internet site: *http://www.u-net.com/~autolog/remindex.html*

SPECIFICATIONS

Temperature range	-40°C to +75°C
Accuracy	± 0.2°C
Measuring interval	0.5 secs to 4.8 hrs
Memory size	2048
Size (lxbxh)	70x60x50 mm.
External materials	glass reinforced plastic
Weight	150 g.
Number per package	5
Power source	battery 1,5 V
Battery life available	2 years

COMMENTS

Test Report: Univalle, 1992
No WHO/UNICEF Standard exists.
Use: Functions as a "reusable cold chain monitor" for use in vaccine refrigerators, shipments and cold chain studies. This version of Tiny TTM has a rugged enclosure. It stores data which can be downloaded by special cable to the serial port of a PC computer with DOS/MS Windows supported software.

1997 PRICES (FOB)

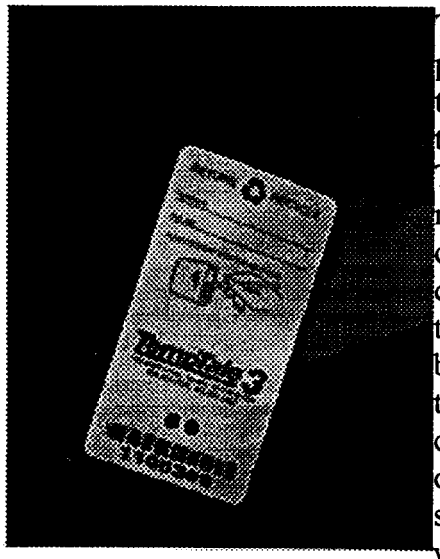
Shipping volume *0.001 m3*

Item	Min. order	Price/unit	
Tiny TTM G IP 68	5	GB£ 115	(US$ 194.92)
I/Fcable & software	1	GB£ 80	(US$ 135.59)
Interface cable	1	GB£ 20	(US$ 33.90)

TempTale3

TempTale3®

Product Description | Benefits | Specifications | Information Request



The TempTale3 is a durable, portable data acquisition unit that records temperature to the tenth of a degree. The TempTale is capable of monitoring both ambient and core temperatures. The data can be easily read either by the TempTale TripStrip® or by your PC. Time and temperature detail can be displayed in either graphical or tabular format. A trip summary provides the user with a snapshot of the trip results.

Validating Product Quality Worldwide is made easy and affordable with the TempTale3.

The TempTale product design has been validated by the world-renowned TUV laboratories. Each TempTale unit is manufactured in the U.S. under ISO 9000 guidelines.

Sensitech has proprietary testing procedures and strict quality control procedures that ensure each TempTale unit is functioning to specification. Our TempTale products have been tested and approved by Fortune 500 customers.

TempTale Benefits

- Monitors and Information Management Available Worldwide
- Industry-Unique, TUV-Verified Design
- Lightweight, Compact, Durable Monitors
- Shock and Vibration Resistant

H. Vaccine Vial Monitors

Vaccine Vial Monitors are time/temperature indicators in the form of small adhesive labels that are applied to individual vials of vaccine. These monitors integrate exposure to heat over a period of time and are designed to meet the heat stability curves of the specific vaccine.

Supplement VI.H., page 800

WHO currently is working with vial monitor manufacturers and vaccine producers to accelerate the introduction of these monitors on all vaccines in the EPI that are degraded by exposure to high temperatures. Currently, all producers of oral polio vaccine supplied to United Nations agencies such as UNICEF and WHO use vial monitors. National producers of oral polio vaccine are also beginning to use them. It is anticipated that the use of Vaccine Vial Monitors may be commenced with other vaccines before the end of 1999. Information on progress in the introduction of these monitors can be obtained from WHO/GPV in Geneva. The introduction of Vaccine Vial Monitors has dictated a change in the manner in which Cold Chain Monitor cards are used. In the case of oral polio vaccine, WHO now recommends that only one cold chain monitor card be used for each shipping box, rather than one for each 3000 doses of vaccine.

(Text resumes on page 806)

VACCINE VIAL MONITORS (VVM) FOR OPV

Specification reference: *E6/IN.5*
Applies to procedures: *E6/PROC/5*
Date of last revision : *29 September 1997*

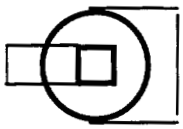
Purpose

Vaccine vial monitors. Serves primarily to warn health workers when the cumulative heat exposure of a vial of vaccine has exceeded a pre-set limit, beyond which the vaccine should not be used. In addition: changes in the appearance of the VVM before this limit is reached, will serve to guide health workers to use more exposed vials of vaccine first .

Format and dimensions:

The VVM is a circle of colour, minimum diameter 7.0mm with a square of colour, minimum dimensions 2.0 x 2.0mm positioned in the centre of the circle (See drawing). The ratio of the area of the square to the area of the circle (including the square) is at least 0.1, whatever dimensions are chosen.

SQUARE minimum 2.0mm:



CIRCLE minimum 7.0mm:

Design :

The circle of the VVM acts as a static, reference colour and the square is a changing, active colour change device. The colour change is limited to a change of shade, from light to dark. Any colour is permitted for the VVM design , but changes in hue are not permitted.

Colour:

The colour density change of the indicator is illustrated in the diagram below. At the start point the colour of the square is lighter than the circle. The end point is indicated when colour of the square matches the circle. The end point is exceeded when the colour of the square is darker than the circle. The following paragraphs describe the colour change in more detail.

The central square is the active surface

- Start point — Square lighter than circle
- End point — Square matches circle
- End point ***exceeded*** — Square darker than circle

Definition of the start-point

1.1 The colour of the active surface of the VVM at the time of labelling of the vaccine vial is called the 'start point'.

1.2 At the start point, the colour density of the square as measured by a colour densitometer1, must be lighter than the colour shade of the circle by at least 0.25 OD densitometer units.

Definition of the end-point

2.1 The colour of the active surface of the VVM at the limit of use of the vaccine vial is called the 'end point'.

2.2 The end point is reached when difference in the average colour density obtained from readings at three different points on the circle and the colour density of the square is 0.00 OD, as measured by the densitometer. The end point is exceeded when the colour of the square is darker than the colour of the circle.

Homogeneity of the colour surface

3.1 The colour density of one 2mm diameter portion of the circle must be within 0.01 OD of the colour density at any other two 2mm diameter portions of the circle, when measured with a colour densitometer.

1 Colour reflection densitometer Xrite Model 404 GS or GSX

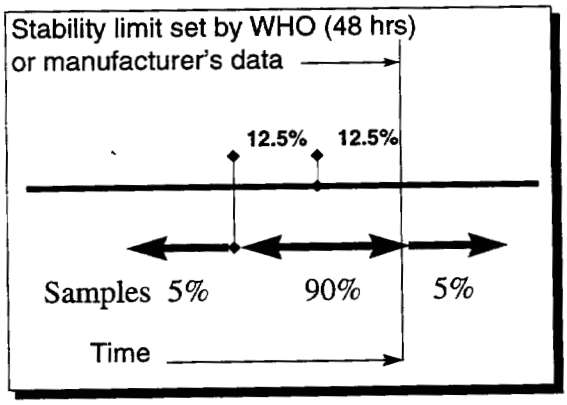
VVM reaction rates: (See Figure below)

4.1 At +37°C and RH 25-35% and RH 55-65%, at least 90% of VVMs tested should reach the end point within a range of time whose upper limit is the WHO stability standard for oral polio vaccine (37°C for 48 hours) or a period set by the vaccine manufacturer, based on published vaccine stability data, and whose lower limit is 25% below the upper limit.

4.2 At +5°C ± 3°C and RH 25-35% and RH 55-65%, at least 90% of VVMs tested should reach the end point within a range of time whose upper limit is the vaccine manufacturer's shelf life of the vaccine at +5°C ± 3°C and whose lower limit is 25% below the upper limit.

4.3 A tolerance is allowed in the above tests for up to 5% of VVM samples tested to reach the end point after the upper limit and 5% before the lower limit.

4.4 The colour change shall be linear in its response to cumulative heat exposure within the limits of the allowed variation. The observer shall be able to distinguish between unchanged, 50% and the end point of the indicator.



Reversion:

5.1 The indicator shall not revert to a lighter colour at any point in its life when exposed to conditions likely to be found during normal use. After the endpoint is reached, the square shall remain the same colour as the circle or become darker than the circle.

Integrity of VVMs

6.1 The VVM shall be permanently attached to the vaccine vial, even after the vial has been opened and remain readily observable before, after and during use.

6.2 The VVM must not be removable from the vaccine vial without self-destruction or indication that it has reached the end point.

6.3 The colour density of the active surface and the reference circle should not change by more than 0.01 OD before and after a standard rub test

6.4 The performance of the VVM should not be changed by exposure to the most aggressive form of human perspiration. They should conform to within +/-0.02 OD units.

Safety

7.1 The exposed surface of the VVM shall not be able to endanger human health. The materials of the VVM shall be non-toxic and non-irritant. The VVM should meet any requirements in force concerning toxicity of labels or packaging in the country of manufacture.

UPDATE
MAY 1996

Expanded Programme on Immunization

TWO SIMPLE WAYS TO USE LESS VACCINE
How to use vaccine vial monitors, and when to use opened vials of vaccine

Vaccine vial monitors (VVMs)

- VVMs are being introduced in 1996. They are now on vials of oral polio vaccine, and will appear on other vaccines in due course.
- VVMs enable field staff to reject vials of vaccine which are heat damaged.

Use of opened* vials of vaccine in subsequent immunization sessions

- Opened vials of OPV, DTP, TT, DT and hepatitis B vaccines may be used in subsequent immunization sessions until a new shipment arrives (provided the expiry date has not passed, the vaccines are kept in the cold chain, and the vials have not been used outside the health centre).
- Opened vials of measles, yellow fever and BCG vaccines MUST be discarded at the end of each immunization session.

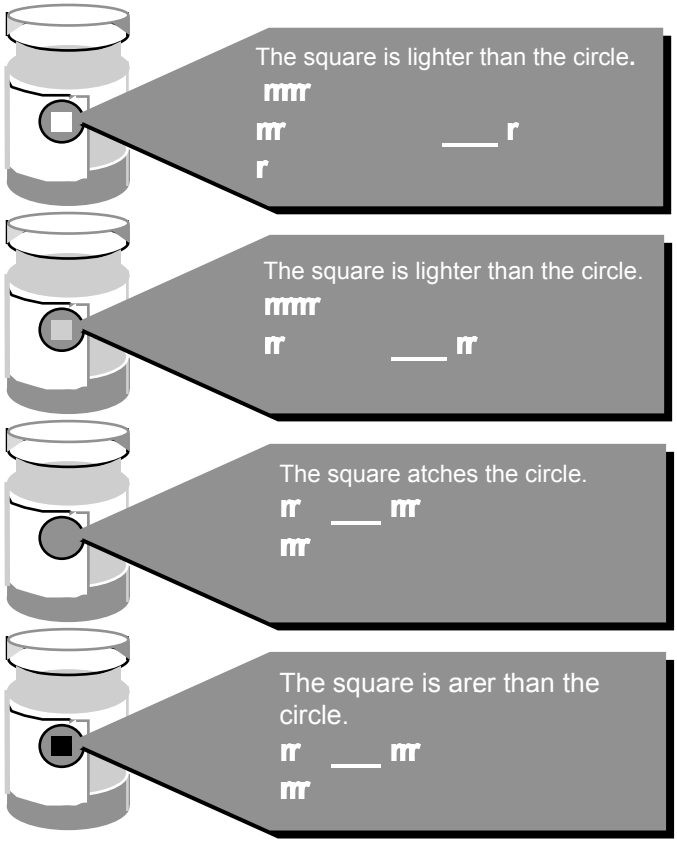
Implementation issues

- Introducing VVMs and adopting the revised policy on the use of opened vaccine vials have training implications for all field and management staff. Neither strategy should be introduced until field staff and supervisors have been trained appropriately.
- Adopting these two new aspects of vaccine handling can help cut wastage by 30% and save up to $40,000,000 a year globally.

* "Opened vials" in this context refer to multidose vials from which one or more doses of vaccine have been used, in line with standard sterile procedures.

H.1

The interpretation of the VVM is simple. Focus on the central square. Its colour will change progressively. The vaccine can be used as long as the colour of this square is lighter than the colour of the ring. The vial should be discarded as soon as the colour of the central square is the same colour as the ring or of a darker colour than the ring.



I. Vaccine-Related Products

1. Selection of injection equipment

The GPV Unit of WHO has published a recommended policy for safety of injections in immunization programmes which includes a section on selection of injection equipment.

Supplement VI.I.1., page 807

2. Syringes and needles

A sterile syringe and a sterile needle must be used for each injection. WHO/UNICEF Product Information Sheets provide information for sterilisable and auto-destruct disposable syringes. The relatively higher price of auto-destruct syringes (US$0.085 compared to US$0.045 for standard disposables) has prevented many countries from following WHO guidance on the selection of syringes for EPI; however, it is anticipated that a range of auto-destruct syringes will become available at a price of US$0.05-0.06.

Supplement VI.I.2., page 813

3. Syringe incinerator box

WHO/UNICEF Product Information Sheets also provide basic information for syringe incinerator boxes.

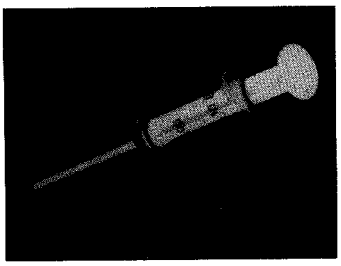
Supplement VI.I.3., page 818

4. Vaccine cold boxes/carriers

The WHO/UNICEF Product Information Sheets include approved cold boxes and vaccine carriers and vaccine packaging.

Supplement VI.I.4., page 820

CODE PIS
E8/01



Sterilizable plastic syringe, 0.05 ml

COMPANY NAME AND ADDRESS

CODAN
Faergejev 4
DK-4970 Roedby
Denmark
Telephone: *45 (54) 607 411*
Fax: *45 (54) 605 101*
Telex: *40 728 ASIK DK*

SPECIFICATIONS

Vaccine capacity	0.05 ml.
Graduations	0/0.05
Material of the syringe	Polypropylene
Needle fixing	Luer
Recommended needle	26G 10 mm.
Max. number of sterilizations	200
External dimensions (i.e. maximum barrel length x external diameter)	70 x 9 mm.

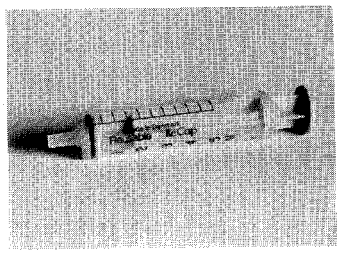
COMMENTS

Test Report: Sprima 82111 (1982), Wolff Lab (1988)
Meets WHO/UNICEF Standard E8/SS.2
Suitable for BCG immunization of infants (0.05ml.).
Syringes delivered peel-packed; ETO sterilized.

1997 PRICES (FOB)

Shipping volume (60 boxes)	*0.032 m3*
Shipping weight (60 boxes)	*2.6 kgs*
Number of syringes per box	*10*
Minimum order:	*300 boxes*
Quantity/Price:	1 box/US$ 3.48

CODE PIS
E8/03



Sterilizable plastic syringe, 5.0 ml

COMPANY NAME AND ADDRESS

CODAN
Faergejev 4
DK-4970 Roedby
Denmark
Telephone: *45 (54) 607 411*
Fax: *45 (54) 605 101*
Telex: *40 728 ASIK DK*

SPECIFICATIONS

Vaccine capacity	5.0 ml. x 0.5
Graduations	0.2 ml or 0.5 ml
Material of the syringe	Polypropylene
Needle fixing	Luer
Recommended needle	18G 50 mm.
Max. number of sterilizations	200
External dimensions (i.e. maximum barrel length x external diameter)	88 x 14 mm.

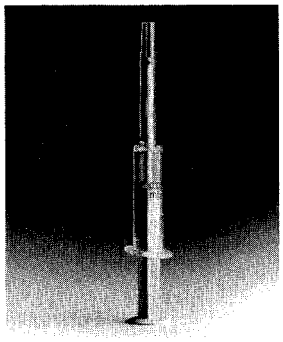
COMMENTS

Test Summary: CCIS 83.7 & 80.6
Meets WHO/UNICEF Standard E8/SS.3.
This is a mixing syringe.
Syringes delivered peel-packed; ETO sterilized.

1997 PRICES (FOB)

Shipping volume (16 boxes)	*0.106 m3*
Shipping weight (16 boxes)	*12.0 kgs*
Number of syringes per box	*100*
Minimum order :	*48 boxes*
Quantity/Price:	US$ 0.259 per syringe

CODE
PIS
E8/04



Sterilizable plastic syringe, 0.05 ml
ref.: 0098NC

COMPANY NAME AND ADDRESS

Sedat S.A.
135 route Neuve
69540 Irigny
France
Telephone: *33 72 39 74 14*
Fax: *33 78 51 89 67*

SPECIFICATIONS

Vaccine capacity	0.05 ml.
Graduations	0.05 only
Material of the syringe	Polypropylene
Needle fixing	Luer
Recommended needle	25 G 10 mm.
Max. number of sterilizations	200
External dimensions (i.e. maximum barrel length x external diameter)	70 x 8 mm.

COMMENTS

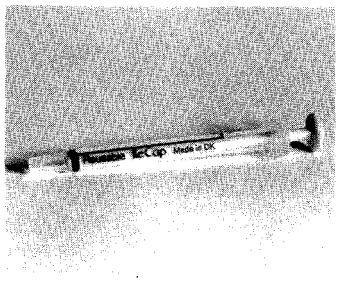
Test Report:SPRIMA 750300 (1986)
Meets WHO/UNICEF Standard E8/SS.2.
Suitable for BCG immunization.
ETO sterile packed.

1997 PRICES (FOB)

Shipping volume (330 boxes)	*0.18 m3*
Shipping weight (330 boxes)	*15.5 kgs*
Number of syringes per box	*10*
Minimum order :	*330 boxes*

Price per box of 10 syringes: XEU 1.69 (US$ 2.08)

CODE
PIS
E8/05



Sterilizable plastic syringe, 0.5 ml

COMPANY NAME AND ADDRESS

CODAN
Faergejev 4
DK-4970 Roedby
Denmark
Telephone: *45 (5460) 5035*
Fax: *45 (5460) 5101*
Telex: *40 728 ASIK DK*

SPECIFICATIONS

Vaccine capacity	0.5 ml.
Graduations	0/0.5
Material of the syringe	Polypropylene
Needle fixing	Luer
Recommended needle	22G 32 mm.
Max. number of sterilizations	200
External dimensions (i.e. maximum barrel length x external diameter)	78 x 6 mm.

COMMENTS

Test Report: SPRIMA 750900 (1986)
Meets WHO/UNICEF Standard E8/SS.1.
Suitable for measles and DPT immunization of infants and tetanus immunization of mothers.
Syringes delivered peel-packed; ETO sterilized.

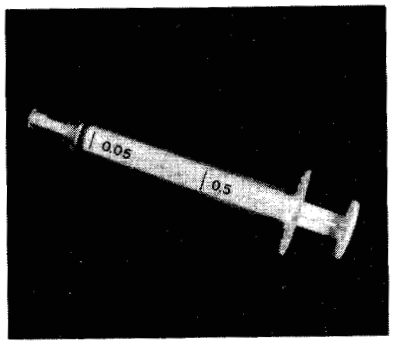
1997 PRICES (FOB)

Shipping volume (320 boxes)	*0.106 m3*
Shipping weight (320 boxes)	*13 kgs*
Number of syringes per box	*10*
Minimum order:	*320 boxes*

Price per box of 10 syringes US$ 3.26

160

CODE
PIS
E8/06



Sterilizable plastic syringe, 0.5 ml
ref.: 0123NA

COMPANY NAME AND ADDRESS

Sedat S.A.
135 route Neuve
69540 Irigny
France
Telephone: *33 4 72 39 74 14*
Fax: *33 4 78 51 89 67*

SPECIFICATIONS

Vaccine capacity	0.5 ml.
Graduations	0/0.5
Material of the syringe	Polypropylene
Needle fixing	Luer
Recommended needle	22G 32 mm.
Max. number of sterilizations	200
External dimensions (i.e. maximum barrel length x external diameter	60 x 7 mm.

COMMENTS

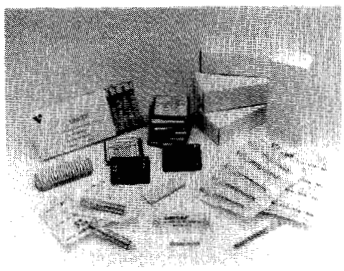
Test Report: BSI 143193 & Sprima 052200 (1990)
Meets WHO/UNICEF Standard E8/SS.1.
Suitable for measles and DPT immunization of infants and tetanus immunization of mothers.
ETO sterile packed.

1997 PRICES (FOB)

Shipping volume (330 boxes)	*0.18 m3*
Shipping weight (330 boxes)	*15.5 kgs*
Number of syringes per box	*10*
Minimum order:	*330 boxes*

Price per box of 10 syringes XEU 1.42 (US$ 1.75)

CODE
PIS
E8/08



Sterilizable syringe Kit B

COMPANY NAME AND ADDRESS

UNICEF (Denmark)
UNICEF Plads
Freeport, DK 2100 Copenhagen 0
Denmark
Telephone: *45 35 27 35 27*
Fax: *45 35 26 94 21*
Telex: *19813 unicef dk*

CONTENTS OF THE KIT

Item	Quantity	Stock no.
Syringes, 0.05 ml.	2 boxes (of 10)	07 822 15
Syringes, 0.5 ml	7 boxes (of 10)	07 822 20
Syringes, 5.0 ml	8 syringes	07 819 05
Needles, 10 mm, 26 gg.	4 boxes (of 12)	07 515 02
Needles, 26 mm, 23 gg.	8 boxes (of 12)	07 507 00
Needles, 32 mm, 22 gg.	4 boxes (of 12)	07 505 00
Needles, 76 mm, 18 gg.	1 box (of 12)	07 488 50
Ampoule files, 50 mm	2	05 050 00
Nail brush	2	05 140 00

COMMENTS

Test Report: —
(No WHO/UNICEF Standard exists)

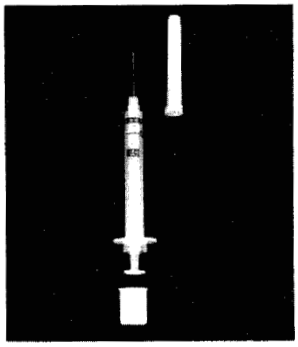
1997 PRICES (FCA)

Shipping weight / volume	*1.3 kgs / 0.006 m3*
Shipping dimensions	*300x200x100 mm*
External materials (package)	*cardboard box**
Minimum order	*1 kit*
Unicef Stock Number	*99 071 00*

Quantity/Price: 1 kit/US$ 32.50

* Overpacked for distribution in triple wall carton.

CODE
PIS
E8/09



Autodestruct syringe, 0.5 ml Soloshot (TM)

COMPANY NAME AND ADDRESS

Becton Dickinson & Company, USA
c/o H. K. Schueler International Ltd.
432 Park Avenue South
New York, N.Y. 10016-8013
United States of America
Telephone: *1 (212) 725 5800*
Fax: *1 (212) 725 2260*
E-mail: *info@hkschueler.com*

SPECIFICATIONS

Vaccine capacity	0.5 ml.
Graduations	0.5 ml
Material of the syringe	Polypropylene
Fixed needle	23G x 25 mm.
Prevented from re-use by	locked/trapped piston

COMMENTS

Test Report: Sprima 951700 (1989)
Meets WHO/UNICEF Standard E8/DS.1
Needle cap and cap over thumb plate make syringe into a sterile unit.
New yellow ring seal : provides of prior opening and use.

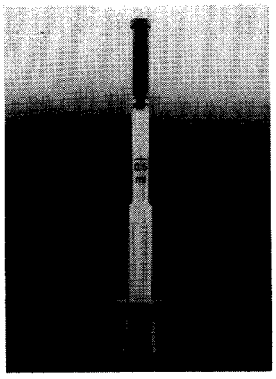
1997 PRICES (FOB)

Shipping volume	*0.0078cbm*
Shipping weight	*1.1. kgs*
Number of syringes per shelfpack	*100*
Number of syringes per carton	*500*
Minimum order:	*500*

Price per shelfpack of 100 syringes: US$ 9.25

162

CODE
PIS
E8/10



Autodestruct syringe, 0.5 ml: DestroJect

COMPANY NAME AND ADDRESS

Bader & Partner Vertriebsgesellschaft mbH
Havelstrasse 1-3
D-24539 Neumuenster
Germany
Telephone: *49 (43) 21 88 00 88*
Fax: *49 (43) 21 81 85 5*

SPECIFICATIONS

Vaccine capacity	0.5 ml.
Graduations	0.5 ml
Material of the syringe	Polypropylene
Fixed needle	23g x 25mm
Prevented from re-use by	locked/trapped piston

COMMENTS

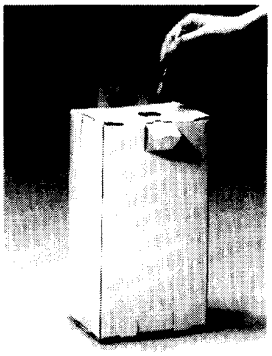
Test Report: Sprima 051400 (1990)
Meets WHO/UNICEF Standard E8/DS.1
Needle cap and cap over thumb plate make syringe into a sterile unit.Supplied and packaged in SyringeSafe (E10/08) triple purpose combustion container.

1997 PRICES (EX WORKS)

Shipping volume (20 containers)	*0.1 m3*
Shipping weight (20 containers)	*12.8 kgs*
Number of syringes per container	*100*
Minimum order:	*30 containers*

Price per container of 100 syringes	DEM 16.42	(US$ 10.39)

CODE
PIS
E10/13



Safety box for the disposal of used syringes and needles, 5 L

COMPANY NAME AND ADDRESS

Pa-Hu OY
Vattuniemenkatu 25
00 210 Helsinki
Finland
Telephone: *358 (0) 673 145*
Fax: *358 (0) 674 818*

SPECIFICATIONS

Volume	5 litres
Capacity	140 autodestr. syr. of 0.5 ml with needles
External dimensions	
Before assembling	590x283x5 mm.
After assembling	290x162x125 mm
Materials	carton
Thickness of walls	1.1-4.4 mm
Weight fully assembled	285.7 grs
Diam. syringe insert hole	34 mm

COMMENTS

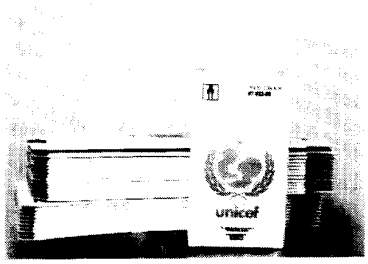
Test Report: Force Institute 59590/m1460-125 (1996)
Meets WHO/UNICEF Standard E10/IC2..
Box also available in 20 litres version. (500 syringes). The latter failed the tumble test (several needles pierced through the walls when the box was dropped from 60-80 cms).

1997 PRICES (FOT Helsinki)

Shipping weight/vol (25 pcs) *9 kgs / 0.022 m3*
Minimum order : *1000*

Quantity/Price: 5 litres box 1/US$ 1.00
20 litres box 1/US$ 2.15

CODE
PIS
E10/14



Safety box for the disposal of used syringes and needles, 5 L

COMPANY NAME AND ADDRESS

POLYNOR
Tordenskjoldsgt. 13-15
PO Box 13
N-2801 Gjovik
Norway
Telephone: *47 (61) 170 180*
Fax: *47 (61) 170 181*

SIZE AND DESCRIPTION

Volume	5 litres
Capacity	122 autodestr. syr. of 0.5 ml with needles
External dimensions	
Before assembling	585x285x5mm
After assembling	285x160x130mm
Materials	recycled board
Thickness of walls	1.5-6.0 mm
Weight fully assembled	343.0 grs
Diam. syringe insert hole	30mm

COMMENTS

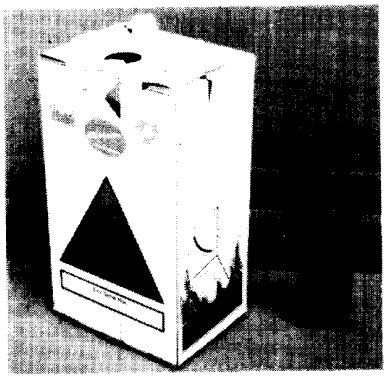
Test Report: Force Institute 65788/m1460-146 (1996)
Meets WHO/UNICEF Standard E10/IC.2.
Directions for use on the box in English, French, Spanish and Arabic.
Price includes the printing of client's logo on the box.
Boxes of 25 pcs. flat packed in robust water resistant outer carton.
Consult manufacturer for nearest distributor.

1997 PRICES (FOB)

Shipping weight / vol (25 pcs) *9.5 kgs / 0.03 m3*
Minimum order : 1900 (2 weeks delivery time; smaller quantities from UNICEF)

Quantity	Price/unit		
1900	1/NOK 10.89	(US$ 1.69)	
other qty	consult manufacturer		

CODE PIS E10/15



Safety box for the disposal of used syringes and needles, 5 L

COMPANY NAME AND ADDRESS

CIP Industries
PO Box 1782
Randburg 2125
South Africa
Telephone: *27 (11) 793 4420*
Fax: *27 (11) 793 5712*

SIZE AND DESCRIPTION

Volume	5 litres
Capacity	117 autodestr. syr. of 0.5 ml with needles
External dimensions	
Before assembling	585x290x7 mm
After assembling	295x165x130mm
Materials	carton plastic
Thickness of walls	
external layer	1.6 mm
internal lining	0.8 mm
Weight fully assembled	301.0 grs
Diam. syringe insert hole	35 mm

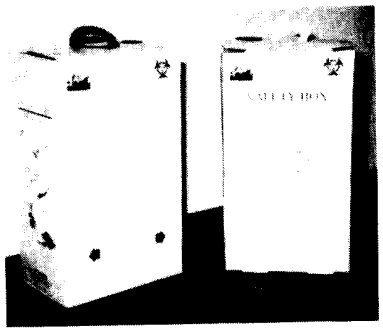
COMMENTS

Test Report: Force Institute 65065/m1460-142.a (1996).
Meets WHO/UNICEF Standard E10/IC.2
Boxes are equipped with a plastic strap as handle, strong enough to withstand the weight of a fully loaded container.
The box is also available in 10 L version (*US$ 1.74*), equally approved by WHO/UNICEF.

1997 PRICES (EX WORKS)

Shipping weight/vol (300 pcs, 1 pallet)	*99 kgs/0.53m3*
Shipping dimensions	*896x610x970 mm*
Minimum order :	*1200 (4 pallets)*
Price/unit	US$ 1.09

CODE PIS E10/16



Safety box for the disposal of used syringes and needles, 5 L

COMPANY NAME AND ADDRESS

Danapak Cartons Ltd
Hill Close
Lodge Farm Industrial Estate
Harlestone Road
Northhampton NN5 7UD
United Kingdom
Telephone: *44(1604) 754 831*
Fax: *44 (1604) 751 569*

SIZE AND DESCRIPTION

Volume	5 litres
Capacity	110 autodestr. syr. of 0.5 ml with needles
External dimensions	
Before assembling	577x283x5 mm
After assembling	285x160x125mm
Materials	carton
Thickness of walls	0.74-2.96 mm
Weight fully assembled	257.0 grs
Diam. syringe insert hole	36mm

COMMENTS

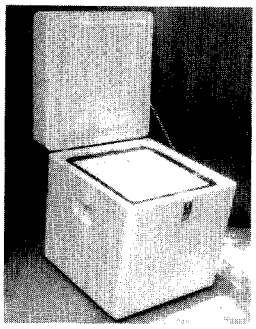
Test Report: Force Institute 65541/m1460-144 (1996).
Meets WHO/UNICEF Standard E10/IC.2
Boxes are equipped with a one finger handle, strong enough to withstand the weight of a fully loaded container.

1997 PRICES (EX WORKS)

Shipping weight / vol (25 pcs)	*10 kgs / 0.02 m3*
Shipping dimensions	*490x140x295*
Minimum order :	*consult manufacturer*
Price/unit	GB£ 0.49 (US$ 0.76)
Large volumes	consult manufacturer

CODE
PIS
E4/10

With CFC



Small vaccine cold box, short range: Model CB/INO/C2/90

COMPANY NAME AND ADDRESS

Hinardi Djajahardja
Jl. Klingkit 3 No 86
Complex Bojong Indah
Jakarta 11740
Indonesia
Tel. / Fax: 62 (21) 581 1531

SPECIFICATIONS

Vaccine storage capacity	6.5 litres
Weight fully loaded	20.6 kgs.
Weight empty	12.5 kgs.
External surface material	Fibreglass
Internal lining material	Fibreglass
Insulation material	Polyurethane
Insulation thickness	105 mm.
External dimensions	47x47x50 cms.
Internal dimensions	26x26x28 cms.
Vaccine storage dimensions	18x18x20 cms.
Lid type & fixings	Hinged
Number of icepacks required	6x0.6l+8x0.4l
Number of icepacks supplied	0
Icepack type	E5/IP.1,2
Robustness in drop test:	Fittings 2; Casing 4
Cold life without openings	+ hrs. at +32°C 81 hrs. at +43°C

COMMENTS

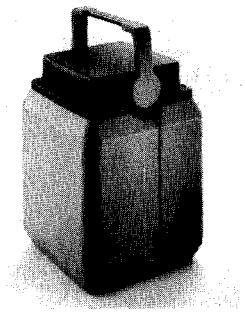
Test Reports: CATR.Z.9423 (1979); AIT/EP/91-4 (1991)
Meets WHO/UNICEF Standard E4/CB.4

1997 PRICES (EX WORKS)

Shipping weight/volume *37 kgs / 0.27 m3*
Minimum order 20

Quantity/Price: 1/US$ 175.00

CODE
PIS
E4/18-M



Large vaccine carrier: Model 3504 UN/CF

COMPANY NAME AND ADDRESS

The Thermos Company
1555 IL Route 75 East
PO Box 600
United States of America
Telephone: 1 (847) 240 3190
Fax: 1 (847) 240 3160

SPECIFICATIONS

Vaccine storage capacity	1.7 litres
Weight fully loaded	5.1 kgs.
Weight empty	2.5 kgs.
External surface material	Polyethylene
Internal lining material	Polyethylene
Insulation material	Polyurethane
Insulation thickness	40 mm.
External dimensions	24x24x33 cms.
Internal dimensions	15x15x19 cms.
Vaccine storage dimensions	10x10x18 cms.
Lid type & fixings	Removable
Number of icepacks required	4
Number of icepacks supplied	4
Icepack type	Thermos*
Robustness in drop test:	Fittings++Casing++
Cold life without openings	48 hrs. at +32°C 34 hrs. at +43°C
Cold life with openings	30 hrs at +32°C

COMMENTS

Test Reports: UNIVALLE C4-95-08 (1995)
Meets WHO/UNICEF Standard E4/VC.2
New model resistant to exposure to direct sunlight. Supplied with soft foam pad insert to hold 6 vaccine vials. Replacement foam pads available on request.

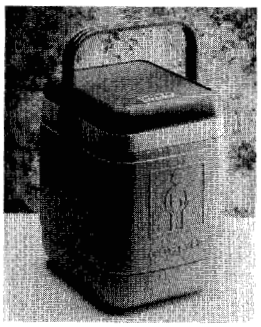
1997 PRICES (FCA USA SEAPORT)

Shipping weight/volume *kgs / m3*
Minimum order for direct shipment from factory: 120

Quantity/Price: 1/US$ 18.50

- *Icepacks supplied by Thermos do not meet WHO/UNICEF standards; may be purchased from Thermos at US$72.96 for 96.*

CODE
PIS
E4/52-M



Large vaccine carrier

COMPANY NAME AND ADDRESS

Quattro Elle Spa
Via Battaina 12
24059 Urgnano (Bergamo), Italy
Telephone: *39 (35) 419 2419*
Fax: *39 (35) 419 2400*

SPECIFICATIONS

Vaccine storage capacity	2.6 litres
Weight fully loaded	6.5 kgs.
Weight empty	1.8 kgs.
External surface material	Polypropylene
Internal lining material	Polypropylene
Insulation material	Polyurethane
Insulation thickness	25-35 mm.
External dimensions	32x27x25 cms.
Internal dimensions	24x18x22 cms.
Vaccine storage dimensions	15x15x12 cms.
Lid type & fixings	Removable
Number of icepacks required	8
Number of icepacks supplied	8
Icepack type	E5/10
Robustness in drop test:	Fittings 1; Casing 5
Cold life without openings	++ hrs. at +32°C 32 hrs. at +43°C

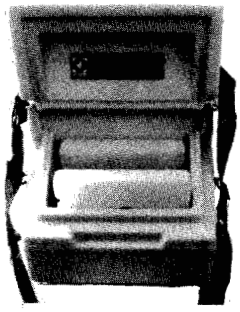
COMMENTS

Test Reports: UNIVALLE E4/3004 (1996).
Meets WHO/UNICEF Standard E4/VC.2 except for its fittings, that are too fragile (rating 1 instead of 2).
Supplied with soft foam insert to hold vaccine vials.
Includes instructions in English and French re correct packing.

1997 PRICES (EX WORKS)

Shipping weight/vol (8 pcs)	*20 kgs / 0.2 m3*		
Minimum order:	*4*		
Quantity/Price:	1/ITL	49 500	(US$ 32.14)
Replacement	1/ITL	800	(US$ .52)
Foam pads			

CODE
PIS
E4/53-M



Small vaccine carrier: Model RCW 2/CF (991.5902.31)

COMPANY NAME AND ADDRESS

Electrolux (Luxembourg)
14, op der Hei
L-9808 Hosingen
Luxembourg
Telephone: *352 920 731*
Fax: *352 920 731 49*
Telex: *3468 ELEX VI*

SPECIFICATIONS

Vaccine storage capacity	0.6 litres
Weight fully loaded	2.2 kgs.
Weight empty	1.3 kgs.
External surface material	Polyethylene
Internal lining material	Polyethylene
Insulation material	Polyurethane foamed with cyclopentane
Insulation thickness	30 mm.
External dimensions	25x15x21 cms.
Internal dimensions	19x 8x13 cms.
Vaccine storage dimensions	18x 5x 7 cms.
Lid type & fixings	Hinged
Number of icepacks required	2
Number of icepacks supplied	2
Icepack type	E5/08, 17
Robustness in drop test:	Fittings(5);Casing(5)
Cold life without openings	++ hrs. at +32°C (17) hrs. at +43°C

COMMENTS

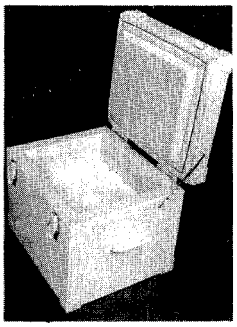
Test Reports: SGS 01.4708.01 (1987), Electrolux (1995)
Meets WHO/UNICEF Standard E4/VC.1

1997 PRICES (FOT Luxembourg)

Shipping weight/vol (1 pce)	*1.6 kgs /0.01 m3*		
Minimum order:	*1*		

Quantity	Price/unit		
1-39	XEU	37.00	(US$ 45.68)
40-99	XEU	35.00	(US$ 43.21)
100+	XEU	33.00	(US$ 40.74)

CODE
PIS
E4/57-M



Small vaccine cold box, short range: Model 55-CF

COMPANY NAME AND ADDRESS

Blow Kings
53 C Mittal Court
Nariman Point, Bombay 400021
India
Telephone: *91 (22) 284 0120/284 0393*
Fax: *91 (22) 283 1412 / 283 652 462*
E-mail: *mkothari@gias01.vsnl.net.in*

SPECIFICATIONS

Vaccine storage capacity	8.6 litres
Weight fully loaded	(22.5) kgs.
Weight empty	(9.5) kgs.
External surface material	Plastic
Internal lining material	Plastic
Insulation material	Polyurethane
Insulation thickness	55 mm.
External dimensions	46x39x41 cms.
Internal dimensions	34x28x26 cms.
Vaccine storage dimensions	++ cms.
Lid type & fixings	Hinged
Number of icepacks required	24
Number of icepacks supplied	24
Icepack type	E5/02, 12
Robustness in drop test:	Fittings 2; Casing (5)
Cold life without openings	++ hrs. at +32°C (58) hrs. at +43°C

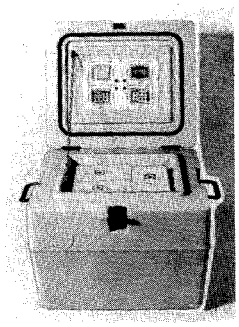
COMMENTS

Test Reports: Blow Kings (1996)
Meets WHO/UNICEF Standard E4/CB.4
Supplied with icepack E5/12 of 0.3 litre. Cold life is 65 hrs when E5/02 (0.4 litre) is used.

1997 PRICES (FOB)

Shipping weight/vol (1 pce)	*10 kgs/0.083 m3*
Minimum order	*1*
Quantity/Price:	1/US$ 80.00

CODE
PIS
E4/62-M



Small vaccine cold box, long range Model RCW 12/CF (991 7701 01)

COMPANY NAME AND ADDRESS

Electrolux (Luxembourg)
14, op der Hei
L-9808 Hosingen
Luxembourg
Telephone: *352 920 731*
Fax: *352 920 731 49*
Telex: *3468 ELEX VI*

SPECIFICATIONS

Vaccine storage capacity	8.9 litres
Weight fully loaded	(21) kgs.
Weight empty	(11.7) kgs.
External surface material	Polyethylene
Internal lining material	Polyethylene
Insulation material	Polyurethane foamed with cyclopentane
Insulation thickness	(105-115) mm.
External dimensions	50x55x47 cms.
Internal dimensions	27x34x26 cms.
Vaccine storage dimensions	19x26x18 cms.
Lid type & fixings	Hinged
Number of icepacks required	14
Number of icepacks supplied	14
Icepack type	E5/09
Robustness in drop test:	Fittings(2);Casing(4)
Cold life without openings	(155) hrs. at +32°C (93) hrs. at +43°C

COMMENTS

Test Reports: CATR.A.9209 (1987) & SGS 01.4708.02(1987), Electrolux (1995)
Meets WHO/UNICEF Standard E4/CB.1

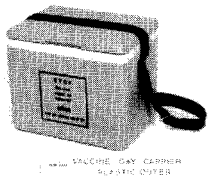
1997 PRICES (FOT Luxembourg)

Shipping weight/volume	*(15.6 kgs /1.5) m3*
Minimum order:	*1*

Quantity	Price/unit			
1-39	XEU	314.00	(US$	387.65)
40-99	XEU	298.00	(US$	367.90)
100+	XEU	283.00	(US$	349.38)

CODE
PIS
E4/68

With CFC



Small vaccine day carrier: Model IVC-4

COMPANY NAME AND ADDRESS

Inalsa Limited
Surya Kiran, 19 Kasturba Gandhi Marg
New Delhi, 110 001 India
Telephone: *91 (11) 331 4214 / 372 2560*
Fax: *91 (11) 371 2108/ 332 0502*
E-mail: *inalsa.edp@elnet.ems.vsnl.in*

SPECIFICATIONS

Vaccine storage capacity	0.85 litres
Weight fully loaded	2.2 kgs.
Weight empty	1.4 kgs.
External surface material	HDPE
Internal lining material	HIP
Insulation material	Polyurethane
Insulation thickness	35/30 mm.
External dimensions	25x18x21 cms.
Internal dimensions	17x10x12 cms.
Vaccine storage dimensions	17x10x5 cms.
Lid type & fixings	Removable
Number of icepacks required	2
Number of icepacks supplied	2
Icepack type	E5/15
Robustness in drop test:	Fittings 3; Casing 4
Cold life without openings	++ hrs. at +32°C 19 hrs. at +43°C

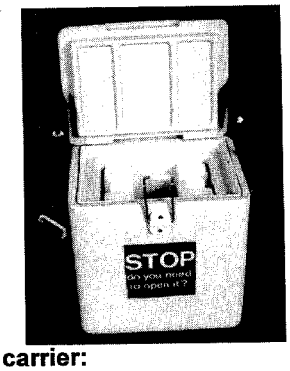
COMMENTS

Test Report: SISIR I 13849/F, January 1989
Meets WHO/UNICEF Standard E4/VC.1
CFC-free model under development.

1997 PRICES (FOB)

Shipping weight/vol (6 pcs)	*10 kgs/0.07 m3*
Minimum order:	*6*
Quantity/Price:	1/US$ 9.20

CODE
PIS
E4/69-M



Small vaccine carrier: Model VDC-24-CF

COMPANY NAME AND ADDRESS

Blow Kings
53 C Mittal Court
Nariman Point
Bombay 400 021, India
Telephone: *91 (22) 284 0120/284 0393*
Fax: *91 (22) 283 1412 / 283 652 462*
E-mail: *mkothari@gias01.vsnl.net.in*

SPECIFICATIONS

Vaccine storage capacity	0.9 litres
Weight fully loaded	2.2 kgs.
Weight empty	1.1 kgs.
External surface material	Plastic
Internal lining material	Plastic
Insulation material	Polyurethane
Insulation thickness	23/35 mm.
External dimensions	22x15x25 cms.
Internal dimensions	13x10x17 cms.
Vaccine storage dimensions	6x10x17 cms.
Lid type & fixings	Removable
Number of icepacks required	2
Number of icepacks supplied	2
Icepack type	E5/12
Robustness in drop test:	Fittings 3; Casing 4
Cold life without openings	++ hrs. at +32°C 15.6 hrs. at +43°C

COMMENTS

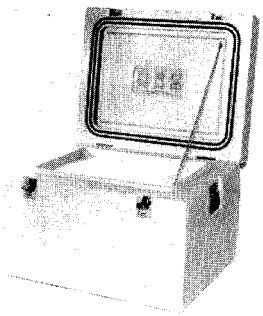
Test Reports: CRL A.11622 (1996)
Meets WHO/UNICEF Standard E4/VC.0

1997 PRICES (FOB)

Shipping weight/vol (12 pcs)	*15 kgs/ 0.12 m3*
Minimum order:	*1*
Quantity/Price:	1/US$ 10.65

CODE
PIS
E4/72

With
CFC



Large vaccine cold box, long range: Model ICB-7

COMPANY NAME AND ADDRESS

Inalsa Limited
Surya Kiran, 19 Kasturba Gandhi Marg
New Delhi, 110 001 India
Telephone: *91 (11) 331 4214 / 372 2560*
Fax: *91 (11) 371 2108/ 332 0502*
E-mail: *inalsa.edp@elnet.ems.vsnl.in*

SPECIFICATIONS

Vaccine storage capacity	25.6 litres
Weight fully loaded	43.2 kgs.
Weight empty	15.4 kgs.
External surface material	LLDPE
Internal lining material	LLDPE
Insulation material	Polyurethane
Insulation thickness	100 mm.
External dimensions (WxDxH)	77x62x52 cms.
Internal dimensions (WxDxH)	53x38x27 cms.
Vaccine storage dimensions	46x31x18 cms.
Lid type & fixings	Hinged
Number of icepacks required	50
Number of icepacks supplied	50
Icepack type	E5/15
Robustness in drop test:	Fittings 2; Casing 3
Cold life without openings	++ hrs. at +32°C 184 hrs. at +43°C

COMMENTS

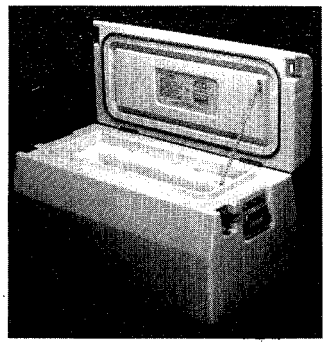
Test Report: SISIR B 00087/TCH/LSG (1989) & B 000377/NTC/TCH (1990).
Meets WHO/UNICEF Standard E4/CB.2
CFC-free model under development.

1997 PRICES (FOB)

Shipping weight/vol (1 pce)	*26.5 kgs/0.28 m3*
Minimum order	*1*
Quantity/Price:	1/US$ 156.00

CODE
PIS
E4/75

With
CFC



Small vaccine cold box, long range: Model ICB-5

COMPANY NAME AND ADDRESS

Inalsa Limited
Surya Kiran, 19 Kasturba Gandhi Marg
New Delhi, 110 001 India
Telephone: *91 (11) 331 4214 / 372 2560*
Fax: *91 (11) 371 2108/ 332 0502*
E-mail: *inalsa.edp@elnet.ems.vsnl.in*

SPECIFICATIONS

Vaccine storage capacity	5 litres
Weight fully loaded	23.5 kgs.
Weight empty	10.8 kgs.
External surface material	LLDPE
Internal lining material	LLDPE
Insulation material	Polyurethane
Insulation thickness	100 mm.
External dimensions (WxDxH)	79x41x43 cms.
Internal dimensions (WxDxH)	58x18x20 cms.
Vaccine storage dimensions	50x10x10 cms.
Lid type & fixings	Hinged
Number of icepacks required	26
Number of icepacks supplied	26
Icepack type	E5/15
Robustness in drop test:	Fittings 3; Casing 4
Cold life without openings	++ hrs. at +32°C 117 hrs. at +43°C

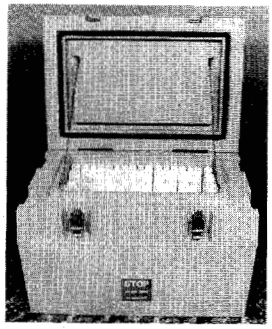
COMMENTS

Test Report: SISIR B 00086/TCH/LSG (1989) & I 14064A (1989)
Meets WHO/UNICEF Standard E4/CB.1
CFC-free model under development.

1997 PRICES (FOB)

Shipping weight/vol (1 pce)	*16.5 kgs/0.17 m3*
Minimum order	*1*
Quantity/Price:	1/US$ 102.00

CODE
PIS
E4/76-M



Small vaccine cold box, long range: Model CB/20/5U -CF

COMPANY NAME AND ADDRESS

Blow Kings
53 C Mittal Court
Nariman Point, Bombay 400 021
India
Telephone: *91 (22) 284 0120/284 0393*
Fax: *91 (22) 283 1412 / 283 652 462*
E-mail: *mkothari@gias01.vsnl.net.in*

SPECIFICATIONS

Vaccine storage capacity	20 litres
Weight fully loaded	(48.9) kgs.
Weight empty	(20.7) kgs.
External surface material	Plastic
Internal lining material	Plastic
Insulation material	Polyurethane
Insulation thickness	110 mm.
External dimensions	77x53x54 cms.
Internal dimensions	54x30x31 cms.
Vaccine storage dimensions	48x24x17 cms.
Lid type & fixings	Hinged
Number of icepacks required	52
Number of icepacks supplied	52
Icepack type	E5/12
Robustness in drop test:	Fittings 2;Casing 5
Cold life without openings	++ hrs. at +32°C (159) hrs. at +43°C

COMMENTS

Test Report: Blow kings (1996)
Meets WHO/UNICEF Standard E4/CB1
Supplied with icepack E5/12 of 0.3 litre. Cold life is 181 hrs when E5/02 (0.4 litre) is used.

1997 PRICES (FOB)

Shipping weight/vol (1pce)	*20 kgs/0.24 m3*
Minimum order	
Quantity/Price:	1/US$ 176.00

CODE
PIS
E4/77-M

Large vaccine carrier: Model VC/42/MOD/2/CF

COMPANY NAME AND ADDRESS

Blow Kings
53 C Mittal Court
Nariman Point, Bombay 400 021
India
Telephone: *91 (22) 284 0120/284 0393*
Fax: *91 (22) 283 1412 / 283 652 462*
E-mail: *mkothari@gias01.vsnl.net.in*

SPECIFICATIONS

Vaccine storage capacity	1.3 litres
Weight fully loaded	3.7 kgs.
Weight empty	1.8 kgs.
External surface material	Plastic
Internal lining material	Plastic
Insulation material	Polyurethane
Insulation thickness	40 mm.
External dimensions	25x25x28 cms.
Internal dimensions	16x16x17 cms.
Vaccine storage dimensions	9x9x17 cms.
Lid type & fixings	Removable
Number of icepacks required	4
Number of icepacks supplied	4
Icepack type	E5/12
Robustness in drop test:	Fittings 3;Casing 4
Cold life without openings	++ hrs. at +32°C 41 hrs. at +43°C

COMMENTS

Test Reports:CRL A.11622 (1996)
Meets WHO/UNICEF Standard E4/VC.2
Supplied with soft foam insert to hold vaccine vials. Replacement foam pads on request.

1997 PRICES (FOB)

Shipping weight/vol (8 pcs)	*20 kgs/0.16 m3*
Minimum order	*100*
Quantity/Price:	1/US$ 13.15

PART VII - PROCUREMENT UNDER WORLD BANK PROCEDURES

A. What the Bank Is 828
B. What the Bank Does 828
C. General Conditions of a World Bank Loan 828
D. How Bank Projects Are Developed 829
E. How Bank Loans Are Linked to Bank Projects 829
F. How Bank Projects Are Implemented 830
G. How Bank Projects Are Monitored 830
H. What the Bank Usually Monitors in the Area of Procurement 830
I. Why the Bank is Concerned About Procurement Methods and Procedures 831
J. General Timeframes Associated With World Bank Projects 831
K. Procurement Guidelines and Standard Bidding Documents 831
L. Special Issues of Vaccine Procurement Under World Bank Projects 832
M. Special Wording for Vaccine Procurement Under World Bank Projects 832

PART VII - Procurement Under World Bank Procedures

As governments move from donor commodity support to financial independence, they are increasingly purchasing critical medical supplies with the financial assistance of the World Bank. Because the World Bank is not a donor but a UN organisation dealing in loans and credits that have covenants, terms and conditions linked to project implementation, it is important for procurement personnel and senior programme managers to understand how the "Bank" works.

A. What the Bank Is

The World Bank consists of two organisations, operating as a single administrative unit, with shared offices and personnel:

- IBRD (International Bank for Reconstruction and Development)
- IDA (International Development Association)

B. What the Bank Does

The World Bank provides loans and credits to promote economic and social progress in developing member countries.

- It lends funds to finance projects that promote economic and social progress.
- It provides advice related to the projects it finances.
- It serves as a catalyst to stimulate investments by others with similar objectives.
- IBRD makes loans to creditworthy borrowers in developing member countries.
- IDA provides credits (soft loans) to the poorer developing countries.

C. General Conditions of a World Bank Loan

- Each loan must be made to, or be guaranteed by, the government concerned.
- The developing-country borrower provides part of the financing for each project from its own resources. In many cases, other lenders and donors also provide assistance.

- The proceeds of a Bank loan must be used:
  - o*nly* for the purposes for which the loan was granted
  - with due attention to considerations of economy and efficiency
  - without regard to political or other non-economic influences or considerations
  - primarily to meet the foreign exchange costs of projects (because most developing countries have an acute shortage of foreign currencies)

D. How Bank Projects Are Developed

Bank projects are developed in close collaboration with the national government and local agencies, and often with other organisations pursuing objectives similar to the Bank's.

- The ***Borrower*** is primarily responsible for proposing and preparing the project, although the Bank, UN agencies and outside consultants frequently assist, particularly when the necessary expertise is unavailable locally.

- Once a project has been identified and is considered feasible, it is formulated by examining the economic, financial and technical requirements and the likelihood of their being realised, and determining what features and conditions will be necessary to ensure the project's success.

- The Bank carries out an appraisal with the assistance of the Borrower and, on occasion, other donors to provide a basis for the decision to support the project with a loan (Staff Appraisal Report).

E. How Bank Loans Are Linked to Bank Projects

Bank loans and Bank projects are linked by the Loan Agreement (IBRD), or the Development Credit Agreement (IDA).

- Formal negotiations between the Bank and the Borrower follow project appraisal. These result in a legal agreement (IDA Development Credit Agreement; IBRD Loan Agreement) between the borrower and the Bank that precisely defines the project and specifies the program to meet its objectives.

- The agreement includes:
  - financial terms and conditions
  - schedule of items to be financed by the Bank
  - procurement methods to be used (usually unrestricted bidding: ICB)
  - covenants on audit, revenue levels, organisational changes, project monitoring and any other points requiring special attention
  - schedule of procurement decisions that are subject to the Bank's review

F. How Bank Projects Are Implemented

- The ***Borrower*** is responsible for implementing projects financed by the Bank, and for satisfying the Bank that the project is being carried out properly and will likely achieve the objectives of the loan.

- The ***Borrower*** is legally responsible for the procurement. The Borrower invites, receives and evaluates bids and awards the contract. The contract is between the Borrower and the Supplier; the Bank is not a party to the contract.

- The ***Borrower*** is solely responsible for the payment of goods, works and services contracted under the project. The Bank, for its part, is required to ensure that funds are paid from a Bank loan only as expenditures are incurred.

- Where Borrowers lack the necessary organisation, resources and experience, they may wish (or be required by the Bank) to employ, as their agent, a firm specialised in handling international procurement.
  - ◊ The agent must strictly follow all the procurement procedures outlined in the Loan Agreement on behalf of the Borrower, including the use of Bank Standard Bidding Documents, review procedures and documentation
  - ◊ This also applies in cases where UN agencies act as procurement agents

G. How Bank Projects Are Monitored

- The Bank supervises the project and reviews its progress regularly to ensure that the Borrower is spending its funds as agreed.

- The Bank assigns a Task Manager for each project who is responsible for reviewing procurement decisions.

- The Borrower reports to the Bank periodically on the project's progress.

H. What the Bank Usually Monitors in the Area of Procurement

- Who purchases the goods
- Methods and procedures used to purchase the goods
- Scheduling and grouping similar items into appropriate "packages" for procurement
- Bidding documents
- Selection of suppliers
- Contract wording
- Contract enforcement

I. Why The Bank Is Concerned About Procurement Methods and Procedures

Four considerations generally guide the Bank's requirements:

- The need for economy and efficiency in the implementation of the project, including the procurement of the goods and works involved.

- The Bank's interest, as a co-operative institution, in giving all eligible bidders from developed and developing member countries an opportunity to compete in providing goods and works financed with Bank funds.

- The Bank's interest, as a development institution, in encouraging the development of domestic contracting and manufacturing industries in the borrowing country.

- The importance of transparency in the procurement process—the Bank needs to be above any suspicion in the way it administers its loan agreements. To this end, it may need to rigidly adhere to the most restrictive clauses in its guidelines.

J. General Timeframes Associated With World Bank Projects

- Project formulation - 1 year

- Loan process - variable

- Project duration - 2 to 5 years

- Procurement under ICB rules - up to 18 months

K. Procurement Guidelines and Standard Bidding Documents

- ***Guidelines for Procurement under IBRD Loans and IDA Credits***

  The World Bank has developed and published this set of guidelines to inform those carrying out a project that is financed in whole or in part by an IBRD loan or an IDA credit of the arrangements to be made for procuring the goods and works required for the project.

- ***World Bank Standard Bidding Documents***

  In addition to the procurement rules and procedures specified in the Bank's "Guidelines" mentioned above and referenced in loan agreements, Borrowers are required to use appropriate Standard Bidding Documents issued by the Bank. These documents incorporate language and clauses that apply to all procurement. Information specific to individual purchases (for example, the name of the Purchaser) is introduced by filling in "Data Sheets" that precede the Instructions to Bidders section and the General Conditions of Contract sections of the documents. Clauses that are necessary to address

country- and project-specific issues are also introduced through Data Sheet entries, allowing the standard language to be left unaltered.

In September 1993 the Bank issued a trial edition of Standard Bidding Documents for Procurement of Pharmaceuticals and Vaccines; in 1995 it followed up with a "Corrigenda". These documents are currently in use, but are undergoing active review. Both are included in the Supplements at the end of Part VII.

Supplement VII.K.1., page 833
Supplement VII.K.2., page 930

L. Special Issues of Vaccine Procurement Under World Bank Projects

The Bank believes that, in most cases, its needs and interests can best be realised through properly administered International Competitive Bidding (ICB). On the other hand, it acknowledges that there are circumstances where ICB would not be the most economic and efficient method of procurement, and where other methods are more appropriate. The particular procurement methods and the categories of goods and works to which they apply are determined by agreement between the Bank and the Borrower, and are specified in the Loan Agreement.

Because ICB procedures are clearly not suited to the procurement of safe, efficacious vaccine, (see discussion in Introduction and Part I Options) it is important for the borrowing government to make sure that alternate procedures, particularly Limited International Bidding (LIB), are specified for vaccine procurement before the Loan Agreement is signed. Once the agreement is in place, it is very difficult to change.

M. Special Wording for Vaccine Procurement Under World Bank Projects[44]

A set of special clauses and data sheet entries consistent with the recommendations of this manual have been developed for use with the 1993 trial edition of Standard Bidding Documents and the 1995 Corrigenda referenced in Section K. above.

Supplement VII.M., page 939

These "special treatment" clauses assume the use of Limited International Bidding (LIB; i.e., restricted bidding) instead of International Competitive Bidding (ICB; advertised, i.e., unrestricted bidding), and put emphasis on pre-qualification of the vaccine rather than the vaccine supplier. Prospective bidders lists are limited to authorised suppliers of vaccines that have been licensed by the National Control Authority of the Purchaser's country. In countries where an NCA with appropriate biological expertise has not yet been established, an alternate approach is discussed in Section IV of this manual.

[44] For use with "Standard Bidding Documents for Procurement of Pharmaceuticals and Vaccines", September 1993.

STANDARD BIDDING DOCUMENTS

FOR

PROCUREMENT OF PHARMACEUTICALS AND VACCINES

THE WORLD BANK

Trial Edition
September 1993

STANDARD BIDDING DOCUMENT FOR PROCUREMENT OF PHARMACEUTICALS AND VACCINES

Trial Edition
September 1993

PREFACE

1. Procurement under projects financed by the International Bank for Reconstruction and Development (IBRD) and its affiliate, the International Development Association (IDA), (hereinafter interchangeably called "the World Bank") is carried out in accordance with policies and procedures laid down in the latest issue of the **Guidelines: Procurement under IBRD Loans and IDA Credits** (hereinafter called "The World Bank Guidelines for Procurement").

2. The purpose of this publication is to provide a tool to borrowers, World Bank Task Managers and other staff working on population and health sector projects to help them avoid the problems inherent in the procurement of pharmaceuticals, vaccines, contraceptives and similar supplies (collectively called "pharmaceuticals and vaccines" in these Standard Bidding Documents). The Standard Bidding Document (SBDs) themselves are drafted for use when international competitive bidding (ICB) procedures are employed. They are based on the World Bank's Sample Bidding Documents for Goods dated March 1986 modified to reflect the experience of the World Bank and its borrowers over 15 years with this kind of procurement as well as related technical literature produced by UN agencies such as WHO, UNICEF, etc. and when used correctly, fully comply with the World Bank Guidelines for Procurement. The contents have been discussed extensively with World Bank Task Managers, client Ministries of Health, interested UN agencies and representatives of the drug manufacturing industry. During a trial period from September 1, 1993 to June 30, 1994, their use is strongly recommended for all procurement of pharmaceuticals, vaccines and similar items under any World Bank-financed loan or credit. Following the trial period user comments will be considered, appropriate revisions made and an official version issued, the use of which will be mandatory, unless otherwise agreed.

3. In order to facilitate use by borrowers and speed up the World Bank review process, the format of the Instructions and General Conditions sections of the documents has been drafted in general terms applicable to all procurement so that all the data specific to each Invitation for Bids (IFB) can be isolated in "Data Sheets" that precede each section allowing the standard language to be left unaltered.

Trial Edition
September 1993

4. The Bank has also prepared a companion Procurement Technical Note (PTN) No. 4.3.2 to address in greater detail the various issues and problems typical in this particular kind of procurement. Bank and borrower staff who will be using these SBDs are encouraged to study the PTN before starting the preparation of Bidding Documents.

5. To obtain further information about procurement under World Bank-financed projects, contact:

Procurement Policy and Coordination Unit
Operations Policy Department
The World Bank
1818 H Street, N.W.
Washington, D.C. 20433, U.S.A.

The World Bank would also welcome any feedback or comments relating to the experience Borrowers and Bank staff have when using these documents.

Trial Edition
September 1993

Table of Contents

PART A	**PREFACE**	
PART B	**SAMPLE BIDDING DOCUMENTS**	
Section No.		**Page No.**
I.	INVITATION FOR BIDS (IFB)	1
II.	ITB DATA SHEET/INSTRUCTIONS TO BIDDERS (ITB)	6
III.	GCC DATA SHEET/SPECIAL CONDITIONS OF CONTRACT AND GENERAL CONDITIONS OF CONTRACT (GCC)	32
IV.	TECHNICAL SPECIFICATIONS	51
V.	SCHEDULE OF REQUIREMENTS	56
VI.	BIDDING AND CONTRACT FORMS AND ATTACHMENTS	57
	List of Forms	
1.	BID FORM	58
2.	PRICE SCHEDULE FOR ITEMS OFFERED LOCALLY	60
3.	PRICE SCHEDULE FOR ITEMS TO BE IMPORTED	61
4.	DOMESTIC VALUE ADDED CALCULATION FORM	62
5.	QUALIFICATION FORM	63
6.	LETTER OF AUTHORITY FROM MANUFACTURER	66
7.	BID SECURITY FORM	67
8.	PERFORMANCE SECURITY FORM	69
9.	SPECIMEN OF LETTER OF CREDIT ISSUED BY THE PURCHASER	70
10.	CONTRACT FORM	73

Trial Edition
September 1993

Table of Contents

Section No. Page No.

Attachments

1. WHO GUIDELINES FOR GOOD MANUFACTURING PRACTICES (GMP) AND CERTIFICATION SCHEME ON THE QUALITY OF PHARMACEUTICALS MOVING IN INTERNATIONAL COMMERCE 76

Trial Edition
September 1993

SECTION I. INVITATION FOR BID (IFB)

LOAN/CREDIT NO:_____ **IFB NO:**_____

PROJECT TITLE:_____

ISSUE DATE:_____

CLOSING DATE:_____

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September 1993

Section I. INVITATION FOR BIDS (IFB)[1] for the Procurement of Pharmaceuticals and Vaccines

Loan No.: _____ **Date:** _____

Project Name: _____ **IFB No.:** _____

1. The (Name of Borrower) has received[2] a loan from the World Bank in various currencies towards the cost of (Name of Project) and it is intended that part of the proceeds of this loan will be applied to eligible payments under this proposed contract.

2. The (Name of Purchaser) hereby invites sealed bids from primary manufacturers or their authorized representatives from member countries of the World Bank and Taiwan, China, for the supply of (give brief description of the pharmaceuticals and vaccines to be procured). A "primary manufacturer" is defined as a company that performs all the manufacturing and fabricating operations needed to produce pharmaceuticals or vaccines in their appropriate dosage forms, including processing, blending, formulating, filling, packing, labelling and quality testing. Bids may be submitted by an authorized agent for and on behalf of the primary manufacturer provided the bid is accompanied by a duly notarized letter of authority from the primary manufacturer authorizing the designated agent to bid solely for and on behalf of the primary manufacturer. Merchant exporters, pre-packers, shippers and traders are not classified as primary manufacturers and bids from them will not be accepted. Documentary evidence will be required of all bidders that they have qualifications, experience and capacity to be able to successfully complete the contract on time for the pharmaceuticals and vaccines offered.

3. Interested eligible Bidders may obtain further information from and inspect the bidding documents at the office of: (Name of Appropriate Purchasing Unit)[3], (Mailing Address of Appropriate Office for Enquiry and Issuance of Bidding Documents and Cable/Telex Numbers).

---

1 The IFB, while included as part of the Bidding Documents, is normally prepared separately and used as the advertisement initiating the ICB process.

2 Substitute "has applied for" if appropriate.

3 The office for enquiry and issuance of bidding documents and that for bid submission may or may not be the same.

4. A complete set of Bidding Documents may be purchased by any interested eligible Bidder on the submission of a written application to the above and upon payment of non-refundable fee of _____[4].

5. All bids must be accompanied by a security of not less than (Fixed Sum or Percentage of Bid Amount)[5] and must be delivered to the above office[6] on or before (Hours) on (Date).

6. Bids will be opened in the presence of Bidders' representatives who choose to attend at (Time and Date) at the offices of (_____).

---

[4] The fee chargeable should only be nominal to defray reproduction and mailing costs and to ensure that only bona fide Bidders will request for Bidding Documents. An amount of between US$200 and US$500 is deemed appropriate.

[5] If bid security is required insert appropriate information from "Instructions to Bidders," Clause 15. (Delete this paragraph if no bid security is required).

[6] Substitute the Purchaser's address for bid submission if different from its address for enquiry and issuance of Bidding Documents.

SECTION II - INSTRUCTIONS TO BIDDERS

SECTION II. INSTRUCTIONS TO BIDDERS

Table of Contents

Part	Clause No.:		Page No.
A.	ITB DATA SHEET		6
B.	INTRODUCTION		
	1.	Source of Funds	10
	2.	Eligible Bidders	10
	3.	Eligible Goods and Services	10
	4.	Cost of Bidding	11
C.	BIDDING DOCUMENTS		
	5.	Content of Bidding Documents	11
	6.	Clarification of Bidding Documents	12
	7.	Amendment of Bidding Documents	12
D.	PREPARATION OF BIDS		
	8.	Language of Bid	13
	9.	Documents Comprising the Bid	13
	10.	Bid Form	14
	11.	Bid Prices	15
	12.	Bid Currencies	16
	13.	Documents Establishing Bidder's Eligibility and Qualifications	17

Table of Contents (Cont'd.)

Part Clause No.: Page No.

D. PREPARATION OF BIDS (Cont'd.)

14. Documents Establishing Goods' Eligibility and Conformity to Bidding Documents 18
15. Bid Security 19
16. Period of Validity of Bids 20
17. Format and Signing of Bids 21

E. SUBMISSION OF BIDS

18. Sealing and Marking of Bids 21
19. Deadline for Submission of Bids 22
20. Late Bids 22
21. Modification and Withdrawal of Bids 23

F. BID OPENING AND EVALUATION

22. Opening of Bids by Purchaser 23
23. Clarification of Bids 23
24. Preliminary Examination 24
25. Conversion to Single Currency 25
26. Evaluation and Comparison of Bids 25
27. Domestic Preference 26
28. Contacting the Purchaser 27

Table of Contents (Cont'd.)

Part. Clause No.: Page No.

G. AWARD OF CONTRACT

29. Post-qualification 27
30. Award Criteria 28
31. Right to Vary Quantities at Time of Award 28
32. Right to Accept any Bid and to Reject any or all Bids 28
33. Notification of Award 28
34. Signing of Contract 29
35. Performance Security 29

Trial Edition
September 1993

IFB NO: _____

ITB DATA SHEET

Line No.	Clause Ref.	Listed Information
1.	ITB 1	The Borrower is: _____ Project Name: _____ Loan/Credit No: _____ Loan/Credit Amount (US$): _____
2.	ITB 4	Name of the Purchaser: _____ Address of the Purchaser: _____ _____ _____ Telephone No: _____ Fax No: _____
3.	ITB 5	The pharmaceuticals and vaccines covered by this ITB fall in the following general categories: _____ _____ _____ _____ _____ _____ _____ _____ (A detailed list is included in Part A of the Technical Specifications).
4.	ITB 8	The language of the bid shall be _____.

ITB DATA SHEET (Cont'd.)

Line No.	Clause Ref.	Listed Information
5.	ITB 12	The alternative international trading currency which Bidders may use to express their Bid Prices for imported Goods is _____.
6.	ITB 13.5	The following additional qualification criteria will also apply: _____ _____ _____ _____ The Bidder must be registered to supply the pharmaceuticals and vaccines offered in the country of the Purchaser. Yes___ No___
7.	ITB 14.3	A sample of the pharmaceutical(s) and vaccine(s) offered by the Bidder shall be submitted along with the bid in the following quantities: Pharmaceutical or Vaccine — Quantity _____ _____ _____ _____ _____ _____ _____ _____
8.	ITB 15.1	A bid security in the amount of not less than _____ (monetary amount and currency or percent of the Bid Price) must accompany the bid, provided the bid security amount is more than $10,000.

ITB DATA SHEET (Cont'd.)

Line No.	Clause Ref.	Listed Information
9.	ITB 16	The period of bid validity specified in SubClause 16.1 shall be _____ days. The factors A and B specified in SubClause 16.3 for possible adjustment of the foreign and local components of the Bid Price shall be: A=_____ B=_____.
10.	ITB 19	Bids shall be opened at _____ on _____, the same day as the last day for submission of bids, at the following location: _____ _____ _____ _____
11.	ITB 17	The bid shall be submitted in _____ copies.
12.	ITB 18.2	Bids shall be submitted to the Purchaser at the following address: _____ _____ _____ Telephone No: _____ Fax No.: _____
13.	ITB 19.1	The deadline for submission of bids is: _____ on _____, 19 ___.
14.	ITB 25	The Purchaser will convert all evaluated Bid Prices into _____ _____ for bid comparison purposes. The exchange rates used for this conversion shall be the selling rates in effect on_____

ITB DATA SHEET (Cont'd.)

Line No.	Clause Ref.	Listed Information
		established or published by _____ _____.

MODIFICATIONS TO THE INSTRUCTIONS TO BIDDERS[7]

15.	ITB _____.	_____ _____ _____
16.	ITB _____.	_____ _____ _____

[7] To be extended, if necessary.

Section II. INSTRUCTIONS TO BIDDERS

A. Introduction

1. Source of Funds:

1.1 The Borrower named in the ITB Data Sheet has applied for/received the loan/credit also identified in the ITB Data Sheet from the World Bank in various currencies equivalent to the US dollar amount given in the ITB Data Sheet towards the cost of the project described thereon. The Borrower intends to apply part of the proceeds of this loan to eligible payments under the Contracts for which this Invitation for Bids (IFB) is issued. Payment by the World Bank will be made only at the request of the Borrower and upon approval by the World Bank in accordance with the terms and conditions of the loan agreement and will be subject in all respects to the terms and conditions of that agreement. Except as the World Bank may specifically otherwise agree, no party other than the Borrower shall derive any rights from the loan agreement or have any claim to the loan proceeds.

2. Eligible Bidders:

2.1 This Invitation for Bids is open to all primary manufacturers and their agents from member countries of the World Bank and Taiwan, China.

3. Eligible Goods and Services:

3.1 All Goods and ancillary Services to be supplied under the Contract shall have their origin in member countries of the World Bank and Taiwan, China, and all expenditures made under the Contract will be limited to such Goods and Services.

3.2 For purposes of this clause, "origin" means the place where the Goods are mined, grown or produced or from which the ancillary Services are supplied. Goods are produced when, through manufacturing, processing or substantial and major assembling of components, a commercially recognized product results that is substantially different in basic characteristics or in purpose or utility from its components.

3.3 The origin of Goods and Services is distinct from the nationality of the Bidder.

4. Cost of Bidding:

4.1 The Bidder shall bear all costs associated with the preparation and submission of its bid, and the Purchaser which is named in the ITB Data Sheet will in no case be responsible or liable for those costs, regardless of the conduct or outcome of the bidding process.

B. The Bidding Documents

5. Content of Bidding Documents:

The Goods required, which are described in the ITB Data Sheet and the Technical Specifications, as well as the bidding procedures and Contract terms are prescribed in the Bidding Documents. In addition to the Invitation for Bids (IFB), the Bidding Documents include:

(a) Instructions to Bidders (ITB);
(b) General Conditions of Contract (GCC);
(c) Special Conditions of Contract (SCC);
(d) Technical Specifications;
(e) Schedule of Requirements;
(f) Domestic Value Added Form (Domestic Pharmaceuticals or Vaccines only);

(g) Bid Form;

(h) Price Schedule for Domestic or Previously Imported Pharmaceuticals or Vaccines;

(i) Price Schedule for Imported Pharmaceuticals or Vaccines;

(j) Contract Form;

(k) Bid Security Form;

(l) Performance Security Form;

The Bidder is expected to examine all instructions, forms, terms and specifications in the Bidding Documents. Failure to furnish all information required by the Bidding Documents or submission of a bid not substantially responsible to the Bidding Documents in every respect will be at the Bidder's risk and may result in the rejection of its bid.

6. Clarification of Bidding Documents:

6.1 A prospective Bidder requiring any clarification of the Bidding Documents may notify the Purchaser in writing or by telex or cable at the Purchaser's mailing address indicated in the ITB Data Sheet. The Purchaser will respond in writing to any request for clarification of the Bidding Documents which it receives no later than 30 days prior to the deadline for the submission of bids prescribed by the Purchaser. Written copies of the Purchaser's response (including an explanation of the query but without identifying its source) will be sent to all prospective Bidders which have received the Bidding Documents.

7. Amendment of Bidding Documents:

7.1 At any time prior to the deadline for submission of bids, the Purchaser may, for any reason, whether at its own initiative or in response to a clarification requested by a prospective Bidder, modify the Bidding Documents by amendment.

7.2 The amendment will be notified in writing or by telex or cable to all prospective Bidders which have received the Bidding Documents and will be binding on them.

7.3 In order to afford prospective Bidders reasonable time in which to take the amendment into account in preparing their bids, the Purchaser may, at its discretion, extend the deadline for the submission of bids.

C. Preparation of Bids

8. Language of Bids:

8.1 The bid prepared by the Bidder and all correspondence and documents relating to the bid exchanged by the Bidder and the Purchaser, shall be written in the language specified in the ITB Data Sheet provided that any printed literature furnished by the Bidder may be written in another language so long as accompanied by a translation of its pertinent passages in that same language in which case, for purposes of interpretation of the bid, the translation in the language given in the ITB Data Sheet shall govern.

9. Documents Comprising the Bid:

9.1 The bid prepared by the Bidder shall comprise the following components:

(a) a Bid Form and a Price Schedule completed in accordance with ITB Clauses 9, 10, 11 and 12;

(b) Documentary evidence established in accordance with ITB Clause 13 that the Bidder is eligible to bid and is qualified to perform the Contract if its bid is accepted;

(c) Documentary evidence established in accordance with ITB Clause 14 that the Goods and ancillary Services to be supplied by the Bidder are eligible Goods and Services and conform to the Bidding Documents; and

(d) Bid security furnished in accordance with ITB Clause 15.

10. Bid Form:

10.1 The Bidder shall complete the Bid Form and the appropriate Price Schedule furnished in the Bidding Documents, indicating for the Goods to be supplied, a brief description of the Goods, their country of origin, quantity and prices.

10.2 For the purpose of granting a margin of domestic preference pursuant to ITB Clause 27, the Purchaser will classify the bid, when submitted, in one of three groups, as follows:

(a) Group A: Bid offering Goods manufactured in the Purchaser's country for which the domestic value added in the manufacturing cost is not less than 20 percent of the ex-factory price;

(b) Group B: Bids offering Goods manufactured in the Purchaser's country for which the domestic value added in the manufacturing cost is less than 20 percent of the ex-factory price, and for Goods of foreign origin already located in the Purchaser's country; and

(c) Group C: Bids offering Goods of foreign origin to be imported by the Purchaser directly or through the successful Bidder's local agent.

10.3 To facilitate this classification by the Purchaser, the Bidder shall complete whichever version of the Price Schedule furnished in the Bidding Documents is appropriate, provided however that the completion of an incorrect version of the Price Schedule by the Bidder will not result

in rejection of its bid but merely in the Purchaser's reclassification of the bid into its appropriate bid group. A Bidder claiming to offer domestic Goods belonging to Group A above shall submit along with its bid a completed Form 4, Domestic Value Added Calculation Form.

11. Bid Prices:

11.1 The Bidder shall indicate on the appropriate Price Schedule (Forms 5 and 6 attached) to these documents the unit prices (if applicable) and total Bid Prices of the Goods it proposes to supply under the Contract.

11.2 Prices indicated on the Price Schedule shall be entered separately in the following manner:

(a) For Goods offered from within the Purchaser's country:

(i) the price of the Goods, quoted ex-factory, ex-showroom, ex-warehouse or off-the-shelf, as applicable, including all customs duties and sales and other taxes already paid or payable:

a. on the components and raw materials used in the manufacture or assembly of Goods quoted ex-factory; or

b. on the previously imported Goods of foreign origin quoted ex-showroom, ex-warehouse or off-the-shelf.

(ii) any Purchaser-country sales and other taxes which will be payable on the Goods if this Contract is awarded; and

(iii) charges for the Incidental Services, if any, listed in the GCC Data Sheet.

(b) For Goods offered from abroad:

(i) the price of the Goods quoted CIF port-of-entry or CIP named destination in the Purchaser's country. In quoting the price, the Bidder shall be free to use surface, air or ocean transportation through vehicles, aircraft or vessels registered in any eligible source country, or through shipping conferences in which the majority of the shipping lines are from such eligible source countries. Similarly, the Bidder may obtain insurance Services from any eligible source country.

(ii) charges for the Incidental Services, if any, listed in the GCC Data Sheet.

11.3 The Bidder's separation of price components in accordance with ITB Sub-Clause 11.2 above will be solely for the purpose of facilitating the comparison of bids by the Purchaser and will not in any way limit the Purchaser's right to contract on any of the terms offered.

11.4 Fixed Price. Prices quoted by the Bidder shall be fixed during the Bidder's performance of the Contract and not subject to variation on any account. A bid submitted with an adjustable price quotation will be treated as non-responsive and rejected, pursuant to ITB Clause 24.

12. Bid Currencies:

12.1 Prices shall be quoted in the following currencies:

(a) for Goods and Services which the Bidder will supply from within the Purchaser's country, the prices shall be quoted in the currency of the Purchaser's country; and

(b) for Goods and Services which the Bidder will supply from outside the Purchaser's country, the prices shall be quoted either in the currency of the Bidder's home country, or in the alternative international trading currency specified in the ITB Data Sheet, or European Currency Units (ECUs).

12.2 Further, a Bidder expecting to incur a portion of its expenditures in the performance of the Contract in more than one currency, and wishing to be paid accordingly, shall so indicate in its bid. In such a case, either (i) the bid shall be expressed in different currencies and the respective amounts in each currency together making up the total price, or (ii) the total Bid Price shall be expressed in one currency and payments required in other currencies expressed as a percentage of the bid price along with the exchange rate used in such calculation.

13. Documents Establishing Bidder's Eligibility and Qualifications:

13.1 Pursuant to ITB Clause 9, the Bidder shall furnish, as part of its bid, documents establishing the Bidder's eligibility to bid and its qualifications to perform the Contract if its bid is accepted.

13.2 The documentary evidence of the Bidder's eligibility to bid shall establish to the Purchaser's satisfaction that the Bidder, at the time of submission of its bid, is from an eligible source country as defined under ITB Clause 2.

13.3 The documentary evidence of the Bidder's qualifications to perform the Contract if its bid is accepted, shall establish to the Purchaser's satisfaction that the Bidder:

(a) is incorporated in the country of manufacture;

(b) has been licensed by the regulatory authority in the country of manufacture to supply the pharmaceuticals and vaccines covered by this IFB;

(c) has manufactured and marketed pharmaceuticals or vaccines for at least five (5) years and the specific items covered by this IFB for at least two (2) years;

(d) has received a satisfactory GMP inspection certificate from the food and drug regulatory authority (FDRA) in the country of manufacture and has not violated any standards for the past two (2) years; and

(e) that, in the case of a Bidder offering to supply Goods under the Contract which the Bidder did not manufacture or otherwise produce, that the Bidder has been duly authorized by a manufacturer of the Goods that meets the above criteria to supply the Goods in the Purchaser's country.

13.4 In addition the Bidder will submit:

(a) a copy of its manufacturing license and a statement of installed manufacturing capacity and

(b) copies of its audited financial statements for the past three fiscal years.

13.5 Any other qualification criteria that will apply are listed in the ITB Data Sheet.

14. Documents Establishing Goods' Eligibility and Conformity to Bidding Documents:

14.1 Pursuant to ITB Clause 9, the Bidder shall furnish, as part of its bid, documents establishing the eligibility and conformity to the Bidding Documents of all Goods and Services which the Bidder proposes to supply under the Contract.

14.2 If the pharmaceuticals or vaccines offered do not meet the specified pharmacopoeial standards, the Bidder will agree to provide testing protocols and alternative reference standards along with the sample required pursuant to ITB para. 14.3 (c). The documentary evidence of the Goods' and Services' eligibility shall consist of a statement in the Price Schedule on the country of origin of the Goods' and Services' offered which shall be confirmed by a certificate of origin issued at the time of shipment.

14.3 The documentary evidence of the Goods' and Services' conformity to the Bidding Documents may be in the form of literature and data, and shall furnish:

(a) a detailed description of the Goods' essential chemical characteristics;

(b) a clause-by-clause commentary on the Purchaser's Technical Specifications demonstrating the Goods' substantial responsiveness to those specifications or a statement of deviations and exceptions to the provisions of the Technical Specifications.

(c) A sample of the pharmaceuticals or vaccines listed and in the quantities specified in the ITB Data Sheet for quality testing purposes. The cost of providing such as a sample will be to the Bidder's account.

14.4 For purposes of the commentary to be furnished pursuant to para. 14.3 (c) above, the Bidder shall note that standards and references to brand names or catalogue numbers designated by the Purchaser in its Technical Specifications are intended to be descriptive only and not restrictive. The Bidder may substitute generic drugs in its bid, provided that it demonstrates to the Purchaser's satisfaction that the substitutions are substantially equivalent or superior to those designated in the Technical Specifications.

15. **Bid Security:**

15.1 Pursuant to ITB Clause 9, the Bidder shall furnish, as part of its bid, bid security of not less than the amount or the percentage of the Bid Price given in the ITB Data Sheet, provided the bid security amount is more than US$10,000 equivalent. For bids of smaller value, no bid security is required.

15.2 The bid security is required to protect the Purchaser against the risk of a Bidder's conduct which would warrant the security's forfeiture, pursuant to ITB Sub-Clause 15.7.

15.3 The bid security shall be denominated in the currency of the bid or another freely convertible currency, and shall be in one of the following forms:

(a) An unconditional bank guarantee or irrevocable Letter of Credit issued directly by a bank located in the Purchaser's country or abroad acceptable to the Purchaser, in the form provided in the Bidding Documents or another form acceptable to the Purchaser and valid for 30 days beyond the validity of the bid; or

(b) A cashier's check, certified check or cash.

15.4 Any bid not secured in accordance with ITB Sub-Clauses 15.1 and 15.3 will be rejected by the Purchaser as non-responsive, pursuant to ITB Sub-Clause 24.3.

15.5 Unsuccessful Bidder's bid security will be discharged/returned as promptly as possible but not later than 30 days after the expiration of the period of bid validity prescribed by the Purchaser, pursuant to ITB Clause 16.

15.6 The successful Bidder's bid security will be discharged upon the Bidder's executing the Contract, pursuant to ITB Clause 34, and furnishing the performance security, pursuant to ITB Clause 35.

15.7 The bid security may be forfeited:

(a) if a Bidder withdraws its bid during the period of bid validity specified by the Bidder on the Bid Form; or

(b) in the case of the successful Bidder, if the Bidder fails:

(i) to sign the Contract in accordance with ITB Clause 34; or

(ii) to furnish performance security in accordance with ITB Clause 35.

16. Period of Validity of Bids:

16.1 Bids shall remain valid for the period specified in the ITB Data Sheet after the date of bid opening prescribed by the Purchaser in the ITB Data Sheet, pursuant to ITB Clause 22. A bid valid for a shorter period may be rejected by the Purchaser as non-responsive.

16.2 In exceptional circumstances, the Purchaser may solicit the Bidder's consent to an extension of the period of validity. The request and the responses thereto shall be made in writing (or by cable or telex). The bid security provided under ITB Clause 15 shall also be suitably extended. A Bidder may refuse the request without forfeiting its bid security. A Bidder granting the request will not be required nor permitted to modify its bid.

16.3 In the event the Purchaser requests and the Bidder agrees to an extension of the validity period, the Contract Price in case the Bidder is selected for award shall be its Bid Price corrected as follows: the foreign and local currency component of the Bid Price shall be increased by the factors A and B given in the ITB Data Sheet respectively for each week, or part of a week, that has elapsed from the expiration of the initial bid validity to the date of notification of award to the successful Bidder.

17. Format and Signing of Bid:

17.1 The Bidder shall prepare the number of copies of the bid specified in the ITB Data Sheet, clearly marking each "Original Bid" and "Copy of Bid," as appropriate. In the event of any discrepancy between them, the original shall govern.

17.2 The original and all copies of the bid shall be typed or written in indelible ink and shall be signed by the Bidder or a person or persons duly authorized to bind the Bidder to the Contract. The latter authorization shall be indicated by written power-of-attorney accompanying the bid. All pages of the bid, except for unamended printed literature, shall be initialed by the person or persons signing the bid.

17.3 The bid shall contain no interlineation, erasures or overwriting except as necessary to correct errors made by the Bidder, in which case such corrections shall be initialed by the person or persons signing the bid.

D. Submission of Bids

18. Sealing and Marking of Bids:

18.1 The Bidders shall seal the original and each copy of the bid in an inner and an outer envelope, duly marking the envelopes as "original" and "copy."

18.2 The inner and outer envelopes shall be:

(a) addressed to the Purchaser at the address given in the ITB Data Sheet, and

(b) bear the Project title given in the ITB Data Sheet, the Invitation for Bids (IFB) number at the top of the ITB Data Sheet and the words **"DO NOT OPEN BEFORE"** (the Time and Date of opening given in the ITB Data Sheet).

18.3 The inner envelope shall indicate the name and address of the Bidder to enable the bid to be returned unopened in case it is declared "late." If the outer envelope is not sealed and marked as required by ITB Sub-Clause 18.2, the Purchaser will assume no responsibility for the bid's misplacement or premature opening.

18.4 Bids should be hand delivered or sent by courier mail to ensure timely arrival.

19. **Deadline for Submission of Bids:**

19.1 Bids must be received by the Purchaser at the address specified in the ITB Data Sheet no later than the date and time specified in the ITB Data Sheet.

19.2 The Purchaser may, at its discretion, extend this deadline for the submission of bids by amending the Bidding Documents in accordance with ITB Clause 7, in which case all rights and obligations of the Purchaser and the Bidders previously subject to the deadline will thereafter be subject to the deadline as extended.

20. **Late Bids:**

20.1 Any bid received by the Purchaser after the deadline for submission of bids prescribed by the Purchaser, pursuant to ITB Clause 19, will be rejected and/or returned unopened by the Bidder.

21. **Modification and Withdrawal of Bids:**

21.1 The Bidder may modify or withdraw its bid after bid submission, provided that written notice of the modification or withdrawal is received by the Purchaser prior to the deadline prescribed for submission of bids.

21.2 The Bidder's modification or withdrawal notice shall be prepared, sealed, marked and dispatched in accordance with the provisions of ITB Clauses 17 and 18. A withdrawal notice may also be sent by telex or cable but followed by a signed confirmation copy, post-marked not later than the deadline for submission of bids.

E. Bid Opening and Evaluation

22. Opening of Bids by Purchaser:

22.1 The Purchaser will open bids, in the presence of Bidders' representatives who choose to attend, at the date and time specified in the ITB Data Sheet and in the location given in the ITB Data Sheet. The Bidders' representatives who are present shall sign a register evidencing their attendance.

22.2 The Bidders' names, bid prices, discounts, modifications, bid withdrawals and the presence or absence of the requisite bid security and such other details as the Purchaser, at its discretion, may consider appropriate will be announced at the opening.

22.3 The Purchaser will prepare minutes of the bid opening.

23. Clarification of Bids:

23.1 To assist in the examination, evaluation and comparison of bids the Purchaser may, at its discretion, ask the Bidder for a clarification of its bid. The request for clarification and the response shall be in writing and no change in the price or substance of the bid shall be sought, offered or permitted.

24. Preliminary Examination:

24.1 The Purchaser will examine the bids to determine whether they are complete, whether any computational errors have been made, whether required sureties have been furnished, whether the documents have been properly signed, and whether the bids are generally in order.

24.2 Arithmetical errors will be rectified on the following basis. If there is a discrepancy between the unit price and the total price that is obtained by multiplying the unit price and quantity, the

unit price shall prevail and the total price shall be corrected. If the Bidder does not accept the correction of the errors, its bid will be rejected. If there is a discrepancy between words and figures, the amount in words will prevail.

24.3 Prior to the detailed evaluation, pursuant to ITB Clause 26, the Purchaser will determine the substantial responsiveness of each bid to the Bidding Documents. For purposes of these clauses, a substantially responsive bid is one which conforms to all the terms and conditions of the Bidding Documents without material deviations. Bids for fewer than the total number of items specified, and for less than the total quantities required for a single item will be considered responsive, provided in the latter case, that at least twenty-five percent (25%) of the specified quantity is offered. The Purchaser's determination of bid responsiveness is to be based on the contents of the bid itself without recourse to extrinsic evidence.

24.4 A bid determined as not substantially responsive will be rejected by the Purchaser and may not subsequently be made responsive by the Bidder by correction of the non-conformity.

24.5 The Purchaser may waive any minor informality or non-conformity or irregularity in a bid which does not constitute a material deviation, provided such waiver does not prejudice or affect the relative ranking of any Bidder.

25. Conversion to Single Currency:

25.1 To facilitate evaluation and comparison, the Purchaser will convert all bid prices expressed in the amounts in various currencies in which the Contract Price would be payable, to the currency specified in the ITB Data Sheet at the selling exchange rates for similar transactions established by the Central Bank in the Purchaser's country or other bank or recognized financial publication named in the ITB Data Sheet on the date specified in the ITB Data Sheet.

26. Evaluation and Comparison of Bids:

26.1 The Purchaser will evaluate and compare the bids previously determined to be substantially responsive, pursuant to ITB Clause 24.

26.2 The Purchaser's evaluation of a bid will exclude and not take into account:

(a) in the case of Goods manufactured in the Purchaser's country or Goods of foreign origin already located in the Purchaser's country, sales and other similar taxes, which will be payable on the Goods if the Contract is awarded to the Bidder;

(b) in the case of Goods of foreign origin offered from abroad, customs duties and other similar import taxes which will be payable on the Goods if the Contract is awarded to the Bidder; and

26.3 The comparison of bids and the determination of the lowest evaluated bid will be made for each individual pharmaceutical or vaccine on the basis of ex-factory prices for the pharmaceuticals or vaccines offered from within the Purchaser's country inclusive of all costs and duties and taxes payable on all raw materials, excipients and packaging material used in the manufacture of the item, and the CIF port-of-entry or CIP named destination prices for such items offered from abroad. Bidders wishing to offer a discount conditional on the award of the Contract covering more than one item, however, are welcome to do so and the discount will be considered in the evaluation.

27. Domestic Preference:

27.1 In the comparison of evaluated bids, the Purchaser will grant a margin of preference to Goods manufactured in the Purchaser's country, in accordance with the following procedures, provided the Bidder shall have established to the satisfaction of the Purchaser and the World Bank that

the manufacturing cost of such Goods includes a domestic value added equal to at least 20 percent of the ex-factory bid price of such Goods.

27.2 The Purchaser will first review the bids and completed Domestic Value Added Calculation Forms to confirm the appropriateness of, and to modify as necessary, the bid group classification for each item offered to which Bidders assigned their bids in preparing their Bid Forms and Price Schedule, pursuant to ITB Clause 10.

27.3 All evaluated bids in each group will then be compared among themselves, to determine the lowest evaluated bid of each group for each item. The lowest evaluated bid of each group will next be compared with the lowest evaluated bids of the other groups for that item. If this comparison results in a bid from Group A or B being the lowest, it will be selected for the award of the Contract for that item provided another Bidder has not offered a discount for the award of more than one item that would offset the results of this comparison.

27.4 If, as a result of the preceding comparison, the lowest bid is from Group C, all Group C bids will then be further compared with the lowest evaluated bid from Group A, after adding to the evaluated bid price of the imported goods offered in each Group C bid for the purpose of this further comparison only:

(a) the amount of customs duties and other import taxes which a non-exempt importer would have to pay for the importation of the Goods offered in each Group C bid; OR

(b) 15 percent of the CIF or CIP Bid Price of such Goods, if the customs duties and taxes exceed 15 percent of the CIF or CIP price of such Goods.

If the Group A bid in the further comparison is the lowest, it will be selected for award. If not, the lowest evaluated bid from Group C, as determined from the comparison under ITB Sub-Clause 27.3 above, will be selected.

28. Contacting the Purchaser:

28.1 Subject to ITB Clause 23, no Bidder shall contact the Purchaser on any manner relating to its bid, from the time of the bid opening to the time the Contract is awarded.

28.2 Any effort by a Bidder to influence the Purchaser in the Purchaser's bid evaluation, bid comparison or Contract award decisions may result in the rejection of the Bidder's bid.

F. Award of Contract

29. Postqualification:

29.1 Based on the qualification criteria listed in ITB Clause 13 and the ITB Data Sheet the Purchaser will determine to its satisfaction whether the Bidder selected as having submitted the lowest evaluated responsive bid is qualified to satisfactorily perform the Contract.

29.2 The determination will take into account the Bidder's financial, technical, quality control and production capabilities. It will be based upon an examination of the documentary evidence of the Bidder's qualifications submitted by the Bidder, pursuant to ITB Clause 13, as well as such other information as the Purchaser deems necessary and appropriate.

29.3 An affirmative determination will be a prerequisite for award of the Contract to the Bidder. A negative determination will result in rejection of the Bidder's bid, in which event, the Purchaser will proceed to the next lowest evaluated bid to make a similar determination of that Bidder's capabilities to perform satisfactorily.

30. Award Criteria:

30.1 Subject to ITB Clause 32, the Purchaser will award the Contract to the successful Bidder whose bid has been determined to be substantially responsive and has been determined as the lowest evaluated bid for each pharmaceutical and vaccine item provided further that the Bidder is determined to be qualified to perform the Contract satisfactorily for the items offered. If the lowest evaluated Bidder has not offered to supply the entire quantities specified in the Schedule of Requirements, the Purchaser will award additional Contracts to the next lowest evaluated Bidder(s) until the specified quantity requirements have been met.

31. Purchaser's Right to Vary Quantities at Time of Award:

31.1 The Purchaser reserves the right at the time of award of Contract to increase or decrease by up to 20 percent the quantity of pharmaceuticals and vaccines specified in the Schedule of Requirements without any change in price or other terms and conditions.

32. Purchaser's Right to Accept Any Bid and to Reject Any or All Bids:

32.1 The Purchaser reserves the right to accept or reject any bid, and to annul the bidding process and reject all bids at any time prior to award of Contract, without thereby incurring any liability to the affected Bidder or Bidders or any obligation to inform the affected Bidder or Bidders of the grounds for the Purchaser's action.

33. Notification of Award:

33.1 Prior to the expiration of the period of bid validity, the Purchaser will notify the successful Bidder in writing by registered letter or by cable or fax or telex, to be confirmed in writing by registered letter, that its bid has been accepted.

33.2 The notification of award will constitute the formation of the Contract.

33.3 Upon the successful Bidder's furnishing of performance security pursuant to ITB Clause 35, the Purchaser will promptly notify each unsuccessful Bidder and will discharge its bid security, pursuant to ITB Clause 15.

34. Signing of Contract:

34.1 At the same time as the Purchaser notifies the successful Bidder that its bid has been accepted, the Purchaser will send the Bidder the Contract Form provided in the Bidding Documents, incorporating all agreements between the parties.

34.2 Within 30 days of receipt of the Contract Form, the successful Bidder shall sign and date the Contract and return it to the Purchaser.

35. Performance Security:

35.1 Within 30 days of the receipt of notification of award from the Purchaser, the successful Bidder shall furnish the performance security in accordance with Clause 7 of the General Conditions of Contract (GCCs), in the Performance Security Form provided in the Bidding Documents.

35.2 Failure of the successful Bidder to comply with the requirements of ITB Clauses 34 or 35 shall constitute sufficient grounds for the annulment of the award and forfeiture of the bid security, in which event the Purchaser may make the award to the next lowest evaluated bidder or call for new bids.

SECTION III.

A. GCC DATA SHEET AND SPECIAL CONDITIONS OF CONTRACT AND

B. GENERAL CONDITIONS OF CONTRACT

SECTION III

Table of Contents

A. GCC DATA SHEET AND SPECIAL CONDITIONS OF CONTRACT

B. GENERAL CONDITIONS OF CONTRACT

CLAUSE	SUBJECT	PAGE NO.
1.	DEFINITIONS	34
2.	APPLICATION	34
3.	COUNTRY OF ORIGIN	35
4.	TECHNICAL SPECIFICATIONS/STANDARDS	35
5.	ASSIGNMENT	35
6.	PATENT RIGHTS	36
7.	PERFORMANCE SECURITY	36
8.	INSPECTION AND TESTS	37
9.	PACKING	38
10.	DELIVERY AND DOCUMENTS	38
11.	INSURANCE	40
12.	TRANSPORTATION	41
13.	INCIDENTAL SERVICES	41
14.	WARRANTY	41
15.	PAYMENT	42
16.	PRICES	43
17.	CHANGE ORDERS	44
18.	CONTRACT AMENDMENTS	44
19.	SUB-CONTRACTS	45
20.	DELAYS IN SUPPLIERS' PERFORMANCE	45
21.	LIQUIDATED DAMAGES	45
22.	TERMINATION FOR DEFAULT	46
23.	FORCE MAJEURE	47
24.	TERMINATION FOR INSOLVENCY	47

Table of Contents (Cont'd.)

B. GENERAL CONDITIONS OF CONTRACT (Cont'd.)

CLAUSE	SUBJECT	PAGE NO.
25.	TERMINATION FOR CONVENIENCE	47
26.	RESOLUTION OF DISPUTES	48
27.	GOVERNING LANGUAGE	49
28.	APPLICABLE LAW	49
29.	NOTICES	49
30.	TAXES AND DUTIES	49

SECTION III A **GCC Data Sheet and Special Conditions of Contract**

No.	GCC Clause	A. Listed Information in the GCCs
1.	1.1 (e)	The Purchaser is: _____
2.	1.1 (f)	The Supplier for this Contract is: _____
3.	10	The port(s) of destination shall be: _____ _____
4.	13	The Supplier is required to provide the following incidental Services under the Contract: _____ _____ _____
5.	14	The currency or currencies of payment will be: _____
6.	26	The language of the Contract shall be: _____
7.	28	The Purchaser's address for notice purposes is: _____ _____ _____ Telephone: _____ Fax: _____

GCC Data Sheet and Special Conditions of Contract

No.	GCC Clause	A. Listed Information in the GCCs (Cont.d)
8.	28	The Supplier's address for notice purposes is:

_____

_____

_____

Telephone: _____

Fax: _____

B. Special Conditions of Contract

9. Special Conditions of Contract:

The following Special Conditions of Contract shall supplement the General Conditions of Contract. Whenever there is a conflict, the provisions herein shall prevail over those in the General Conditions of Contract. The corresponding clause number of the General Conditions is indicated in parentheses.

9. (a) _____

_____

_____

(b) _____

_____

_____

(c) _____

_____

Section III. B GENERAL CONDITIONS OF CONTRACT

1. **Definitions:**

1.1 In this Contract, the following terms shall be interpreted as indicated:

(a) "The Contract" means the agreement entered into between the Purchaser and the Supplier, as recorded in the Contract Form signed by the parties, including all attachments and appendices thereto and all documents incorporated by reference therein;

(b) "The Contract Price" means the price payable to the Supplier under the Contract for the full and proper performance of its contractual obligations;

(c) "The Goods" means all drugs, pharmaceuticals, contraceptives, etc., which the Supplier is required to supply in various dosage forms to the Purchaser under the Contract;

(d) "Services" means services ancillary to the supply of the Goods, such as transportation and insurance;

(e) "The Purchaser" means the organization purchasing the Goods defined on Line 1 of the GCC Data Sheet; and

(f) "The Supplier" means the individual or firm supplying the Goods under this Contract as defined on Line 2 of the GCC Data Sheet.

2. **Application:**

2.1 These General Conditions shall apply to the extent that they are not superseded by provisions in other parts of the Contract.

3. **Country of Origin:**

3.1 All Goods and Services supplied under the Contract shall have their origin in the member countries of the World Bank and Taiwan, China.

3.2 For purposes of this Clause "origin" means the place where the Goods were mined, produced, or manufactured. Goods are produced when, through manufacturing, processing, a commercially recognized new product results that is substantially different in basic characteristics or utility from its components.

3.3 The origin of Goods and Services is distinct from the nationality of the Supplier.

4. **Standards:**

4.1 The Goods supplied under this Contract shall conform to the standards mentioned in the Technical Specifications, and, when no applicable standard is mentioned, to the authoritative standard appropriate to the Goods' country of origin and such standards shall be the latest issued by the concerned institution.

5. **Assignment:**

5.1 The Supplier shall not assign, in whole or in part, its obligations to perform under the Contract, except with the Purchaser's prior written consent. Such consent shall be granted only if the Purchaser is satisfied that the assignee meets all the qualification criteria established by the Bidding Documents.

6. **Patent Rights:**

6.1 The Supplier shall indemnify the Purchaser against all third party claims of infringement of patent or trademark rights arising from use of the Goods or any part thereof in the Purchaser's country.

7. **Performance Security:**

7.1 Within 30 days after the Supplier's receipt of notification of award of the Contract, the Supplier shall furnish performance security to the Purchaser in the amount of ten percent of the Contract Price.

7.2 The proceeds of the performance security shall be payable to the Purchaser as compensation for any loss resulting from the Supplier's failure to complete its obligations under the Contract.

7.3 The Performance Security shall be denominated in the currency of the Contract or in a freely convertible currency acceptable to the Purchaser, and shall be in one of the following forms:

(a) An unconditional bank guarantee or irrevocable Letter of Credit, issued directly by a bank located in the Purchaser's country or abroad acceptable to the Purchaser, and in the form provided in the Bidding Documents or another form acceptable to the Purchaser; or

(b) A cashier's check, certified check, or cash.

7.4 The performance security will be discharged by the Purchaser and returned to the Supplier not later than four (4) months following the date of delivery to the final destination indicated in the Contract.

8. **Inspections and Tests:**

8.1 The Purchaser or its representative shall have the right to inspect and/or to test the Goods to confirm their conformity to the Contract. The Technical Specifications shall specify what inspections and tests the Purchaser requires and where they are to be conducted. The cost of such inspections and tests shall be to the Purchaser's account. The Purchaser shall notify the Supplier in writing of the identity of any representatives retained for these purposes within 21 days of signing of the Contract. The Supplier shall notify the Purchaser or its representative at least ten days prior to the time when Goods are available for inspection. The Goods shall not be shipped unless a satisfactory inspection and quality assurance report is provided to the Supplier.

8.2 The Supplier will provide to the Purchaser or its representative all reasonable facilities for the performance of such inspections and tests at no additional cost to the Purchaser. The Supplier may have an independent quality test conducted on a batch ready for shipment. The cost of such tests will be borne by the Supplier.

8.3 Should any inspected or tested Goods fail to conform to the Technical Specifications, the Purchaser may reject them and the Supplier shall either replace the rejected Goods or make all alterations necessary to meet the specified requirements free of cost to the Purchaser.

8.4 Where the Supplier contests the validity of the rejection by the Purchaser or his representative, whether based on product or packing grounds, a sample drawn jointly by

the Supplier and Purchaser or his representative and authenticated by both, will be forwarded for umpire analysis to an independent agency mutually agreed by the Purchaser and Supplier. The umpire's finding, which will be promptly obtained, will be final and binding on both parties. The cost of umpire analysis will be borne by the losing party.

8.5 The cost of quality assurance testing in the Purchaser's country will be borne by the Purchaser. If the Goods fail the test, the Purchaser shall inform the Supplier of the results in writing, and the Purchaser reserves the right to forward samples to a mutually acceptable independent laboratory for testing, the costs of which shall be borne by the Supplier.

8.6 The Purchaser's right to inspect, test and, where necessary, reject the Goods after the Goods' arrival in the Purchaser's country shall in no way be limited or waived by reason of the Goods having previously been inspected, tested and passed by the Purchaser or its representative prior to the Goods' shipment from their country of origin.

8.7 Nothing in this GCC Clause 8 shall in any way release the Supplier from any warranty or other obligations under the Contract.

9. **Packing:**

9.1 The Supplier shall provide such packing of the Goods as is required to prevent their damage or deterioration during transit to their final destination as indicated in the

Contract. The packing shall be sufficient to withstand, without limitation, rough handling during transit and exposure to extreme temperatures sunlight, humidity, salt and precipitation during transit and storage. Packing case size and weights shall take into consideration, when appropriate, the remoteness of the Goods' final destination. All primary packaging containers which come in contact with the pharmaceutical or drug content shall strictly conform to the specifications included in the relevant pharmacopoeia to protect the quality and integrity of the Goods.

9.2 The packing, marking and documentation within and outside the packages shall comply strictly with such special requirements as shall be expressly provided for in the Technical Specifications and, subject to GCC Clause 17, any subsequent instructions ordered by the Purchaser.

10. Delivery and Documents:

10.1 Delivery of the Goods shall be made by the Supplier to the port(s) specified on Line 3 of the GCC Data Sheet in accordance with the schedule and terms specified by the Purchaser in its Schedule of Requirements and this GCC Clause 10.

10.2 For Imported Goods: Immediately on shipment of the contracted Goods, the Supplier will advise the Purchaser by telex, fax, or cable of the following details:

(i) Name of the vessel or carrier

(ii) Contract number

(iii) Date and time of departure from port of shipment

(iv) Quantity of Goods on board

(v) Invoiced value of the Goods

(vi) Bills of lading number(s)

(vii) Expected time of arrival at port of discharge.

10.3 The Supplier will also dispatch to the Purchaser one set of the following documents by courier service and another set through the Master of the vessel, aircraft or other means of transport:

(i) One negotiable copy of the clean bill of lading with non-negotiable copies (marked "freight prepaid" in CIF, CFR or CIP Contracts).

(ii) Certified commercial invoice and ten copies.

(iii) Original copy of the Certificate of Origin and ten copies.

(iv) Original copy of the certificate of inspection furnished to Supplier by the nominated inspection agency and six copies.

(v) Certificate of in-house analysis

(vi) Original copy of the certificate of weight issued by the port authority/licensed authority and ten copies

(vii) Original copy of the packing list

(viii) Insurance Certificate

(ix) Supplier's/manufacturer's warrantee

(x) Copy of telex/fax/cable sent to Purchaser by Supplier upon the departure of the vessel, aircraft or other means of transport

10.4 For domestic Goods:

(i) Copies of the Supplier's invoice showing a description of the Goods, quantity, unit price, total amount:

(ii) Delivery note/railway receipt/truck receipt;

(iii) Supplier's/manufacturer's warrantee;

(iv) Inspection Certificate issued by the nominated inspection agency, and the Supplier's factory inspection report; and

(v) Certificate of origin.

One identical set consisting of copies of the above documents will be mailed by the Supplier to the Purchaser immediately after presentation for payment to the bank where the letter of credit has been opened.

10.5 For purposes of the Contract, "FOB," "CFR," "CIF," "CIP" and other trade terms used to describe the obligations of the parties shall have the meanings assigned to them by the current edition of the International Rules for the Interpretation of the Trade Terms published by the International Chamber of Commerce, Paris, and commonly referred to as "INCOTERMS".

11. **Insurance:**

11.1 The Goods supplied under the Contract shall be fully insured in a freely convertible currency against loss or damage incidental to manufacture or acquisition, transportation, storage and delivery.

11.2 Where delivery of the Goods is required by the Purchaser on a CIF or CIP basis, the Supplier shall arrange and pay for marine or other transportation insurance, naming the Purchaser as the beneficiary. The insurance shall be in an amount equal to 110 percent of the CIF or CIP value of the Goods from "warehouse to warehouse" on an "All Risks" basis including War Risks and Strike clauses. Where delivery is on an FOB or CFR basis, transportation insurance shall be the responsibility of the Purchaser.

12. **Transportation:**

12.1 Where the Supplier is required under the Contract to deliver the Goods FOB, transport of the Goods, up to and including the point of putting the Goods on board the vessel at the specified port of loading, shall be arranged and paid for by the Supplier, and the cost thereof shall be included in the Contract Price.

12.2 Where the Supplier is required under the Contract to deliver the Goods CIF or CIP, transport of the Goods to the port, railway terminal or airport of discharge shall be arranged and paid for by the Supplier, and the cost thereof shall be included in the Contract Price.

12.3 Transportation of the Goods after delivery shall be the responsibility of the Purchaser.

12.4 Where the Supplier is required under the Contract to deliver the Goods CIF or CIP, no further restriction shall be placed on the choice of carrier.

12.5 All items must be stored and shipped under appropriate storage conditions at all times during the delivery process as specified in the Technical Specifications.

13. Incidental Services:

13.1 As specified in the GCC Data Sheet, the Supplier may be required to provide inland transportation, insurance or other Services under the Contract.

13.2 Prices charged by the Supplier for the preceding incidental Services, if not included in the Contract Price for the Goods, shall be agreed upon in advance by the parties and shall not exceed the prevailing rates charged other parties by the Supplier for similar services.

14. **Warranty:**

14.1 The Supplier warrants that all Goods supplied under the Contract will have remaining a minimum of five-sixths (5/6ths) of the specified shelf life upon delivery, have "overages" within the ranges set forth in the Technical Specifications, and are not subject to recall by the applicable regulatory authority due to unacceptable quality or an adverse drug reaction, and in every other respect will fully comply in all respects with the Technical Specifications and with the conditions laid down in the Contract. In the event any of the Goods are recalled, the Supplier shall notify the Purchaser within fourteen (14) days and promptly replace the items covered by the recall at its own cost.

14.2 The Purchaser shall have the right to make claims under the above warranty for only three months after the Goods have been delivered to the final destination indicated in the Contract.

14.3 The Purchaser shall promptly notify the Supplier in writing of any claims arising under this warranty.

14.4 Upon receipt of such notice, the Supplier shall, with all reasonable speed, replace the defective Goods without cost to the Purchaser. The Supplier will be entitled to remove, at his own risk and cost, the defective Goods once the replacement Goods have been delivered.

14.5 If the Supplier, having been notified, fails to replace the defective Goods within a reasonable period, the Purchaser may proceed to take such remedial action as may be necessary, at the Supplier's risk and expense and without prejudice to any other rights which the Purchaser may have against the Supplier under the Contract.

15. **Payment:**

15.1 (a) Payment for imported Goods. Payment shall be made in the currency(ies) of the Contract specified on Line 5 of the GCC Data Sheet in the following manner:

(i) On shipment: 90 percent of the Contract Price of the Goods shipped shall be paid through irrevocable confirmed Letter of Credit established in favor of the Supplier in a bank in his country, on submission of documents specified in GCC Clause 10; and

(ii) On Receipt of Goods: 10 percent of the Contract Price of Goods received shall be paid within 30 days of receipt of Goods on submission of an invoice supported by documentary evidence issued by the Purchaser's representative that the Goods have been received.

(b) Payment for domestic Goods and Services shall be made in the currency(ies) of the Contract specified on Line 5 of the GCC Data Sheet.

(i) On Delivery: 90 percent of the Contract Price shall be paid on receipt of the Goods and upon submission of the documents specified in Clause 10; and

(ii) On Final Acceptance: the remaining 10 percent of the Contract Price shall be paid to the Supplier within thirty (30) days after the date of receipt of the Goods confirmed by documentary evidence signed by the Purchaser's representative that the Goods have been received.

15.2 The Supplier's requests(s) for payment shall be made to the Purchaser in writing, accompanied by an invoice describing, as appropriate, the Goods delivered and Services performed, and by shipping documents, submitted pursuant to GCC Clause 10, and upon fulfillment of other obligations stipulated in the Contract.

15.3 Payments shall be made promptly by the Purchaser within sixty (60) days of submission of an invoice by the Supplier.

16. Prices:

16.1 Prices charged by the Supplier for Goods delivered and Services performed under the Contract shall not vary from the prices quoted by the Supplier in its bid.

17. Change Orders:

17.1 The Purchaser may at any time, by a written order given to the Supplier pursuant to GCC Clause 29, make changes within the general scope of the Contract in any one or more of the following aspects:

(a) the specifications, where Goods to be furnished under the Contract are to be specifically manufactured for the Purchaser;

(b) the method of shipment or packing;

(c) the place of delivery; or

(d) the Services to be provided by the Supplier.

17.2 If any such change causes an increase or decrease in the cost of, or the time required for, the Supplier's performance of any part of the Contract, whether changed or not changed by the order, an equitable adjustment shall be made in the Contract Price or delivery schedule, or both, and the Contract shall accordingly be amended. Any claims by the Supplier for adjustment under this clause must be asserted within thirty (30) days from the date of the Supplier's receipt of the Purchaser's change order.

18. Contract Amendments:

18.1 Subject to GCC Clause 17, no variation in or modification of the terms of the Contract shall be made except by written amendment signed by the parties.

19. Subcontracts:

19.1 The Supplier shall notify the Purchaser in writing of all subcontracts awarded under the Contract if not already specified in his bid. Such notification, in his original bid or later, shall not relieve the Supplier from any liability or obligation under the Contract.

20. Delays in the Supplier's Performance:

20.1 Delivery of the Goods and performance of Services shall be made by the Supplier in accordance with the time schedule specified by the Purchaser in its Schedule of Requirements.

20.2 An unexcused delay by the Supplier in the performance of its delivery obligations shall render the Supplier liable to any or all of the following sanctions: forfeiture of its performance security, imposition of liquidated damages, and/or termination of the Contract for default.

20.3 If at any time during performance of the Contract, the Supplier or its subcontractor(s) should encounter conditions impeding timely delivery of the Goods and performance of Services, the Supplier shall promptly notify the Purchaser in writing of the fact of the delay, its likely duration and its cause(s). As soon as practicable after receipt of the Supplier's notice, the Purchaser shall evaluate the situation and may at its discretion extend the Supplier's time for performance, in which case the extension shall be ratified by the parties by amendment of the Contract.

21. Liquidated Damages:

21.1 Subject to GCC Clause 23, if the Supplier fails to deliver any or all of the Goods or perform the Services within the time period(s) specified in the Schedule of Requirements, the Purchaser shall, without prejudice to its other remedies under the Contract, deduct from the Contract Price, as liquidated damages, a sum equivalent to 0.5 percent of the delivered Contract Price of the delayed Goods or unperformed Services for each week of delay until actual delivery or performance, up to a maximum deduction of 10 percent of the delayed Goods or Services Contract Price. Once the maximum is reached, the Purchaser may consider termination of the Contract.

22. Termination for Default:

22.1 The Purchaser may, without prejudice to any other remedy for breach of contract, by written notice of default sent to the Supplier, terminate the Contract in whole or in part:

(a) if the Supplier fails to deliver any or all of the Goods within the time period(s) specified in the Contract, or any extension thereof granted by the Purchaser pursuant to GCC Clause 20;

(b) if the Supplier fails to promptly replace any Goods rejected when submitted for testing or subject to a recall ordered by the applicable regulatory authority in the country of manufacture due to unacceptable quality or reports of adverse drug reactions after giving prompt notice of the recall; or

(c) if the Supplier fails to perform any other obligation(s) under the Contract.

2.2 In the event the Purchaser terminates the Contract in whole or in part, pursuant to GCC Sub-Clause 22.1 above, the Purchaser may procure, upon such terms and in such manner as it deems appropriate, Goods similar to those undelivered, and the Supplier shall be liable to the Purchaser for any excess costs for such similar Goods. If the Supplier fails to reimburse the Purchaser for such excess costs within a reasonable period, the Purchaser may have recourse to the performance security. However, the Supplier shall continue performance of the Contract to the extent not terminated.

23. Force Majeure:

23.1 Notwithstanding the provisions of GCC Clauses 20, 21 and 22, the Supplier shall not be liable for forfeiture of its performance security, liquidated damages or termination for default, if and to the extent that, its delay in performance or other failure to perform its obligations under the Contract is the result of an event of Force Majeure.

23.2 For purposes of this clause, "Force Majeure" means an event beyond the control of the Supplier and not involving the Supplier's fault or negligence and not foreseeable. Such events may include, but are not restricted to, acts of the Purchaser in its sovereign capacity, wars or revolutions, fires, floods, epidemics, quarantine restrictions and freight embargoes.

23.3 If a Force Majeure situation arises, the Supplier shall promptly notify the Purchaser in writing of such condition and the cause thereof. Unless otherwise directed by the Purchaser in writing, the Supplier shall continue to perform its obligations under the Contract as far as is reasonably practical, and shall seek all reasonable alternative means for performance not prevented by the Force Majeure event.

24. Termination for Insolvency:

24.1 The Purchaser may at any time terminate the Contract by giving written notice to the Supplier, without compensation to the Supplier, if the Supplier becomes bankrupt or

otherwise insolvent, provided that such termination will not prejudice or affect any right of action or remedy which has accrued or will accrue thereafter to the Purchaser.

25. Termination for Convenience:

25.1 The Purchaser may, by written notice sent to the Supplier, terminate the Contract, in whole or in part, at any time for its convenience. The notice of termination shall specify that termination is for the Purchaser's convenience, the extent to which performance of the Contract is terminated, and the date upon which such termination becomes effective.

25.2 The Goods that are complete and ready for shipment within 30 days after the Supplier's receipt of notice of termination shall be purchased by the Purchaser at the Contract terms and prices. For the remaining Goods, the Purchaser may elect:

(a) to have any portion completed and delivered at the Contract terms and prices; and/or

(b) to cancel the remainder and pay to the Supplier an agreed amount for partially completed Goods and for materials previously procured by the Supplier.

26. **Resolution of Disputes:**

26.1 The Purchaser and the Supplier shall make every effort to resolve amicably by direct informal negotiation any disagreement or dispute arising between them under or in connection with the Contract.

26.2 If, after thirty (30) days from the commencement of such informal negotiations, the Purchaser and the Supplier have been unable to resolve amicably a Contract dispute, either party may require that the dispute be referred for resolution to the formal mechanisms specified below:

(a) in the case of a dispute between the Purchaser and a Supplier which is a national of the Purchaser's country, the dispute shall be referred to adjudication/arbitration in accordance with the laws of the Purchaser's country; and

(b) in the case of a dispute between the Purchaser and a foreign Supplier, the dispute shall be settled by arbitration in accordance with the provisions of the UNCITRAL Arbitration Rules.

27. **Governing Language:**

27.1 The Contract shall be written in the language of the bid, as specified by the Purchaser on Line 6 of the ITB Data Sheet. Subject to GCC Clause 28, that language version of the

Contract shall govern its interpretation. All correspondence and other documents pertaining to the Contract which are exchanged by the parties shall be written in that same language.

28. **Applicable Law:**

28.1 The Contract shall be interpreted in accordance with the laws of the Purchaser's country.

29. **Notices:**

29.1 Any notice given by the Supplier to the Purchaser pursuant to the Contract shall be sent in writing or by telegram or telex/fax/cable and confirmed in writing to the address specified for that purpose on Line 7 of the GCC Data Sheet. The Supplier's address for notice purposes shall be given on Line 8 of the GCC Data Sheet.

29.2 A notice shall be effective when delivered or on the notice's effective date, whichever is later.

30. **Taxes and Duties:**

30.1 A foreign Supplier shall be entirely responsible for all taxes, stamp duties, license fees, and other such levies imposed outside the Purchaser's country.

30.2 A local Supplier shall be entirely responsible for all taxes, duties, license fees, etc., incurred until delivery of the contracted Goods to the Purchaser.

SECTION IV. TECHNICAL SPECIFICATIONS

PART A: TECHNICAL SPECIFICATIONS

Specific Products Covered by this IFB Section IV

No.	Code No.	Product Name	INN/GENERIC NAME	Pharmacopoeial Standard	Strength	Dosage form	No. of Units in Pack	Labelling Instructions	Logo	Remarks
	e.g.	Paracetamol	Acetominophen	B.P.	500 mg per tablet	tablets	1,000	Overprinting For Govt. Use Only	M	
1.										
2.										
3.										
4.										
5.										
6.										
7.										
8.										
9.										

1. Product Name/INN may be same in some cases.
2. Labelling -- standard labelling instructions are at Part B. Here indicate any overprinting requirement like "For FMOH-TO90."

PART B: GENERAL TECHNICAL SPECIFICATIONS

1. PRODUCT AND PACKAGE SPECIFICATIONS

1.1 The pharmaceuticals and vaccines to be purchased by the Purchaser under this Invitation for Bids are included in the Purchaser's national essential drugs list or national formulary. The required packing standards and labelling must meet the WHO Good Manufacturing Practices ("GMP") standards in all respects. These standards are contained in Attachment A (Good Practices in the Manufacture and Quality Control of Drugs).

1.2 Product specifications indicate dosage form (e.g., tablet, liquid, injectable, emulsion, suspension, etc.), and the drug content (exact number of mg or % v/v with acceptable range). The products should conform to standards specified in one of the following compendia: the British Pharmacopoeia, the United States Pharmacopoeia, the French VIPAL Pharmacopoeia or the International Pharmacopoeia. In case the pharmaceutical or vaccine product is not included in the specified compendium, the Supplier, upon award of the Contract, must provide the reference standards and testing protocols to allow for quality control testing.

1.3 Not only the pharmaceutical or vaccine item, but also the packaging components (e.g., bottles and closures) should also meet specifications suitable for use in a climate similar to that prevailing in the country of the Purchaser. All packaging must be properly sealed and tamper-proof.

1.4 Pharmaceuticals and drugs requiring refrigeration or freezing for stability must specifically indicate storage requirements on labels and containers and be shipped in special containers to ensure stability in transit from point of shipment to port of entry.

2. PRODUCT INFORMATION

2.1 The following information will be required for each pharmaceutical and vaccine product offered by the Bidder:

(i) INN (International Non-proprietary Name)
(ii) Brand name (if it appears on the label)
(iii) Name and address of the manufacturer
(iv) Country of Origin
(v) Compendia standards

2.2 Upon award, the successful Bidder shall on demand provide a translated version in the language of the bid of the prescriber's information for any specific product the Purchaser may request.

2.3 Failure to include any of this information may, at the discretion of the Purchaser, render the bid non-responsive.

3. EXPIRATION DATE:

All products must indicate the dates of manufacture and expiry. In addition, unless otherwise stated in Part A of these Specifications, all products must arrive at the port of entry (for imported pharmaceuticals or vaccines) or ex-factory warehouse (for local purchases) with a remaining shelf life of at least five-sixths (5/6ths) of the total stipulated shelf life at the time of manufacture.

4. RECALLS:

If products must be recalled because of problems with product quality or adverse reactions to the pharmaceutical or vaccine, the Supplier will be obligated to notify the Purchaser,

providing full details about the reason leading to the recall, and shall take steps to replace the product in question at its own cost with a fresh batch of acceptable pharmaceuticals or vaccines, or withdraw and give a full refund if the product has been taken off the market due to safety problems.

5. LABELLING INSTRUCTIONS:

The label for each pharmaceutical and vaccine products shall meet the W210 GMP standard and include:

i) the INN or generic name prominently displayed and above the brand name, where a brand name has been given. Brand names should not be bolder or larger than the generic name

ii) the active ingredient "per unit, dose, tablet or capsule, etc.

iii) the applicable pharmacopoeial standard

iv) the Purchaser's logo and code number if required in Part A of these Specifications

v) content per pack

vi) instructions for use

vii) special storage requirements

viii) batch number

ix) date of manufacture and date of expiry.

5.2 The outer carton should also display the above information.

6. All cases should prominently indicate the following:

i) Purchaser's Part A line and Code numbers

ii) the generic name of the product

iii) date of manufacture and expiry

iv) batch number

v) quantity per case

No case should contain pharmaceutical or vaccine products from more than one batch.

7. UNIQUE IDENTIFIERS:

The Purchaser shall have the right to request the Supplier to imprint a logo on the containers used for packaging and in certain dosage forms, such as tablets, and this will be indicated in Part A of the Technical Specifications. The design of such logo shall be provided to the Supplier at the time of Contract award.

8. QUALIFICATIONS OF MANUFACTURER

The Bidder shall furnish a certificate from the competent FDRA that the manufacturer of the pharmaceutical or vaccine product covered by this Invitation for Bids is licensed to manufacture these products.

9. STANDARDS AND QUALITY ASSURANCE FOR SUPPLY:

9.1 All products must:

(a) meet the requirements of manufacturing legislation and regulation of pharmaceuticals or vaccines in the country of origin;

(b) conform to all the specifications contained herein; and

(c) be certified by a competent authority in the manufacturer's country according to resolution WHO 28-65B, of the World Health Organization "Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce" (see Attachment A).[8]

[8] It is recognized that reputable manufacturers of drugs for certain tropical medical conditions (e.g., schistosomiasis, onchocerciasis, etc.) may not always need to go through the drug registration procedures in their home countries before being permitted to make such drugs available for use in tropical countries. This is justified in that such items are not intended for sale in domestic markets. In these cases, a certificate of drug registration or free sale in the country of origin, though desirable, may be waived at the option of the Purchaser.

9.2 The successful Bidder will be required to furnish to the Purchaser:

(a) With each consignment, a certificate of quality assurance test results in conformity with the WHO Certification Scheme concerning quantitative assay, chemical analysis, sterility, pyrogen content uniformity, microbial limit and other tests, as applicable to the product being supplied and Part A of these Specifications.

(b) Assay methodology of any or all tests if requested.

(c) Evidence of bio-availability and/or bio-equivalence for certain critical pharmaceuticals or vaccines upon request.

(d) Evidence of basis for expiration dating and other stability data concerning the commercial final package upon request.

9.3 The successful Bidder will also be required to provide the Purchaser with access to its manufacturing facilities to inspect its facilities, quality control procedures for raw materials, test methods, in-process tests, and finished dosage forms.

SECTION V. SCHEDULE OF REQUIREMENTS

SECTION V

Schedule of Requirements

Product Code or No.	Product Description	Strength	Required Quantity	Delivery Schedule	Remarks

List of Bidding and Contract Forms

1. BID FORM
2. BID SCHEDULE OF PRICES FOR DOMESTIC DRUGS
3. BID SCHEDULE OF PRICES FOR IMPORTED DRUGS
4. DOMESTIC VALUE ADDED CALCULATION FORM
5. QUALIFICATIONS FORM
6. LETTER OF AUTHORITY FROM MANUFACTURER
7. BID SECURITY FORM
8. PERFORMANCE SECURITY FORM
9. SPECIMEN LETTER OF CREDIT
10. CONTRACT FORM

ATTACHMENTS

A WHO GUIDELINES ON GOOD MANUFACTURING PRACTICE (GMP) AND CERTIFICATION SCHEME ON THE QUALITY OF DRUGS MOVING IN THE INTERNATIONAL COMMERCE

Bid Form

Form 1

Date: _____
IFB No: _____

Gentlemen:

Having examined the Bidding Documents, including Addenda Nos. _____ the receipt of which is hereby duly acknowledged, we, the undersigned, offer the supply and deliver the drugs specified in and in conformity with the said Bidding Documents for the sum of _____ - _____ (Total Bid Amount in Words and Figures) or such other sums as may be ascertained in accordance with the Schedule of Prices attached herewith and made part of this bid.

We undertake, if our bid is accepted, to commence delivery within _____ days and to complete delivery of all the items specified in the Contract within _____ days calculated from the date of receipt of your Notification of Award/Letter of Credit.

If our bid is accepted, we will obtain an unconditional guarantee of a first class bank in the sum of ten percent of the Contract Price for the due performance of the Contract.

We agree to abide by this bid for a period of _____ days from the date fixed for bid opening under ITB Clause 16 of the Instructions to Bidders, and it shall remain binding upon us and may be accepted at any time before the expiration of that period.

Until a formal Contract is prepared and executed, this bid, together with your written acceptance thereof and your notification of award, shall constitute a binding Contract between us.

We understand that you are not bound to accept the lowest or any bid you may receive.

Dated this _____ day of _____, 19_____.

_____
Signature

_____
(in the capacity of)

Duly Authorized to sign bid for and on behalf of _____

Attachment.

Documentation to Accompany this Qualifications Form

a. Certified copy of current license in country of legal domicile as a primary manufacture of pharmaceuticals or vaccines.

b. Certified copy of license in country of manufacturer as a primary manufacturer of the range of pharmaceuticals or vaccines to be offered.

c. Duly authorized letter of authority for the Contract period from the manufacturer authorizing the agent to bid for and on behalf of the manufacturer, if applicable.

d. Full description of on-site quality assurance laboratory facilities and services, and the range of tests conducted.

e. A certified statement of satisfactory inspection of Good Manufacturing Practices for the last 5 years.

f. A list of major supply contracts completed within the last five years.

g. In addition the following detailed information regarding contracts from the above list valued in total at the equivalent of US$50,000. These would be large institutional contracts with national or private sector purchasers, e.g., WHO, UNICEF, etc., for developing countries over the past five years:

(1) Names and address of responsible persons in the purchasing organizations.

(2) Total value of each contract with dates of commencement and completion.

(3) Names, quantities and value of pharmaceuticals or vaccines supplied on each contract.

(4) Letters of reference from clients previously serviced, if available.

h. List of pharmaceuticals or vaccines being manufactured by the firm with information about product registration/license number and date.

i. A list of the pharmaceuticals or vaccines that the firm proposes to offer.

j. Audited financial statements for the past three years.

k. The original and three copies of this application and attachments.

Note: Documents in languages other than the language of the bid must include a translation into that language of all certificates in addition to the original untranslated document.

Form 2

Price Schedule for Items Offered Locally
(To be Completed by Group A or B Bidders)

Location: _____ Date: _____

Name of Bidder: _____ IFB Number: _____ Page ___ of ___

Address of Bidder: _____ Currency of Bid: _____

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Product Code	Product	Strength	Dosage Form	Unit Pack Size	Unit Price Ex-factory	Quantity Offered	Total Price	Estimated Delivery Time	*Domestic Value Added	Name of Manufacturer	Pharmacopoeial Standard	For Official Use	For Official Use

* The Domestic Value Added in the Manufacturing Process should be given as a Percentage of the Ex-factory Price.

SIGNATURE OF BIDDER: _____

DATE: _____

Form 3

Price Schedule for Items to be Imported
(To be completed by Foreign Suppliers or their Local Agents)

Location: _____ Date: _____

Name of Bidder: _____ IFB Number: _____ Page ___ of ___

Address of Bidder: _____ Currency of Bid: _____

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Product Code	Product	Strength	Dosage form	Unit Pack Size	Unit Price CIF Port of Entry	Quantity Offered	Total CIF Price	Estimated Lead Time	Name of Manufac-turer	County of Origin	Pharma-copoeial standard	For Offi-cial Use Only	For Official Use Only

SIGNATURE OF BIDDER: _____ DATE: _____

Form 4

Domestic Value Added Calculation Form

Name of Bidder:	Factory Location:

IFB Number: _____

- To be completed by manufacturers located in Purchaser's country only.
- To be completed for Goods manufactured in Purchaser's country which have at least 20 percent domestic value added in the ex-factory bid price.
- Manufacturers may be required to provide further evidence to verify domestic value-added claims, the amount of customs duty on finished Goods, and details about any associations established with foreign or local firms that would affect the manufacturing process.

Product: _____

Strength: _____

Dose: _____

Pack Size: _____

Ex-factory bid price: _____

	Component Cost			
Product Component	Imported	Local	Total	% of Local Component Cost to Ex-Factory Bid Price
Active Raw Material				
Excipients				
Packing				
Local Materials				
Local Labor				
Finance/Cost of Capital				
Estimated Duties and Taxes				
Overhead				
Profit				
Other				
TOTAL:				

Signature of Bidder: _____

Date: _____

Qualification Form

Capacity and Quality Certification from Relevant

Country Authority

IFB No. Date

1. Name of the Firm:

Address:

Telephone: Telex:

Telefax: Cable:

2. Name of Principals or Owner(s):

Address:

Telephone: Telex:

Telefax: Cable:

3. _____ (Name of firm) is properly registered to supply pharmaceuticals or vaccines in _____ _____ (Name of Country), is in good legal and statutory standing with the responsible health authorities in that country, and is licensed as a primary manufacturer of the range of pharmaceuticals or vaccines to be offered. (The list of items to be offered is attached.)

4. The production capacities for _____ (Name of Firm) follow:

The prequalified Installed Capacity for this firm is as follows:

	Annual Capacity Non-Sterile	Annual Capacity Sterile
Dry:	Tablets Capsules Sachets	Vials Bottles
Wet: (Liquids, and Colloids)	Internal Syrups Suppositories Aerosols	Ampoules I.V. Fluids
	External Liquids Creams Ointments	Drops/Ointments

5. _____ (Name of Firm) retains full records of production batches and quality control test results, and will exhibit these on request.

6. _____ (Name of Firm) has at least five years experience in the manufacturing of specific dosage forms it will bid on, and has two years or more experience in producing any product covered by this Invitation for Bids.

7. _____ (Name of Firm) has experience with and knowledge of modes of packaging, distribution, and transportation of pharmaceuticals or vaccines in countries similar to that of the Purchaser in terms of level of development, climate, etc. The following countries have been supplied pharmaceuticals or vaccines worth at least US$50,000 within the past five years:

_____

_____

_____

---

8. We hereby certify that the above information is true and accurate to the best of our knowledge. We understand that the provision of information that is later found to be false is sufficient justification for disqualification.

Signature: _____ Date: _____

Full Name (printed): _____

Position of Officer: _____

Form 6

Letter of Authority from Manufacturer

_____
(Name of Manufacturer)

To Whom it May Concern:

We _____ (Name of Manufacturer), a manufacturer duly organized under the laws of _____ (Name of Country) and having its principal place of business at _____ (Address of Manufacturer), hereby make, constitute and appoint _____ _____ (Name of Agent), a company duly organized under the laws of _____ _____ (Name of Eligible Source Country) and having its principal place of business at _____. _____ (Address of Trading Company), to be our true and lawful attorney in fact to do the following:

(1) To represent and bind us in the Purchaser's country for the Purchaser's Invitation for Bids (IFB) No. _____ for supply of the Goods proposed in the bid which we manufacture or produce.

(2) That, as a manufacturer, we bind ourselves as co-maker of the bid and are jointly and severally responsible for the compliance of the said bid.

(3) That we hereby give and grant to the said _____ (Name of Agent) full power and authority to do and perform all and every act and thing whatsoever, requisite, necessary and proper to be done in the premises, as fully, to all intents and purposes as we might or could do, with full power of substitution and renovation, hereby ratifying and confirming all that _____ (Name of Agent) or its duly authorized representative shall lawfully do, or cause to be done by virtue hereof.

IN TESTIMONY WHEREOF WE HAVE HERETO SIGNED THIS DOCUMENT ON_____ _____, 19___.

Accepted on _____, 19 __.

NAME OF TRADING COMPANY	NAME OF ISSUING MANUFACTURER
_____	_____
_____	_____
(Name of duly authorized representative to sign, rank or position)	(Name of duly authorized representative to sign, rank or position and department)

Bid Security Form

Whereas _____
(hereinafter called "the Bidder") has submitted its bid dated _____ for thesupplyof_____(hereinafter called "the Bid") _____
KNOW ALL MEN by these presents that WE _____ of _____ having our registered office at _____ (hereinafter called "the Bank") are bound unto _____ (hereinafter called "the Purchaser") in the sum of _____ for which payment well and truly to be made to the said Purchaser, the Bank binds itself, its successors and assigns by these presents. Sealed with the Common Seal of the said Bank this _____ day of _____, 19____.

THE CONDITIONS of this obligation are:

1. If the Bidder withdraws its Bid during the period of bid validity specified by the Bidder on the Bid Form; or

2. If the Bidder, having been notified of the acceptance of its bid by the Purchaser during the period of bid validity:

   (a) fails or refuses to execute the Contract Form, if required; or

   (b) fails or refuses to furnish the Performance Security, in accordance with the Instructions to Bidders;

we undertake to pay to the Purchaser up to the above amount upon receipt of its first written demand, without the Purchaser having to substantiate its demand, provided that, in its demand, the Purchaser will note that the amount claimed by it is due to it owing to the occurrence of one or both of the two conditions, specifying the occurred condition or conditions.

This guarantee will remain in force up to and including thirty (30) days after the period of bid validity, and any demand in respect thereof should reach the Bank not later than the above date.

(Signature of the Bank)

Form 8

Performance Security Form

To: _____ (Name of Purchaser) _____

To: _____ (Name of Purchaser) _____

WHEREAS _____

hereinafter called "the Supplier" has undertaken, in pursuance of Contract No. _____-_____ dated, _____ 19____ to supply (Description of Goods and Services) hereinafter called "the Contract."

AND WHEREAS it has been stipulated by you in the said Contract that the Supplier shall furnish you with a Bank Guarantee by a recognized bank for the sum specified therein as security for compliance with the Supplier's performance obligations in accordance with the Contract.

AND WHEREAS we have agreed to give the Supplier a Guarantee:

THEREFORE WE hereby affirm that we are Guarantors and responsible to you, on behalf of the Supplier, up to a total of (Amount of the Guarantee in Words and Figures) and we undertake to pay you, upon your first written demand declaring the Supplier to be in default under the Contract and without cavil or argument, any sum or sums within the limits of (Amount of Guarantee) as aforesaid, without your needing to prove or to show grounds or reasons for your demand or the sum specified therein.

This guarantee is valid until the _____ day of _____ 19____

Signature and Seal of the Guarantors

_____

_____

_____

Date _____

Address _____

Specimen of Letter of Credit Issued by the Purchaser

Date: _____

To: _____

This Letter of Credit is forwarded through _____

We open an irrevocable Letter of Credit No._____ in your favor by order of _____ - _____ (Name of the Purchaser).
For account of _____
To the extent of _____
Available against your drafts drawn at sight on _____ (Name of the Purchaser) (for account of _____) and permit the payments as follows:

1. Payment of 90 percent of the total Contract Price prorated as 90 percent of the value of each shipment on presentation of the following documents:

   A. Three original and two copies of clean on board Ocean Bill of Lading marked "freight prepaid" in CIF Contract and made out to order, bland endorsed and notifying the Purchaser's representative at the port of destination.

   B. Five copies of commercial invoice covering 90 percent of the relevant amounts for the delivered Goods of the Contract.

   C. Five copies of detailed packing list.

   D. Five copies of quality certificate issued by the manufacturer.

   E. Sight draft to be drawn on the Purchaser to the _____ _____ (bank nominated by the Purchaser).

   F. A copy of telex advising shipment according to GCC Clause 10 of this Contract.

   G. One original and four copies of insurance policy covering 110 percent of the total invoice value against all risks with the Purchaser as beneficiary.

   H. Five copies of certificate of country of origin issued by the Supplier.

2. Payment of 10 percent of the total Contract Price after the acceptance of all Contract Goods on presentation of the following documents:

   A. Five copies of commercial invoice.

   B. Five copies of acceptance certificate of the Contract Goods signed by the Purchaser and Supplier.

   C. Sight draft to be drawn on the Buyer to _____ (bank nominated by the Purchaser).

EVIDENCING SHIPMENT OF: Goods under Contract No: _____

An additional certificate issued by yourselves is required, certifying that details of all the documents presented under this Letter of Credit are in conformity with the terms of the above mentioned Contract.

Shipment from _____ to _____

Not later than _____

Partial shipment _____ allowed. Transshipment _____ allowed.

This Credit remains valid in _____ until _____ and all drafts must be marked that they are drawn under this Letter of Credit.

We hereby undertake that against presentation of the drafts and shipping documents drawn under and in compliance with the terms of this Credit, the same shall be negotiated subject to special instructions item 1 and 2 stated hereunder.

SPECIAL INSTRUCTIONS:

1. This Letter of Credit is established within the framework of the _____ _____ Project/Loan No. _____ financed by the World Bank. This Letter of Credit will become effective only if and when the World Bank has issued its Special Commitment to the said advising bank.

2. Negotiations of drafts under this Credit are restricted to the advising bank _____. Upon negotiation, the negotiating bank should claim reimbursement from _____ by cable provided all terms and conditions

stipulated herein have been complied with and simultaneously the negotiation bank should dispatch all documents to us in one lot by first available airmail.

For Banker's Office Division.

Contract Form

THIS AGREEMENT made _____ day of _____, 19___
Between (Name of Purchaser) of (Country of Purchaser) (hereinafter "the Purchaser") of the one part and (Name of Supplier) of (City and Country of Supplier) (hereinafter "the Supplier") of the other part:
WHEREAS the Purchaser is desirous that certain Goods and ancillary Services should be provided by the Supplier, viz, (Brief Description of Goods and Services) and has accepted a bid by the Supplier for the supply of those Goods and Services in the sum of (Contract Price in Words and Figures) (hereinafter "the Contract Price").

NOW THIS AGREEMENT WITNESSETH AS FOLLOWS:

1. In this Agreement words and expressions shall have the same meanings as are respectively assigned to them in the Conditions of Contract referred to.

2. The following documents shall be deemed to form and be read and construed as part of this Agreement, viz:

(a) the Bid Form and the Price Schedule submitted by the Bidder;
(b) the Schedule of Requirements;
(c) the Technical Specifications;
(d) the General Conditions of Contract;
(e) the GCC Data Sheet and Special Conditions of Contract; and
(f) the Purchaser's Notification of Award.

3. In consideration of the payments to be made by the Purchaser to the Supplier as hereinafter mentioned, the Supplier hereby covenants with the Purchaser to provide the Goods and Services and to remedy defects therein in conformity in all respects with the provisions the Contract.

4. The Purchaser hereby covenants to pay the Supplier in consideration of the provision of the Goods and Services and the remedying of defects therein, the Contract Price or such other sum as may become payable under the provisions of the Contract at the times and in the manner prescribed by the Contract.

IN WITNESS whereof the parties hereto have caused this Agreement to be executed in accordance with their respective laws the day and year first above written.

Signed, Sealed and Delivered by the

said _____ (For the Purchaser)

in the presence of: _____

Signed, Sealed and Delivered by the

said _____ (For the Supplier)

in the presence of: _____

ATTACHMENTS

A WHO GUIDELINES ON GOOD MANUFACTURING PRACTICE (GMP) AND CERTIFICATION SCHEME ON THE QUALITY OF DRUGS MOVING IN THE INTERNATIONAL COMMERCE

Trial Edition
September 1993

The Alphabetical List of Addresses of Competent Authorities of Participating Countries, and the Text of Replies of Participating Countries included in the original WHO Certification Scheme are not reproduced in this SBD. They are available upon request directly from WHO.

STANDARD BIDDING DOCUMENTS
PROCUREMENT OF PHARMACEUTICALS AND VACCINES
Trial Edition, September 1993

CORRIGENDA
to incorporate the Guidelines,
Procurement Under IBRD Loans and IDA Credits,
dated January 1995

The provisions in the Corrigenda result from modifications required in the bidding documents following the publication by the World Bank of the revised ***Guidelines: Procurement Under IBRD Loans and IDA Credits****, January 1995 edition. For procurement under World Bank-financed projects, Borrowers or Purchasers shall henceforth include the new provisions in their bidding documents, either in modifications to the concerned Clauses or in the form of Corrigenda.*

ITB Data Sheet

Substitute Item 5. ITB 12, with the following new provision:

5. ITB 12 *[If the Bidder is permitted to use foreign currencies for pricing and payment for goods and services from within the Purchaser's country, state the following:]*

Clause 12.1 (a) is not applicable and Clause 12.1 (b) applies to all bidders and the words "from outside the Purchaser's country" shall not apply.

Substitute Item 14. ITB 25 with the following new provision:

14. ITB 25.2 Currency chosen for the purpose of converting to a common currency.

[Specify either the local currency or a convertible currency, for example, U.S. dollars.]

Source of exchange rate.

[If the common currency is other than the local currency, for example, U.S. dollars, indicate the name of an internationally circulated newspaper that lists daily currency exchange rates (e.g., Financial

Times), *which will be used for converting prices in foreign currencies. For prices in local currency and if the common currency selected above is the local currency, specify either the central bank or a commercial bank in the Purchaser's country.]*

Date of exchange rate.

[Select a date that shall not be earlier than four (4) weeks prior to the original deadline for the receipt of bids, specified in ITB Clause 22.1, and no later than the original date for expiry of the period of bid validity, specified in ITB Clause 16.1.]

Section II. Instructions to Bidders

Insert a new Sub-Clause 1.2, as follows:

1.2 The Loan Agreement prohibits a withdrawal from the loan account for the purpose of any payment to persons or entities, or for any import of goods, if such payment or import, to the knowledge of the Bank, is prohibited by a decision of the United Nations Security Council taken under Chapter VII of the Charter of the United Nations.

Substitute Clause 2 with the following provisions:

2. Eligible Bidders

2.1 This Invitation for Bids is open to all suppliers from eligible source countries as defined in *Guidelines: Procurement under IBRD Loans and IDA Credits*, dated January 1995, hereinafter referred to as the *IBRD Guidelines for Procurement*, except as provided hereinafter.

2.2 Bidders should not be associated, or have been associated in the past, directly or indirectly, with a firm or any of its affiliates which have been engaged by the Purchaser to provide consulting services for the preparation of the design, specifications, and other documents to be used for the procurement of the goods to be purchased under this Invitation for Bids.

2.3 Government-owned enterprises in the Purchaser's country may participate only if they are legally and financially autonomous, if they operate under commercial law, and if they are not a dependent agency of the Purchaser.

Substitute Sub-Clause 3.1 with the following new provisions:

3.1 All goods and related services to be supplied under the contract shall have their origin in eligible source countries, defined in the *IBRD Guidelines for*

Procurement, and all expenditures made under the contract will be limited to such goods and services.

Substitute Sub-Clause 10.2 with the following new provisions:

10.2. For the purpose of granting a margin of domestic preference, bids will be classified in one of three groups, as follows:

(a) **Group A:** Bids offering goods manufactured in the Purchaser's country, for which (i) labor, raw materials and components from within the Purchaser's country account for more than thirty (30) percent of the EXW price; and (ii) the production facility in which they will be manufactured or assembled has been engaged in manufacturing or assembling such goods at least since the date of bid submission.

(b) **Group B:** All other bids offering goods from within the country of the Purchaser.

(c) **Group C:** Bids offering goods of foreign origin to be imported by the Purchaser directly or through the Supplier's local Agent.

Substitute Clause 12 with the following new provisions:

12. Bid Currencies

12.1 Prices shall be quoted in the following currencies:

(a) For goods and services that the Bidder will supply from within the Purchaser's country, the prices shall be quoted in the currency of the Purchaser's country, unless otherwise specified in the ITB Data Sheet.

(b) For goods and services that the Bidder will supply from outside the Purchaser's country, the prices shall be quoted in any currency of a Bank member country. If the Bidder wishes to be paid in a combination of amounts in different currencies, it may quote its price accordingly but use no more than three foreign currencies. For the purpose of this clause, the European Currency Unit (ECU) is also considered an eligible currency.

Substitute Sub-Clause 15.3 with the following new provisions:

15.3 The bid security shall be denominated in the currency of the bid or in another freely convertible currency, and shall be in one of the following forms:

(a) a bank guarantee or an irrevocable letter of credit issued by a reputable bank located in the Purchaser's country or abroad, in

the form provided in the bidding documents or another form acceptable to the Purchaser and valid for thirty (30) days beyond the validity of the bid; or

(b) a cashier's or certified check.

Insert at the end of Sub-Clause 22.2 the following sentence:

"No bid shall be rejected at bid opening, except for late bids, which shall be returned unopened to the Bidder pursuant to ITB Clause 20."

Insert a new Sub-Clause 22.4 as follows:

22.4 Bids (and modifications) that are not opened and read out at bid opening shall not be considered further for evaluation, irrespective of the circumstances. Withdrawn bids will be returned unopened to the bidders.

Substitute Clause 25 with the following new provisions:

25. Conversion to Single Currency

25.1 To facilitate evaluation and comparison, the Purchaser will convert all bid prices expressed in the amounts in various currencies in which the bid prices are payable to either:

(a) the currency of the Purchaser's country at the selling exchange rate established for similar transactions by the Central Bank or a commercial bank in the Purchaser's country;

or

(b) a currency widely used in international trade, such as U.S. dollars, at the rate published in the international press for the amount payable in foreign currency; and at the selling rate of exchange established for similar transactions by the Central Bank in the Purchaser's country for the amount payable in the currency of the Purchaser's country.

25.2 The currency selected for converting bid prices to a common base for the purpose of evaluation, along with the source and date of the exchange rate, are specified in the ITB Data Sheet.

Substitute Sub-Clauses 27.1 and 27.2 with the following new provisions:

27.1 The Purchaser will grant a margin of preference to goods manufactured in the Purchaser's country for the purpose of bid comparison, in accordance with the

procedures outlined in subsequent paragraphs, provided the Bidder shall have established to the satisfaction of the Purchaser and of the Bank that its bid complies with the criteria specified in ITB Clause 10.2 (a).

27.2 The Purchaser will first review the bids to confirm the appropriateness of, and to modify as necessary, the bid group classification to which bidders assigned their bids in preparing their Bid Forms and Price Schedules, pursuant to ITB Clauses 10 and 11.

Section III. A GCC Data Sheet and Special Conditions of Contract

Insert under Item 9 (a) the following provision:

Country of Origin (GCC Clause 3)

All countries and territories as indicated in Appendix 3 of the Corrigenda, "Eligibility for the Provision of Goods, Works, and Services in Bank-Financed Procurement".

Section III. B. General Conditions of Contract

Substitute Sub-Clause 3.1 with the following provisions:

3.1 All Goods and Services supplied under the Contract shall have their origin in the countries and territories eligible under the rules of the World Bank, as further elaborated in the GCC Data Sheet.

Section V. Schedule of Requirements

Replace the table Schedule of Requirements with the table attached herewith as Appendix 1.

List of Bidding and Contract Forms

Replace the table Price Schedule for Items offered locally (Form 2, page 74) and the Domestic Value Added Calculation Form (Form 4, page 76) with the tables attached herewith as Appendix 2.

ELIGIBILITY FOR THE PROVISION OF GOODS, WORKS, AND SERVICES IN BANK-FINANCED PROCUREMENT

Add the page attached herewith as Appendix 3 at the end of the bidding documents.

Section V

Schedule of Requirements

The delivery schedule expressed as weeks/months stipulates hereafter a delivery date which is the date of delivery (I) at EXW premises, or (ii) to the carrier at the port of shipment when the contract is placed on FOB or CIF terms, or (iii) to the first carrier when the contract is placed on FCA or CIP terms. In order to determine the correct date of delivery hereafter specified, the Purchaser has taken into account the additional time that will be needed for international or national transit to the Project Site or to another common place.[1]

Product Code or No.	Product Description	Strength	Required Quantity	Delivery Schedule (shipment) in weeks/months from [2]	Remarks

[1] The delivery may be specified for a single shipment, or for several partial shipments, for a specific date, or for a range of acceptable delivery periods.

[2] The Purchaser must specify here the date from which the delivery schedule will start. That date should be either the date of contract award, or the date of contract signature, the date of opening of the letter of credit, or the date of confirmation of the letter of credit, as appropriate. The Bid Form should include only a cross-reference to this Schedule.

Price Schedule for Items Offered Locally
(To be Completed by Group A or Group B Bidders)

Location: _____
Name of Bidder: _____
Address of Bidder: _____

Date: _____
IFB Number: _____ **Page** ___ **of** ___
Currency of Bid: _____

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Product Code	Product	Strength	Dosage Form	Unit Pack Size	Unit Price Ex-Factory	Quantity Offered	Total Price	Esti-mated Delivery Time	*Cost of local labor, raw material and compon-ents	Name of Manu-facturer	Pharma-copoeial Standard	For Official Use	For Official Use

*Indicated as a Percentage of the Ex-Factory Price

Signature of Bidder: _____
Date: _____

Cost of Local Labor, Raw Material and Components Calculation Form

Name of Bidder:	Factory Location:

IFB Number: _____

- To be completed by manufacturers located in Purchaser's country only.
- To be completed for Goods manufactured in Purchaser's country which have at least 30 percent of local labor, raw material and components in the ex-factory bid price.
- Manufacturers may be required to provide further evidence to verify claims regarding the content of local labor, raw material and components, the amount of customs duty on finished Goods, and details about any associations established with foreign or local firms that would affect the manufacturing process.

Product: _____

Strength: _____

Dose: _____

Pack Size: _____

Ex-factory bid price: _____

	Item Cost			
Product Item	Imported	Local	Total	% of Local Component Cost to Ex-Factory Bid Price
Active Raw Material*				
Excipients*				
Packing*				
Local Materials*				
Local Labor*				
Finance/Cost of Capital				
Estimated Duties and Taxes				
Overhead				
Profit				
Other				
TOTAL:				

Components marked with an * will be the only input considered for domestic preference; their total as a percentage of the total price shall account for more than 30 percent.

Signature of Bidder: _____

Date: _____

Appendix 3

Public Information Center

ELIGIBILITY FOR THE PROVISION OF GOODS, WORKS AND SERVICES IN BANK-FINANCED PROCUREMENT

as of January 1995

For the information of borrowers and bidders, and with reference to paragraph 1.6, footnote 9, of the *Guidelines: Procurement under IBRD Loans and IDA Credits*, dated January 1995, set forth below is a list of countries from which bidders, goods and services are not eligible to participate in procurement financed by the World Bank or IDA.[1]

- Andorra
- Brunei Darussalam
- Cuba
- Democratic People's Republic of Korea (North Korea)
- Liechtenstein
- Monaco
- Nauru
- San Marino
- Tuvalu

In addition, bidders, goods and services from other countries or territories may be declared ineligible by a provision in the bidding documents if the borrower's country has excluded them by a law, official regulation, or act of compliance meeting the requirements of paragraph 1.8 (a) of the *Guidelines: Procurement under IBRD Loans and IDA Credits*.

[1] Any questions regarding this list should be addressed to the Chief, Procurement Policy and Coordination Unit, Operations Policy Department, the World Bank.

Special Clauses for use with World Bank *Standard Bidding Documents for Procurement of Pharmaceuticals and Vaccines,* Trial Edition, September 1993 and Corrigenda 1995 (Supplements VII.K.1. and VII.K.2.)

The following "special treatment" clauses take into consideration the unique qualities and requirements of vaccine. They are based on Limited International Bidding (LIB) rather than International Competitive Bidding (ICB) and pre-qualification of the vaccine rather than pre-qualification of the vaccine Supplier. Prospective bidders are limited to authorised suppliers of vaccines that have been licensed by the National Control Authority of the Purchaser's country. Editors notes provide comment and clarification as necessary. A supplementary general specification for vaccine is offered in Part IV of this manual. All of the clauses are numbered with the Trial Edition section and pages to which they apply, including the page number of the data sheet and the page number of the referenced clause.

Section I - Invitation for Bids (IFB)

Page 1, Paragraph 2 of 6:

"give brief description of the pharmaceuticals and vaccines to be procured"

insert: supply of ***[—named—] vaccine produced under the control of a recognized, National Control Authority which performs all six critical functions for control of biological products as defined by the World Health Organization:***

1. ***licensing based on published set of requirements***
2. ***surveillance of vaccine field performance***
3. ***system of lot release for vaccines***
4. ***use of laboratory when needed***
5. ***regular inspections for GMP (Good Manufacturing Practices)***
6. ***evaluation of clinical performance***

Page 2, Paragraph 4:

"A complete set of Bidding Documents may be purchased by any interested eligible Bidder on the submission of a written application to the above and upon payment of non-refundable fee of $____."

Add: ***For vaccine procurement under LIB procedures, Bidding Documents are provided with this ITB at no charge.***

Section II - Instructions to Bidders (ITB) Data Sheets

Page 8, Line No. 6, Clause Ref. ITB 13.5 (appearing on page 19):

The following additional qualification criteria will also apply:

Insert: ***For vaccine purchases, the Bidder must furnish a certificate from the NCA in the Manufacturer's country stating that the Manufacturer of the vaccine covered by this Invitation for Bids is licensed to manufacture these products.***

Page 10, Modifications to the Instructions to Bidders, Line No. 15, Clause Ref. ITB 14.1 (appearing on page 19):

Add: ***Documentary evidence that the offered vaccine has been licensed by the NCAs of both the Manufacturer's country and the Purchaser's country shall accompany bids.***

Page 10, Modifications to the Instructions to Bidders, Line No. 16, Clause Ref. ITB 14.2 (appearing on page 19):

Replace existing text with: ***If the vaccines offered have not been licensed by the NCA in the Purchaser's country, the Bidder shall furnish evidence that such licensing application has been submitted, along with all required fees and documents. If there is no NCA with specific biologics expertise in the Purchaser's country, the Bidder shall furnish evidence that the vaccines offered meet the qualification criteria listed in Part B, General Technical Specifications, Section 1 (page 62). The documentary evidence of the Goods' and Services' eligibility also shall include a statement in the Price Schedule on the country of origin of the Goods' and Services' offered which shall be confirmed by a Certificate of Origin issued at the time of shipment.***

Page 10, Modifications to the Instructions to Bidders, Line No. 17, Clause Ref. ITB 14.3 (appearing on pages 19 and 20):

Modify existing text as follows: The documentary evidence of the Goods' and Services' conformity to the Bidding Documents may be in the form of literature, ~~and~~ data and ***copies of relevant licenses*** and shall furnish:

a) a detailed description of the Goods' essential ***biological*** ~~chemical~~ characteristics;
b) a clause-by-clause commentary on the Purchaser's Technical Specifications demonstrating the Goods' substantial responsiveness to those specification or a statement of deviations and exceptions to the provisions of the Technical Specifications.
c) a sample of ~~the pharmaceuticals or~~ vaccines listed and in the quantities specified in the ITB Data Sheet ~~for quality testing purposes~~ ***for examination of primary packaging and labelling.*** The cost of providing such a sample will be to the Bidder's account.

Page 10, Modifications to the Instructions to Bidders, Line No. 18, Clause Ref. ITB 14.4 (appearing on page 20):

Add: ***Any proposed substitution of vaccine must comply with the licensing requirements and qualifications criteria listed in Part B, General Technical Specifications, Section 1 (page 62).***

Page 10, Modifications to the Instructions to Bidders, Line No. 19, Clause Ref. ITB 29.1 (appearing on page 29):

Modify existing text as follows: Based on the qualification criteria listed in ITB Clause**s** 13 ***and 14*** and the ITB Data Sheets, the Purchaser will determine to its satisfaction whether the Bidder selected as having submitted the lowest evaluated responsive bid is qualified to satisfactorily perform the Contract.

Page 10, Modifications to the Instructions to Bidders, Line No. 20, Clause Ref. ITB 29.2 (appearing on page 29):

Modify existing text as follows: The determination will take into account the Bidder's financial, ***and*** technical, ~~quality control~~, ~~and production~~ capabilities ***and the licensing status of the offered vaccine.*** It will be based upon an examination of the documentary evidence ~~of the Bidder's qualifications~~ submitted by the Bidder, pursuant to ITB Clauses 13 ***and 14,*** as well as such other information as the Purchaser deems necessary and appropriate.

<u>Section III - General Conditions of Contract (GCC) Data Sheet and Special Conditions of Contract</u>

Page 36, Line No. 9.a, GCC Clause 8.1 (appearing on page 41):

Modify existing text as follows: The Purchaser or its representative shall have the right to inspect and~~/or to~~ test…

…The Goods shall not be shipped unless a satisfactory inspection and quality assurance report is provided to the Supplier ***as a result of inspections and tests conducted by the Purchaser or its representative.***

Page 36, Line No. 9.b, GCC Clause 10.1 (appearing on page 43):

Add: ***All vaccine must be shipped via air.***

Page 36, Line No. 9.c, GCC Clause 10.2 (appearing on pages 43 and 44):

Modify existing text as follows: For Imported Goods: Immediately on shipment of the contracted Goods, the Supplier will advise the Purchaser by telex, fax or cable of the following details:

(i) Name of ***carrier and flight number***

(iv) ***Description and*** quantity of Goods on board

(vi) ***Air Waybill number***

(vii) Expected time of arrival ***and*** port of discharge

Page 36, Line No. 9.d, GCC Clause 10.3 (appearing on page 44):

Replace existing text of (i) and (iv) with the following: The Supplier will also dispatch to the Purchaser one set of the following documents by courier service and another set through the Master of the vessel, aircraft or other means of transport:

(i) One copy of Air Waybill (marked "freight prepaid" in CIF, CFR or CIP contracts)"

(iv) Original and six copies of satisfactory inspection and quality assurance certificate furnished to Supplier by the nominated inspection agency of Purchaser

Add:

(xi) One copy of the Lot Release Certificate issued by the National Control Authority of the country of manufacture for each lot shipped.

Page 36, Line No. 9.e, GCC Clause 10.4 (appearing on page 45):

Add:

(vi) Packing list

(vii) Certificate of in-house analysis

(viii) Copy of Lot Release Certificate issued by the National Control Authority of the country of manufacture for each lot shipped.

Page 36, Line No. 9.f, GCC Clause 14.2 (appearing on page 48):

Modify existing text as follows: The Purchaser shall have the right to make claims under the above warranty ***for 12 months*** after the Goods have been delivered to the final destination indicated in the Contract.

Page 36, Line No. 9.g, GCC Clause 14.5 (appearing on page 49):

Move text from Part B-General Technical Specification, "4. Recalls" appearing on page 63 to GCC 14.5: If products must be recalled because of problems with product quality or adverse reactions to the vaccine, the Supplier will be obligated to notify the Purchaser, providing full details about the reason leading to the recall, and shall take steps to replace the product in question at its own cost with a fresh batch of acceptable vaccines, or withdraw and give a full refund.

Add to existing text: ***If an Adverse Event Following Immunization (AEFI) occurs in the Purchaser's country and the cause of such event cannot be***

immediately established, the Purchaser will, with all urgency and in accordance with the procedures laid down by the NCA of the Purchaser's country, take steps to advise the Supplier in order that an investigation may be launched immediately. If the vaccine has been supplied through an agency of the United Nations, the procedure laid down by the WHO for such situations will be used.

Page 36, Line No. 9.h, GCC Clause 15.3 (appearing on page 50):

Delete the existing text. This clause conflicts with GCC 15.1.

Section IV - Technical Specifications

Page 61, Part A - Specific Products Covered by this IFB Section IV:

"No. of Units in Pack" column should read *"**Doses Per Vial/Ampoule**"*

Page 62, Part B - General Technical Specifications:

Editors' note: See attached General Technical Specifications for Vaccine, located on pages 945-949.

Section V - Schedule of Requirements

Page 68

"Strength" column is not applicable for Vaccines.

Section VI - Bidding and Contract Forms and Attachments

Page 70, Bid Form, first paragraph of letter:.

Substitute: "deliver the ***vaccine*** specified " for "deliver the drugs specified"

Page 72, Documentation to Accompany this Qualifications Form:

Replace existing text with:

b. Certified copy of license issued by the National Control Authority of the country of manufacture for primary manufacture of the vaccines to be offered AND copy of vaccine license/registration issued by NCA of Purchaser's country.

Pages 74 and 75, Price Schedules - Forms 2 & 3:

Unit Pack Size shall be defined as adult or infant doses per vial or ampoule as appropriate

Editors' note: Where shipments of vaccine in bulk or in large volumes are undertaken the "pack size" will be a matter of negotiation between the Purchaser and the Supplier.

Page 76, Domestic Value Added Calculation - Form 4:

Pack Size shall be defined as adult or infant doses per vial or ampoule as appropriate for the vaccine. *See above for the note regarding shipments of vaccine in bulk.*

Page 76, Domestic Value Added Calculation - Form 4, Product Components Chart:

Substitute: "***Source Material***" for "Active Raw Material" and "***Preservatives, Adjuvant, Diluent***" for "Excipients"

Add: "***Quality Assurance Costs***"

Page 77, Qualification Form - Form 5, page 2 of 3:

In item 4, under Annual Capacity Sterile column, add:
Dry: "***ampoules***"
Wet: "***vials***"

Page 84, Specimen of Letter of Credit Issued by the Purchaser - Form 9:

1.A., substitute: "...two copies of ***Air Waybill*** marked "freight prepaid" in ***CIP*** Contract..."

Insert after H:

I. [number] copies of lot release certificate issued by NCA in country of manufacture

J. [number] copies of certificate of satisfactory pre-shipment inspection and quality assurance report issued by Purchaser or its representative.

Editors' note: at existing 1.A., second line should read "blank" endorsed, not "bland" endorsed.

General Technical Specification for Vaccine

1. Product Qualification Requirements

1.1 The vaccines to be purchased by the Purchaser under this Invitation for Bids must be produced under the control of a recognized, well functioning National Control Authority for biologics which performs all six critical functions as defined by the World Health Organization:

(i) licensing based on published set of requirements

(ii) surveillance of vaccine field performance

(iii) system of lot release for vaccines

(iv) use of laboratory when needed

(v) regular inspections for GMP (Good Manufacturing Practices)

(vi) evaluation of clinical performance

1.2 The vaccines to be purchased by the Purchaser under this Invitation for Bids must be produced in accordance with internationally recognized Good Manufacturing Practices such as WHO Good Manufacturing Practices for Biological Products.

1.3 [The vaccine to be purchased by the Purchaser under this Invitation for Bids must be licensed by the National Control Authority (NCA) of the Purchaser's country]

2. Product Specifications

2.1 Dosage form (for example: Oral or Injectable; Liquid or Freeze Dried with sterile diluent packed separately, etc.)

2.2 Type (for example: "live attenuated", "manufactured from purified inactivated […]obtained from human plasma or manufactured using recombinant DNA technology", etc.)

2.3 Administration (for example: "intended for intramuscular injection", etc.)

2.4 Description of intended use (for example: "immunization of newborn infants", etc.)

2.5 Dosage size (if not restrictive), or expected immunogenic reaction (for example: each dose shall contain that amount of Hbsag protein with micrograms/ml specified by the manufacturer for newborn dosage, that when given as part of a primary immunization series [3 doses], is capable of producing specific humeral antibody [anti HBs] at a level of at least 10 milli international units in >-90 percent of recipients", etc.)

2.6 Dose package (for example: "5 infant dose sterile glass vials", etc.)

2.7 Filling volume (for example: "final product should contain 15% overfill", etc.)

2.8 Closures (for example: "vaccine vials shall be fitted with closures that conform to ISO standard 8362-2")

2.9 Storage Temperature (for example: "2-8 degrees C. Do not freeze", etc.)

2.10 Shelf life (for example: "at the time of delivery to the country of destination, eighteen months of shelf life shall remain", etc. *Editor's note: This varies with the vaccine. Polio, for example, has a maximum shelf life of 12 months at the correct storage temperature.*)

2.11 Stability (for example: "stable for a minimum of two years after production at 2 to 8 degrees C", etc.)

2.12 Standards (for example, "The vaccine should conform to standards established by the Purchaser's country or, where no standard has been adopted, meet current requirements published by the WHO Expert Committee on Biological Standardization, or requirements of an established body of equivalent stature such as the US Pharmacopoeia, the British Pharmacopoeia, the French VIPAL Pharmacopoeia, or the International Pharmacopoeia".)

3. Labelling Requirements

3.1 Each vial or ampoule shall carry the manufacturer's standard label in [...*language of Purchaser's country*...], if available, at no extra charge; otherwise, the label shall be in English.

3.2 Each vial or ampoule label shall state the name of the vaccine, name of the manufacturer, place of manufacture, lot number, composition, concentration, dose and mode for administration, expiration date, storage temperature and any other information that is appropriate.

3.3 All labelling shall withstand immersion in water and remain intact.

4. Packing Requirements

4.1 Inner Boxes: Inner boxes shall contain not more than [*number*] individual [*vials/ampoules*] and shall be constructed of sturdy white cardboard outfitted with individual segments for protecting and separating each [*vial/ampoule*].

4.2 Printed Materials: Each inner box shall contain at least [*number*] manufacturer's standard package inserts in [*language of Purchaser's country*] if available at no extra charge; otherwise, package insert shall be in English. *(Editor's note: See 3.1 above.)*

4.3 Over-packing: Inner Boxes shall be over-packed so that the vaccine remains refrigerated as designated in Section 2.9 above. The over-packing must be suitable for export handling and be in accordance with WHO EPI Guidelines on International Packaging and Shipping of Vaccines including all measures needed to maintain required temperatures for 72 hours. It must have adequate insulation and sufficient refrigerant to ensure that the warmest storage temperature of the vaccine does not rise above that designated in Section 2.9 when exposed to continuous outside ambient temperature of +43 degrees C, nor fall below that specified in Section 2.9 when exposed to continuous outside ambient temperature of -20 degrees C during transit and for a period of at least 24 hours after arrival at the airport of destination. Additional cushioning shall be provided sufficient to protect the [*vials/ampoules*] from breakage during transit and handling.

4.4 Exterior Shipping Cartons: Product and printed materials, packaged as described above, shall be packed in weather-resistant, triple-wall corrugated fibreboard cartons with a bursting test strength of not less than 1,900 kPa. The overall dimensions of the exterior shipping cartons should be such that the product does not become damaged during transportation and storage.

No shipping carton should contain vaccine from more than one lot.

4.5 Cold Chain Monitor Cards: Each insulated shipping container must include appropriate temperature monitoring devices designated by the Buyer.

a) At least two suitable cold chain monitor cards, as approved by the Purchaser, shall be packed in each transport case of vaccine.

b) Freeze watch indicators shall be included in each transport case at the direction of Purchaser.

5. Marking Requirements

5.1 All containers and invoices must bear the name of the vaccine, expiation date of the vaccine and appropriate storage temperature.

5.2 Inner Boxes: The inner boxes containing vaccine [*vials/ampoules*] shall be marked with the following information in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Manufacturer's name and registered address
- Manufacturer's national registration number
- Lot or batch number
- Composition and concentration
- Number of vials contained in box
- Expiration date (month and year)
- Instructions for storage and handling
- Place of manufacture (Made in _____)

5.2 Exterior Shipping Cartons: The following information shall be stencilled or labelled on the exterior shipping cartons on two opposing sides in bold letters at least 30mm high with waterproof ink in a clearly legible manner which is acceptable to the Purchaser:

- Generic name and trade name of the vaccine
- Lot or batch number
- Expiration date (month and year)
- Manufacturer's name and registered address
- Manufacturer's national registration number
- Destination airport and routing
- Consignee's name and address in full
- Consignee contact name and telephone number
- Number of [vials] [ampoules] contained in the carton
- Gross weight of each carton (in kg)
- Carton #___ of ___
- Instructions for storage and handling
- Contract number
- Place of manufacture (Made in ____)

6. Expiration Date

All vaccine must indicate the expiry date; the manufacturing date is not required.

Unless otherwise stated in *Part A* of these Specifications, all vaccine must arrive at the port of entry with a remaining shelf life of at least 18 months.

7. Quality Assurance for Supply

7.1 All vaccine must:

a) meet the requirements of manufacturing legislation and regulation of vaccines in the country of origin,

b) meet internationally recognized standards for safety, efficacy and quality;

c) conform to all the specifications and related documents contained herein;

d) be fit for the purposes expressly made known to the Seller by the Purchaser;

e) be free from defects in workmanship and materials and

f) be certified by a competent authority in the manufacturer's country according to resolution WHO 28-65B, of the World Health Organization "Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce".

7.2 The successful Bidder will be required to furnish to the Purchaser with each consignment:

a) A certificate of quality assurance and test results in conformity with the WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce

b) Assay methodology of any or all tests if requested

c) Evidence of bio-availability and/or bio-equivalence for certain critical pharmaceuticals or vaccines upon request

d) Evidence of basis for expiration dating and other stability data concerning the commercial final package upon request

7.3 Pre-shipment Inspection and Testing: The successful Bidder will be required to provide the Purchaser or his representative with access to the product as packed for shipment at the Seller's factory and/or warehouse at a mutually agreeable time prior to shipment of the product.

a) The Purchaser may inspect and sample, or cause to be sampled, such product.

b) The Purchaser may cause independent laboratory testing to be performed as deemed necessary to assure that the goods conform to prescribed requirements. Testing laboratory shall be of the Purchaser's choice and suitably equipped and qualified to conduct quality assurance tests on biological products.

Editors' note: Vaccines are not normally sampled in their finished form as tests do not provide assurance of quality. Laboratory tests may be carried out in exceptional circumstances to verify potency and confirm that the vaccines have been correctly stored, i.e. , that they have remained in the cold chain.

ACRONYMS

BCG	Bacillus Calmette Guerin vaccine (for tuberculosis)
CDC	United States Centers for Disease Control and Prevention
CIF	Cost, Insurance and Freight
CIP	Carriage and Insurance Paid*
CFR	Cost and Freight*
CPT	Carriage Paid To*
DAF	Delivered at Frontier*
DDP	Delivered Duty Paid*
DDU	Delivered Duty Unpaid*
DEQ	Delivered Ex Quay*
DES	Delivered Ex Ship*
DPT	Diphtheria, pertussis, tetanus vaccine (U.S. terminology)
DTP	Diphtheria, tetanus, pertussis vaccine
EPI	Expanded Programme on Immunization
ETA	Estimated time of arrival
EXW	Ex Works*
FCA	Free Carrier*
FAS	Free Alongside Ship*
FOB	Free On Board*
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GPV	Global Programme for Vaccines and Immunization
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B Virus
HIV	Human immunodeficiency virus
IBRD	International Bank for Reconstruction and Development
ICB	International Competitive Bidding**
ICC	International Chamber of Commerce
IDA	International Development Association
IFPMA	International Federation of Pharmaceutical Manufacturers Association
IMR	Infant Mortality Rate
ISO	International Organisation for Standardisation
ITB	Invitation to Bid
L/C	Letter of Credit
LCB	Local Competitive Bidding**
LIB	Limited International Bidding**
MOH	Ministry of Health
NCA	National Control Authority
NCB	National Competitive Bidding
NCL	National Control Laboratory
NID	National Immunization Day
NRA	National Regulatory Authority

* See definitions in INCOTERMS, Part VI.D.

** See discussion in Part I.A.

OPV	Oral Polio Vaccine
PATH	Program for Appropriate Technology in Health
PO	Purchase Order
QA	Quality Assurance
QC	Quality Control
RFQ	Request for Quotation**
TT	Tetanus toxoid
UCP	Uniform Customs and Practice for Documentary Credits
UN	United Nations
UNICEF	United Nations Children's Fund
USFDA	United States Food and Drug Administration
VAT	Value added tax
VII	Vaccine Independence Initiative
VSQ	Vaccine Supply and Quality
VVM	Vaccine vial monitor
WHO	World Health Organization
W/V	Weight to volume

GLOSSARY

Accountee A legal term used in banking to describe the party who is responsible for paying an amount guaranteed through a commercial "Letter of Credit".

Agent A supply term for an independent contractor authorised by a manufacturer to promote and sell the manufacturer's products within a designated geographic area. Often an agent will contract to represent several manufacturers of non-competing products.

Air Waybill A shipping document issued by air freight carriers that contains a description of the commodity being shipped, shipping instructions, terms and conditions of the shipment and applicable transportation charges.

Batch A manufacturing term meaning a single, uniform and homogeneous quantity (of vaccine) produced from one compounding formulation, in one manufacturing and production operation and which has received entirely the same processing treatment. Used interchangeably with (production) Lot.

Batch Number See "Lot or Batch Number".

Beneficiary A legal term used in banking to describe the party who is entitled to collect funds guaranteed by a commercial Letter of Credit upon presentation of stipulated documents, usually shipping and quality assurance documents.

Bid A procurement term describing an offer for goods or services submitted or received in response to an Invitation to Bid.

Bidder An individual or organisation making an offer to sell goods or services, usually in response to an Invitation to Bid or another form of solicitation.

Bid Security A procurement term describing a financial instrument that is sometimes used to guarantee compensation to the prospective buyer for inconvenience and expense if a winning bidder rescinds his offer after the bid is closed and an award is made to him. Each bidder provides an amount stated in the bidding documents with their bid submission; bid security is refunded promptly to all unsuccessful bidders.

Bill of Lading A shipping document issued by a carrier (usually an ocean freight line) to a shipper that provides a written receipt for the goods, describes the conditions on which transport is made and includes a written commitment to deliver goods at a stated destination to the lawful holder of the Bill of Lading.

Biologicals A term used in medicine, immunization and regulatory circles describing products that have been produced with living organisms; for example, vaccine and certain laboratory materials.

Biologicals Committee A regulatory/quality assurance term referring to a sub-group of the national medicines control body with special responsibilities and expertise in vaccines and other biologicals.

Buffer Stock A term used in vaccine supply to describe extra quantities of stock kept on hand to cover unanticipated shortages—25% above expected usage is common.

Bulk Vaccine A manufacturing term that describes the homogenous container of finished vaccine before it is packaged into vials or ampoules. See "Final Bulk".

Buyer Also referred to as Purchaser. A procurement/contractual term used to define one of the parties to a purchase transaction; normally Buyer and Seller. The Buyer does not necessarily have to be the recipient or consignee.

Campaign See "Mass Campaign".

Census Data Statistics gathered about individuals in a national population; primarily numbers. Used by immunization programmes to estimate annual vaccination requirements and, thus, determine the amount of vaccine needed to meet these requirements. The most important numbers are births cohorts (individuals born in a particular year) and birth rates.

Certificate of Free Sale See "Lot Release Certificate".

Certificate of Inspection A document often required with shipments of perishable or other goods; certification attests to the good condition of the merchandise immediately prior to shipment.

Certificate of No Objection See "No Objection Certificate".

Certificate of Origin A shipping and import document declaring and certifying the country of origin of the merchandise; required by certain for-

eign countries for tariff purposes. It sometimes requires the signature of the consul of the country to which it is destined.

Clean Report of Findings A certificate issued by an inspection company stating that no discrepancies were found between specified criteria and the vaccine as prepared for shipment. Pre-shipment inspection at the manufacturer's facility is recommended for vaccine shipments. Some importing countries require routine (cursory) visual inspections at the port of loading.

Cold Chain A system to ensure the potency of a vaccine from the time of manufacture until it is given to a child or adult by maintaining the temperature of the vaccine within the correct range for the vaccine. All vaccines are sensitive to too much heat and some are sensitive to freezing.

Cold Chain Monitor A device, usually a printed card, that is included with shipments of vaccine to indicate if the temperature has remained within safe bounds.

Commercial Invoice A shipping document issued by the Seller, that identifies the Buyer, and provides a description of the goods, the agreed price, delivery and payment terms, shipping date, mode of transport and an assigned invoice number.

Commercial Merit A procurement term that represents a judgement as to whether or not, or how well, a company making an offer or bid meets requirements for stability, financial viability, good references, etc.

Commodity Any piece of tangible property, supplies or equipment that is the subject of a procurement activity; commonly used to describe consumable products.

Competitive Bidding Procurement process in which clearly stated product specifications and contract requirements are issued to multiple suppliers to solicit pricing and performance responses (bids). The purpose is to generate competition among several suppliers which theoretically elicits the lowest possible prices. There are several types of competitive bidding procedure. See Limited International Bidding, International Competitive Bidding and Local Competitive Bidding.

Consignee A term used in shipping goods that describes the party to whom something is entrusted, e.g., the "ship-to" party.

Contractual Merit A procurement term that represents a judgement as to whether or not, or how well, a bid matches the contract requirements of the Purchaser such as payment terms, delivery date, etc.

Correspondent Relationship A banking term that describes the relationship between two banks when they have formally agreed to perform services for each other.

Coverage A vaccination programme term for the estimated number of individuals actually vaccinated as a percentage of the target population.

Defects - Critical, Major, Minor Quality assurance terminology used in evaluating vaccine appearance, packaging and packing through visual examination and comparison with a precise description of requirements. The examination results in a classification of any defects according to importance. There are published standards for how many defects can be allowed in a particular lot size under different assumptions.

Direct Purchase Used in EPI systems to mean purchasing vaccine (and other items) directly from a manufacturer rather than through UNICEF or another third party. More broadly used to describe purchasing an item from a pre-selected source without considering competitive offers.

Domestic Preference Allowance A term used in World Bank procurement documents to describe a competitive advantage, expressed in a percentage, that is sometimes given to local manufacturers of goods competing for contracts against international sources. It is not appropriate for vaccine procurement because there are very few local manufacturers who meet quality requirements.

Entity A business and legal term to describe something that exists and functions as a separate and distinct body, for example, a corporation, a ministry of health or a committee.

Estimate of Procurement Requirements A judgement or approximate calculation of future commodity needs; quantification based on forecast of use plus buffer stock requirements less existing stock and undelivered purchases.

Expanded Programme on Immunization (EPI) A programme initiated by the World Health Organization in 1974 and adopted by many countries since then. It is aimed at achieving universal childhood immunization against six initial target diseases: diphtheria, tetanus, pertussis (whooping cough), polio, measles and tuberculosis. The basic EPI vaccines are BCG (Bacillus of Calmette and Guerin for immunization against tuberculosis), DTP (diphtheria-tetanus-pertussis and variations), measles and OPV (oral polio vaccine). EPI-Plus adds yellow fever and hepatitis B to the initial target diseases.

Expiry Date A supply term for a date established by the manufacturer that appears on a drug or vaccine, beyond which the manufacturer

will not guarantee the potency, purity, uniformity or bio-availability of the product.

Final Bulk (Vaccine) A manufacturing term that describes the final homogenous vaccine present in a single container from which the final vials or ampoules are filled, either directly or through one or more intermediate containers.

Final Lot A manufacturing term that describes a collection of sealed final containers (vials or ampoules) that are homogeneous with respect to the risk of contamination during filling. A final lot is filled from a single container in one continuous working session.

Forecast of Use A term used in vaccination programmes to describe a rational projection of future commodity demand based on population, birth rate and past consumption data.

Good Manufacturing Practice (GMP) An organized set of activities and performance standards covering personnel, premises and equipment, animal quarters and care, production, labelling, lot processing records and distribution records, quality assurance and quality control. A facility where GMP is followed can be relied upon to consistently produce good quality products that conform to established specifications because it maintains high standards of performance and adheres to written procedures.

Immunization Policy Commonly used to describe the delivery strategies, schedules, guidelines and recommendations that a country and/or immunization initiative or programme are following.

Immunization Session An EPI expression that refers to a time interval starting from removal of a quantity of vaccine from storage, through its administration to individuals and ending at disposal or re-storage of the remaining quantity.

Immunization Strategy A term relating to how vaccination will be carried out; the two main strategies are routine immunization (infants and children are vaccinated according to a standardised schedule for delivery of specific antigens at specific ages) and mass campaign (see below).

INCOTERMS International rules for the interpretation of the most commonly used terms in foreign trade published by the International Chamber of Commerce (ICC). The terms most commonly associated with vaccine procurement are:

CIP: Carriage and Insurance Paid to (…named place of destination). Seller must clear goods for export and pay all costs and transportation plus insurance necessary to deliver to des-

tination named by Buyer. Buyer bears all risks from the time goods are delivered into custody of the first carrier.

CPT: Carriage Paid To (…named port of destination). Seller must clear goods for export and pay for all costs and transportation necessary to deliver to place of destination named by Buyer. Buyer bears all risks from the time goods are delivered into custody of first carrier.

CIF: Cost, Insurance and Freight. Seller pays these costs. Old term, but still seen in practise.

Inspection Criteria In the context of vaccine procurement and quality assurance, a term for the set of instructions and related specifications against which an inspection agent will examine a shipment of vaccine, usually before it leaves the manufacturer's site.

International Chamber of Commerce (ICC) A non-governmental organisation that serves world business by harmonising trade practices, formulating terminology and establishing guidelines for importers and exporters.

International Competitive Bidding (ICB) A procurement method that is initiated with a widely advertised notice of the bidding opportunity. Sealed bids are required based on clearly stated product specifications and performance expectations. Submissions are evaluated on their technical, commercial, contractual and financial merit, with awards going to the Supplier making the most advantageous and cost-effective offer. All bids are final and no negotiation is allowed, except in regard to minor contractual points, after selection of a winning bid. The objective of the ICB is to provide all eligible prospective bidders with an equal opportunity to participate in the competition. Also known as open or unrestricted bidding.

International Shopping A term used by the World Bank and others to describe a procurement process that relies on informal quotations and catalogue pricing to establish a minimum level of competition. See "Request for Quotation".

Invitation to Bid (ITB) A procurement term that describes the formal written document used to solicit offers for the supply of specified goods and/or services. In addition to detailed specifications, it contains contractual conditions that apply to the proposed procurement and instructions to the Bidders. Used with International Competitive Bidding (open/unrestricted bidding) and Limited International Bidding (restricted bidding).

Labelling Used in the context of vaccines and medicines to describe written text on vials or ampoules, on boxes containing vials or ampoules and in accompanying leaflets. Labelling is consid-

ered an important part of the product and changes must be approved by the licensing authority.

Lead Time A procurement term that describes the time interval needed to complete a procurement cycle. It begins when the need for new stock is recognised and ends when that stock is received and available for issue. Alternate definition: Time from order to delivery; e.g., manufacturing and shipping time.

Letter of Credit An arrangement by banks for settling commercial transactions; specifically, a written promise by a bank given to the Seller in accordance with the instructions (and cash deposit) of the Buyer to pay up to a given sum of money within a prescribed time limit when and if the Seller presents specified documents that give evidence of his performance.

Licensed Vaccine In the context of importing countries, a vaccine that has been evaluated and formally approved by the National Control Authority of the country of manufacture and by the National Control Authority of the country where it will be used. In the context of vaccine producing countries, a vaccine that has been evaluated and formally approved by the National Control Authority for production and use in that country. Licensing implies a quality standard based on verified Good Manufacturing Practices, quality assurance data and appropriate regulation.

Limited International Bidding (LIB) A procurement term describing the bidding process that limits participation to international and domestic suppliers that have been pre-qualified or pre-selected in some manner by the Purchaser. See "Restricted Tender"," Pre-qualification" and "Licensed Vaccine".

Local Competitive Bidding (LCB) A procurement method that follows the same format as International Competitive Bidding, but is limited to local participants.

Lot A supply term that can be used in two ways: Production Lot (see "Batch") and Shipping Lot.

Lot or Batch Number A manufacturing term that describes the series of numbers or letters or both established to record production and control of a single, uniform and homogeneous quantity of drugs, chemicals or biologicals produced from one formulation, in one manufacturing and production operation and which has received entirely the same processing treatment.

Lot Release A regulatory activity employed in both manufacturing and non-manufacturing (purchasing) countries that independently checks each lot of vaccine to determine/confirm that it is in

compliance with the regulations of the country. In manufacturing countries this step is done by the NCA (of the manufacturing country) prior to shipment; in non-manufacturing (purchasing) countries this step is done by the NCA (of the purchasing country) prior to distribution. In most cases, the NCA of a purchasing country does not perform additional laboratory testing but relies upon the quality assurance documentation provided by the manufacturer and the lot release certificate provided by the NCA of the manufacturing country.

Lot Release Certificate A regulatory term describing a certificate issued by the National Control Authority of the country of manufacture that states the lot number being shipped has been tested by the government's laboratory or checked in some other manner and found to be in conformity with the regulations of the country of manufacture and is released for sale. In some cases this document may be titled "Certificate of Free Sale".

Manufacturer's Representative A supply term used to describe a direct employee of a vaccine manufacturer with responsibility to promote the use of, provide information on and sell the manufacturer's products. In some cases, the representative also facilitates importation.

Marking A term used in packing and shipping for the application of numbers, letters, labels, tags, symbols or colours for handling and identification during shipment and storage.

Mass Campaign In the context of immunization programmes, vaccination of large numbers of individuals against a specific disease within a very short time. Primarily used for epidemic situations and disease eradication.

Material Deviation A procurement term that is used in evaluating bids to describe a significant and unacceptable difference from the requirements stated in bidding documents. More precisely, a material deviation is one that affects, in any way, the price, quantity, quality or delivery of the goods as required in the bid documents, or limits in any way the responsibilities, duties or liabilities of the Bidder or any rights of the Purchaser.

Merit Point System A procurement term that describes a numerical system used to evaluate and compare offers or bids. Points (based on a total of 100) are assigned according to how well an offer is judged to match requirements and preferences (which are stated by the Purchaser in the original bidding documents) and its relative standing in the range of prices offered.

Middleman A procurement term for an independent broker who purchases product from a manufacturer or wholesaler and re-sells the product. This adds to the final cost of the product as the middleman's revenue from the transaction is the difference be-

tween his acquisition and holding cost and his sales price. Purchase of vaccine through a middleman increases the risk of receiving poor quality, mishandled or counterfeit product unless shipments are made directly from the manufacturer to the purchaser with appropriate original documentation.

National Control Authority (NCA) An independent government entity responsible for establishing procedures to ensure that medicines (and biological products) intended for use in the country are safe, potent and effective.

National Control Laboratory (NCL) A laboratory advisory to the National Control Authority.

National Immunization Days (NIDs) An immunization programme term describing a type of mass campaign (see above), usually lasting one or a few days and conducted over specified time intervals (e.g., a few months per year or annually).

National Regulatory Authority (NRA) See National Control Authority—both terms are currently in use.

No Objection Certificate A shipping/import document sometimes required by a country's customs, tax or other laws certifying that domestic manufacturers of vaccine/medicine have "no objection" to the import of a competing, similar or identical vaccine/medicine.

Offer Same as "bid" where the procurement is advertised or invited; same as "proposal" where the procurement is solicited by a "Request for Quotation".

Open Tender A formal procurement procedure in which bids are accepted from any interested local or international source of the required product. If it must be used for vaccine procurement, the tender invitation should include a requirement that the vaccine must be licensed by the Purchaser's NCA.

Open Vial Policy A new immunization programme policy initiated by WHO advising that opened vials of some vaccines (OPV, DPT, DT, TT, Hepatitis B) may be retained and safely used on succeeding days, as long as they are kept under refrigeration and are not contaminated.

Outreach In the context of EPI, a term for immunization services provided from mobile or temporary locations to hard-to-reach populations.

Packaging A product's containers and coverings. In the context of vaccine, vials and ampoules are the primary packaging, boxes and

bags containing several—up to 100—vials or ampoules are secondary packaging.

Packaging for Bidding A term used by the World Bank and others for organising very large, diverse schedules of goods to be purchased into grouping of like items for bidding purposes.

Packaging (requirements) Identifies how product should be packaged to withstand the handling and climatic conditions it will be subject to during transit. For vaccines this includes instructions on the specific temperature range in which the product must ship and whether it can or cannot be frozen as well as information on the type of packaging and strength of packaging material to be used and inclusion of cold chain monitoring devices.

Packing Assembling of items into a unit for shipment; carton, overwrapping and insulation for protecting products against damage or deterioration during shipment.

Payment Terms In the context of vaccine purchases, a description of how, where and when payment will be made; for example, Letter of Credit, Cash in Advance, Open Account.

Performance Security A procurement term describing the financial instrument used to guarantee compensation to the Buyer for inconvenience and expense if the Seller does not perform, i.e., does not produce and ship the contracted goods or provide the contracted services within the stated period. The Seller puts up his own funds, often through a bank or an insurance company, to be held in reserve until the contract terms have been met.

Potency In vaccine terminology, the specific ability of the product to give protection against disease as confirmed by appropriate laboratory tests or by adequately controlled clinical data.

Pre-qualification (of supplier) A process of pre-approving suppliers for participation in vaccine tenders based on a judgement of reliability, technical competence and financial stability. Referenced as second-level pre-qualification.

Pre-qualification (of vaccine) A process of pre-determining that a specific vaccine of a specific manufacturer meets stated requirements and may be considered for purchase contracts in the approving country. Licensing by the National Control Authority in the purchasing country automatically confers pre-qualification status. Referenced as first-level pre-qualification.

Procurement Agent An individual or an organisation hired to act on behalf of a purchaser.

Procurement Entity Body functioning as the Purchaser in a commercial transaction (see "Entity").

Procurement Requirements A complete description of the product to be purchased; used in the context of vaccine procurement to describe technical attributes (including manufacturing and quality assurance norms), programme specifications (including packaging, packing), shipping terms, payment terms, port of delivery, delivery date, quantity, documentation and any other relevant detail of the expected purchase.

Procurement Team In broadest terms, includes all individuals who actively contribute to a specified procurement, particularly those who cooperate together and are accountable for some part of the transaction. A typical procurement team is made up of the procurement unit, the EPI manager, and representatives from the Tender Board, the National Control Authority, the Ministry of Finance, and the Ministry of Health.

Procurement Transaction Agreements and actions of a Buyer and a Seller around a specific purchase; usually documented and legally binding.

Procurement Unit Individuals organized around procurement duties within a larger entity.

Proposal A procurement term that describes an offer of goods or services where (1) the procurement has not been advertised, or (2) an offer to supply goods or services is made in response to a specific Request for Proposal (RFP). Less formal in structure and process than sealed bidding (ICB, LCB and LIB).

Public-Sector Supply Service An organisation that contracts annually with manufacturers for large quantities of product which it then supplies in smaller quantities to individual clients in the public sector on a reimbursable, but non-profit basis. UNICEF is the largest public-sector supply service for vaccine.

Pull System A term used in vaccine distribution systems to indicate that peripheral levels request deliveries of specific kinds and amounts of vaccine from a central level.

Purchaser See "Buyer".

Purity A laboratory/quality assurance term describing the degree of freedom from extraneous matter whether or not harmful to the recipient or deleterious to the product. In most instances, the concepts of purity and safety coincide, and purity relates to freedom from such materials as pyrogens, contaminants and residual chemicals from the manufacturing process.

Push System A term used in vaccine distribution systems to indicate that a central authority is sending vaccine to lower levels based on its own calculations of need rather than specific requests from the lower levels; i.e., it "pushes" vaccine to the lower levels.

Quality Assurance The combination of organised activities performed to demonstrate that a product meets quality criteria and specifications for its intended application. Quality assurance in contractual or other external situations provides confidence to the customers. In the context of vaccine, it is typically undertaken before a shipment leaves the manufacturer's facility and/or before the product is released for use in a country. Quality assurance within the manufacturing organisation provides confidence to the management.

Quality Control A manufacturing term that describes operational techniques and activities aimed at monitoring the process and eliminating causes of unsatisfactory performance. Some quality control and quality assurance actions are interrelated.

Reference Box A marker in the main text of this manual indicating the presence of related information and where it is located.

Registration A term used in regulating vaccine; exact usage varies from country to country. It is often synonymous with Licensing but it can mean simply that the particulars about a vaccine shipment are recorded as it enters a country.

Relative Potency A laboratory/quality assurance term describing potency relative to a reference material that is estimated as the ratio between equivalent (equipotent) doses of a test preparation and a standard preparation.

Request for Quotation (RFQ) A procurement process where offers (quotations) are requested from several prospective suppliers without employing formal sealed bidding procedures. Offers can be made by telex, fax or e-mail. Some degree of negotiation may be included.

Restricted Tender Also known as Selective Tender and Limited International Bidding. A formal procurement process that limits participation to international and domestic Suppliers that have been prequalified or pre-selected in some manner by the Purchaser.

In vaccine procurement, a purchasing method in which participation in bidding is limited to manufacturers of vaccine that has been pre-qualified based on technical criteria through licensing with a competent NCA in the country of use. Restricted tenders may also include suppliers who have been preapproved by the purchasing entity based on commercial criteria and a verifiable legal relationship with the manufacturer

of licensed vaccine. In countries without a specific biologicals committee within the National Control Authority, pre-qualification is based on surrogate approval of competent international regulatory experts such as WHO/GPV/VSQ and USFDA. See also "Pre-qualification (of vaccine)" and "Pre-qualification (of supplier)".

Safety A quality assurance term used to characterise a vaccine's relative freedom from harmful effect to the recipient, when correctly and appropriately administered, taking into consideration the character of the product and the condition of the recipient at the time.

Sealed Bids A procurement process where formal bids are submitted in sealed envelopes and held unopened until an appointed date and time, then opened and read out in public with bidders in attendance. See "International Competitive Bidding", "Local Competitive Bidding", "Limited International Bidding".

Selective Tender See "Restricted Tender".

Seller A procurement term used to describe the party to a contract who offers goods, commits to seeing that they come into the Buyer's possession and (usually) receives payment from the Buyer. The Seller does not necessarily have to be the Supplier of the goods. Used in contracts to define one of the parties to a purchase transaction; normally, Buyer - Seller. The Seller is usually the Bidder in a tender process.

Session See "Immunization Session".

Shelf life A supply term for the length of time designated by the manufacturer that vaccine may be stored without affecting its usability, safety, purity or potency. Shelf life varies from vaccine to vaccine.

Shipping Lot A manufacturing term used to describe a collection of like items assembled together for shipment to one customer under one transport document. A shipping lot may be comprised of items (vaccine) from several different batches or productoin lots.

Shipping Terms A description of how goods will be shipped, who is responsible for them at each stage of the process and who pays which costs. See INCOTERMS.

Sole Source A procurement term used to describe purchasing from a single manufacturer without competition among potential suppliers; most often applies to items that are not available from any other source.

Solicitation A procurement term for the process of inviting bids or requesting proposals for the supply of a product or service; also used to refer to the document requesting bids or proposals.

Special Commitment Form 1931 Used when purchasing goods with proceeds of a World Bank loan; application initiates guarantee of funds from a World Bank account to a commercial bank for the purpose of opening a commercial "Letter of Credit".

Specification Detailed, precise written description; in the context of vaccine procurement, there are two types: (1) technical specifications cover the physical properties of the vaccine such as type, relative potency, purity, thermostability, dosage, quality assurance norms and required storage temperatures; (2) programme specifications cover acceptable variables in vial size, packaging, packing, etc. and may call for a shelf life beyond the minimum established by the technical specifications.

Standard Something that is established by authority as a rule for the measure of a quantity, weight, extent, value or quality; for example, the International Organization for Standardization (ISO) establishes "rules" for the vial closures commonly used for vaccine.

Standard Preparation A laboratory term used to describe a reference preparation, such as bacterial or viral antigen, or serum with a defined specific activity, that can be used in evaluating relative potency of a biological product when compared to it. There are international, national and in-house standard preparations. The reference standard (for example, virus strain) must match the product.

Supplier A procurement term describing the party who transfers goods out of his own control to a named recipient.

Target Population A vaccination programme term for the total number of intended clients based on expected coverage rates.

Technical Merit A procurement term that represents a judgement as to whether or not, or how well, a bid matches the programme specifications and any related requirements of the purchaser; for example, vaccine and syringes packaged together. Technical specifications are normally set by a National Control Authority through licensing, and bids that offer unlicensed products are not accepted.

Tender A procurement process by which competing offers are requested for a particular good or service. Same as "Invitation to Bid".

Tender Committee A committee of senior ministry and programme representatives, often including the ministry of finance, that oversees, guides and approves key steps in the procurement process, including final selection. Also known as Tender Review Committee.

Tender Offer A procurement term meaning an offer for goods or services submitted or received in response to a specific Invitation to Bid (Tender).

Tender Review Committee See "Tender Committee".

Thermostability A quality assurance term that describes the limits of a vaccine's ability to retain potency over time at prescribed temperatures.

Uniform Customs and Practice for Documentary Credits (UCP) A set of rules for cross-border transactions relating to Letters of Credit (also known as Documentary Credits and Documentary Letters of Credit) codified by the International Chamber of Commerce (ICC). The latest version is UCP 500, which has been in effect since 1993.

United Nations Children's Fund (UNICEF) A semi-autonomous UN agency working for sustainable human development to ensure the survival, protection, and development of children. Co-operates with governments in the developing world and promotes universal immunization against six childhood diseases, in part, by operating a global supply service that purchases and distributes vaccine as well as many other health related items. Formerly - United Nations International Children's Emergency Fund.

Unresponsive Bid A procurement term used describe an offer that does not comply with the most basic instructions and requirements stated in the bidding documents provided by the purchasing organisation. For example, an unresponsive bid may be one that is not signed, is tendered in the wrong language, or does not offer the required (licensed) product(s).

Unrestricted Bidding See "Open Tender", "International Competitive Bidding".

Vaccination Schedule A term used in immunization programme management to describe the national plan for routine vaccination: the list of vaccines to be given, the number of doses required for each vaccine and the age at which each dose should be given.

Vaccine Independence Initiative (VII) A financial mechanism established by UNICEF to help low- to middle-income countries purchase EPI vaccine from UNICEF. A donor pays into a revolving fund for a specific

participating country and the participating country repays the fund after the vaccine is received. The cash on deposit satisfies UNICEF's requirement for cash in advance and entitles the participating country to re-order. In addition, the participating country receives assistance with forecasting annual vaccine needs. Commonly known as VII.

Vaccine Vial Monitor (VVM) A small indicator dot affixed to a vaccine vial that signals with a colour change when a pre-set limit of heat exposure over time has been exceeded indicating that the vaccine should not be used.

Wastage In vaccination programmes, the amount of vaccine that remains in an opened vial when its safe usage period has ended. Wastage may also occur because of cold chain failure, expiration of vaccine or breakage.

Wastage Factor In vaccination programmes, a coefficient or a correction factor which takes into account the expected wastage in calculating the amount of vaccine required to immunize a specified number of individuals. It is also known as the "index of vaccine use" or the "number of doses used per immunization". The wastage factor is directly calculated by using the following formula: wastage factor = number of doses of vaccine used during a certain time period/number of immunizations given during a certain time period.

For example: If 1500 doses of measles were used to give 1000 vaccinations (as in the example described in the definition of wastage rate), then the wastage factor is 1500/1000 = 1.5. The wastage factor also can be calculated if the wastage rate is known or presumed by using the following formula: w a s t - age factor = 1/(1-Wastage Rate). Thus, again using the wastage rate of 33% (from the example described in the definition of wastage rate), the wastage factor is 1/(1-.33) = 1.5. Therefore, to immunize 1000 children, 1500 doses of measles vaccine will be needed (1000 x 1.5 = 1500 doses).

Wastage Rate In vaccination programmes, the portion of vaccine used beyond the doses given to children. It is defined as: Wastage % = Doses used - children vaccinated/Doses used x 100.

For example: 75 20-dose vials (75 x 20 = 1500 doses) are used to vaccinate 1000 children. The wastage rate is (1500 - 1000)/1500 x 100 = 33%. Wastage rates are sometimes broken down into wastage occurring in closed vials due to cold chain problems and wastage occurring in opened vials during administration.

Wholesaler A supply term for a dealer who purchases supplies from a manufacturer on his own behalf and re-sells them for a profit.

Whooping Cough Pertussis.

GENERAL INDEX

Adjudicating bids 106, 119
Adjudication worksheet 106, 107, 121
Authorisation for shipment 154, 155
Award letter 124, 125, 126
Award process 123
Awarding contracts 123
BCG (Bacillus Calmette Guerin) vaccine 239
Bid comparison 105
Bid documents, preparation of 57
Bid evaluation 105
Bid evaluation, preparation for 93
Bid examination 98, 101
Bid opening 98, 100
Bid package 58, 62, 64, 95
Bid responsiveness 98
Bid security 64, 75, 136, 137, 953
Bid summary 106, 109
Bidder's list 59
Bid (see also Offers) 953
Budget estimate worksheet 48, 52, 53, 691
Buyer 15, 954
Cash in advance 705
Closeout activities 161
Comparison of purchasing methods 25
Competitive bidding, ten principles of 23
Conditional bid acceptance letter 125
Contract 135
Contract monitoring system 148, 150
Contract performance 147, 150, 152
Contracting ..... see Procurement
Cost data ..... see Price data
Customs clearance 157
Donations and special offers of vaccine 572
Down payment 705
DTP (Diphtheria, Tetanus, Pertussis) vaccine 273
Evaluation criteria 105
Financial arrangements 139
Forecasting vaccine needs 30, 31

Forms 7, 975
Glossary of terms and phrases 953
Good Manufacturing Practice (GMP) 957
Hepatitis B vaccine 457
Import controls 571
INCOTERMS 712, 713, 957
Injection equipment, selection of 806, 807
Inspection agents (partial list) 574
Inspection and testing 570, 574
Inspection, pre-shipment 570
Inspection, QA and costs 576
Inspection upon receipt 571
Insurance certificate 62
International Competitive Bidding (ICB) 13, 50, 958
International Competitive Bidding (ICB) with pre-qualification 13
International inspection agents 574
International shopping 14, 958
Invitation to bid 66, 68
Laboratories, testing (partial list) 578
Letter of Credit 140, 142, 143, 707, 710, 711, 959
Licensing a vaccine 600, 959
Limited International Bidding (LIB) 14, 50, 959
Local Competitive Bidding (LCB) 14, 959
Manufacturer 15
Manufacturer's representative 16, 960
Material deviation 116, 960
Measles vaccine 336
Merit point system 106, 107, 112, 117, 119, 960
Middleman 16
Model Certificate for Release of Vaccines Acquired by United Nations Agencies 676
Modified International Competitive Bidding (ICB) 13
National Competitive Bidding (NCB) 14
National Control Authority (NCA) 3, 17, 961
Needles 806, 813
Offers, receipt (LIB) 97
Offers, solicitation (LIB) 97
Open account 705
Options 11
OPV (Oral Polio Vaccine) 406
Payment against documents 706
Payment options 705

Performance guarantee, performance security 962
Planning 30
Pre-qualification 36
Pre-qualification announcement 39, 45
Pre-qualification criteria for vaccines 77
Pre-qualification of supplier 36, 962
Pre-qualification of vaccines 36, 962
Pre-qualification questionnaire 37, 38, 41
Pre-shipment compliance 573
Price data 681
Procedures 27
Procurement activity timeline 26
Procurement Agent (commercial or state) 16, 19, 962
Procurement, competitive 12
Procurement, definitions and roles 15
Procurement entities 19, 963
Procurement file 48
Procurement, in-house 19
Procurement methods 12, 25
Procurement, official request 48
Procurement participants 15
Procurement preparation 47
Procurement request form 49, 54, 55
Procurement requirements 963
Procurement, selecting the supplier 115
Procurement, sole source 14, 50, 965
Procurement team 94, 963
Procurement through UNICEF 21, 643
Procurement unit 963
Procurement through WHO 21
Purchaser 17
Purchaser's rights 18
Purchasing methods, comparison of 25
Quality assurance 8, 567, 964
Quality assurance for procurement staff 568
Quality assurance inspection (pre-shipment) 574
Quality assurance, pre-shipment compliance 573
Quality assurance testing (pre-shipment) 575
Quality control 964
Receipt of offers 97
Recommendations for Handling and Storage of Selected Biologicals 751, 774
Record of bid examination 99, 101, 103

Record of bid opening 98, 100
Reference box 5, 7
Registration 964
Regulator (country of manufacture) 15
Regulator (in purchaser's country) 17
Request for Letter of Credit 140, 145, 708
Request for quotation 14, 50, 77, 79, 964
Sample procurement activity timeline 26
Samples 7
Sampling 573
Sealed bids 965
Seller 15, 965
Seller's expectations 18
Shipment 153
Shipping instructions 124, 132
Solicitation 97, 966
Specifications 8, 165, 569, 966
Specifications, BCG vaccine 239
Specifications, DTP vaccine 273
Specifications, general requirements 168
Specifications, hepatitis B vaccine 457
Specifications, measles vaccine 336
Specifications, OPV vaccine 406
Specifications, tetanus toxoid 556
Specifications, yellow fever vaccine 507
Specifications for vaccines 165
Standard Bidding Documents for Procurement of Pharmaceuticals and Vaccines 831, 833
Statement of known good quality 36, 38, 60
Storage of vaccines 751, 772, 774
Summary cover sheet 106, 107, 113
Supplements 7, 976
Supplier 966
Supplier history form 162, 163
Syringe incinerator box 806, 818
Syringes 806, 813
Temperature monitoring 772, 782, 783, 784, 794, 795, 796, 799
Tender Review Committee 94, 95, 96, 116, 967
Testing ..... see Quality assurance
Tetanus toxoid 556
Transportation 750
Unconditional bid acceptance/award letter 126

UNICEF (United Nations Children's Fund) 17
UNICEF, general conditions 645
UNICEF, minimum requirements for vaccines 653
UNICEF option 643
UNICEF pricing 677
UNICEF purchase agreement 645
UNICEF quality assurance program 662
UNICEF standard procurement services 644
UNICEF Vaccine Independence Initiative (VII) 644, 645
Unrestricted tender ..... See International Competitive Bidding
Unsuccessful bidder notification 136, 137
Vaccine arrival report 758
Vaccine cold boxes/carriers 806, 820
Vaccine cold chain monitor 782, 783, 784, 955
Vaccine cost components 682
Vaccine Independence Initiative (VII) 644, 645, 952, 967
Vaccine potency tests 576
Vaccine price 681, 683
Vaccine sources 692, 694, 699, 701
Vaccine storage 751
Vaccine supply survey 692, 703
Vaccine tests, identity 577
Vaccine tests, other 577
Vaccine tests, potency 576
Vaccine vial monitors (VVM) 677, 799, 800, 952, 968
WHO Guidelines for Implementation of the WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce 608
WHO Expanded Programme on Immunization (EPI) 951, 956
WHO Guidelines on the International Packaging and Shipping of Vaccines . 754
WHO, role of 567
World Bank Guidelines for Procurement Under IBRD Loans and IDA Credits 831
Yellow fever 507

NOTES

INDEX OF FORMS, SAMPLES AND SUPPLEMENTS

FORMS

II.C.3. Budget Estimate Worksheet 52
II.C.6. Procurement Request Form 54
II.D.9.a. Invitation to Bid 66
II.D.9.i. Bid Security Form 75
II.D.9.k. Performance Guarantee Form 76
II.D.13.a. Request for Quotation 77
II.F.4.d. Record of Bid Opening 100
II.F.7. Record of Bid Examination 101
II.G.5.b. Tender Review Committee Summary Cover Sheet 113
II.I.1.a. Conditional Bid Acceptance Letter 125
II.I.4.a. Unconditional Bid Acceptance/Award Letter 126
II.I.4.a. (Enclosure)
Contract Form for Enclosure with Unconditional Bid Acceptance/ Award Letter 127
II.I.4.b. Bid Award Letter 129
II.I.4.b. (Enclosure)
Contract Form for Enclosure with Bid Award Letter 130
II.I.5. General Shipping Instructions 132
II.J.3. Unsuccessful Bidder Notification 137
II.K.3.a. Instructions for Letter of Credit 143
II.M.6. Authorisation for Shipment 155
II.O.2. Supplier History 163
VI.A.2. Budget Estimate Worksheet 691

SAMPLES

II.B.6. Pre-qualification Questionnaire 41
II.B.7.c. Pre-qualification Announcement 45
II.C.3. Budget Estimate Worksheet 53
II.C.6. Procurement Request Form 55
II.D.9.a. Invitation to Bid 68
II.D.13.a. Request for Quotation 79

II.D.13.b. Draft Model Contract for Supply of Vaccines 85
II.F.7. Record of Bid Examination 103
II.G.1. Bid Summary 109
II.G.1.a. Bid Summary—Financial Adjustment Sheet 111
II.G.3. Merit Point Form 112
II.H.1.c. Adjudication Worksheet 121
II.K.3.b. Application and Agreement for Commercial Letter of Credit 145
II.L.1.a. Responsibilities for Contract Performance 150
II.L.1.c. Sample Contract Performance Timeline Vaccine Procurement 152

SUPPLEMENTS

I.A. Ten Principles of Competitive Bidding 23
I.D.1. Comparison of Purchasing Methods 25
I.D.2. Sample Procurement Activity Timeline 26
II.A.1. Forecasting Vaccine Need 31
II.D.9.c. General and Special Conditions for Supply Contracts 72
II.H.1.c. Adjudicating Bids 119
II.K.3. Letter of Credit 142
IV.D.1. Sample Compliance Programme 579
VI.A.1. Price History 683
VI.B.1. Address List of Suppliers Approved in Principle for the Supply of Vaccines to United Nations Agencies 694
VI.B.2.a. Vaccine and Biologicals Manufacturers Contact Information 699
VI.B.2.b. Biologicals Manufacturers Members of the International Federation of Pharmaceutical Manufacturers Association 701
VI.B.2.c. Vaccine Supply Survey 703
VI.B.3. An Example of the Consolidation of Vaccine Manufacturers 704
VI.C.5.d. Issuing a Letter of Credit 710
VI.C.5.e. Settlement of a Letter of Credit 711
VI.D.1. INCOTERMS 713
VI.D.2.a. Common Terms 719
VI.D.2.b. Symbols 720
VI.E.1. Normally Required Import Documents 722
VI.E.2. Common Import Documents 732
VI.E.3. Additional Import Documents 739
VI.F.1. Vaccine Stability 752
VI.F.2.a. Guidelines on the International Packaging and Shipping of Vaccines (EPI/CCIS/81.4 Rev.6) 754
VI.F.2.b. A Guide to Estimating Capacity of Equipment Required for Storage and Transporting EPI Vaccine (WHO/EPI/CCIS/86.3) 760
VI.F.3.a. Temperature Monitoring 772

VI.F.3.b. Vaccine Management, Recommendations for Handling and Storage of Selected Biologicals 774
VI.G.1.a. Vaccine Cold Chain Monitor Card 783
VI.G.1.b. Vaccine Cold Chain Monitor (WHO/EPI/CCIS/85.1 Rev. 5) 784
VI.G.2. DT and TT Shipping Indicator 794
VI.G.3. Freeze Watch Indicator 795
VI.G.4. Electronic Time/Temperature Recorders 796
VI.H. Vaccine Vial Monitors (VVM) for OPV 800
VI.I.1. Safety of Injections in Immunization Programmes (WHO/EPI/LHIS/96.05 Rev.1) 807
VI.I.2. Syringes and Needles 813
VI.I.3. Safety Box for the Disposal of Used Syringes and Needles 818
VI.I.4 Vaccine Cold Boxes/Carriers 820
VII.K.1. Standard Bidding Documents for Procurement of Pharmaceuticals and Vaccines 833
VII.K.2. Corrigenda 930
VII.M. Special Treatment Clauses 939

NOTES

INDEX OF WHO DOCUMENTS

A Guide to Estimating Capacity of Equipment Required for Storage and Transporting EPI Vaccine, WHO/EPI/CCIS/86.3 760

General Requirements for the Sterility of Biological Substances (Requirements for Biological Substances No. 6, revised 1973, amendment 1995), WHO *Technical Report Series*, No. 872, 1998 202

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