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Diagnostic Technology for Community Health

Briefing Document
for
Program Evaluation

February 1990

Diagnostic Technology for Community Health
(DiaTech)

Briefing Document for Program Evaluation

Cooperative Agreement DPE-5935-A-00-5065-00

Submitted to

United States Agency for International Development
(A.I.D.)

By

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(PATH)
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I. INTRODUCTION

The Diagnostic Technology for Community Health (DiaTech) program (Cooperative Agreement DPE-5935-A-00-5065-00) is administered by the Program for Appropriate Technology in Health (PATH), in collaboration with the United States Agency for International Development (A.I.D.). The primary purpose of the DiaTech program is to improve the health status of developing country populations through the development and transfer of simple, cost-effective diagnostic technologies for infectious diseases. By focussing on methods for diagnosis of disease in the individual to improve health care, as well as for identification of disease burden in selected populations or communities, a rational basis can be provided for design of treatment and control programs to advance the health sector strategy of A.I.D. for promotion of child survival.

When the project was first conceived by A.I.D. in 1985, A.I.D. personnel responsible for framing the project believed that leading-edge research in biotechnology, in the form of monoclonal antibody and recombinant DNA technology, could be applied to the development of simple, rapid diagnostics appropriate for use in the developing world. Even though few relevant diagnostic opportunities using biotechnology-derived products could be immediately identified or specified in the initial proposal outlining the cooperative agreement, there was, nevertheless, much promise for the application of monoclonal antibody or DNA probe-based technologies for diagnosis of relevant infectious diseases. Furthermore, it was recognized that the development of diagnostic health products from concept to application requires a time frame which averages five years and often as long as ten years. This lengthy development cycle is required even when significant funds, generally on the order of $1 million or more per product for typical commercial diagnostics, are expended on the project. It was further recognized that, to be successful, DiaTech would need to be driven by technological opportunities. DiaTech has considered development of diagnostics for a broad spectrum of important diseases, emphasizing three areas: malaria, diarrheal diseases, and acute respiratory infections (ARI), since these disease entities are responsible for the greatest numbers of morbidity and mortality in children in the developing world.

In 1985, the expectation of A.I.D. and PATH was that relevant core technologies, or even specific product entities, were approaching maturity in the laboratories of the more than 200 biotechnology companies operating in the United States and overseas. In addition, it was also presumed that research on other promising approaches to diagnosis of infectious disease was occurring in numerous university laboratories, where advances in cell and gene cloning had been underway since the mid-1970s. It was hoped, therefore, that the DiaTech project could identify these maturing technologies and provide funding for their continued development. This hope was quickly dispelled when the search for mature technologies was carried out during the first four months of the

DiaTech program. Furthermore, consensus on the priority disease organisms or priority points of application for diagnostics was hard to reach, even among the internationally known infectious diseases experts assembled by DiaTech for the technical advisory and consultative workshop groups.

Recognizing this reality, and with the full collaboration of the A.I.D.-assigned cognizant technical officers (CTOs), DiaTech then turned its attention to the funding of "upstream" research. As an alternative, the DiaTech program would foster and facilitate discovery through research activities, confident that, given sufficient time and resources, a subset of these creative activities would prove successful. DiaTech anticipated that the resulting technologies could then be directed into focussed product development pipelines within a reasonable number of years. To maximize the probability of a successful outcome for any one disease group, DiaTech was compelled to: (1) allow a broad diversity of types of technologies addressing important pathogens within each disease group; (2) identify and support partially proven technologies ("leveraged" projects) whenever possible in order to conserve resources; (3) manage research and discovery in a recognized and effective way by allowing a balance between self-direction and interaction with DiaTech management among the collaborating investigators; and (4) evolve into a more interactive, directed, and tightly managed mode of operation upon the emergence of promising technologies waiting to move into a phase of objective validation and field testing.

The DiaTech project can thus be viewed as operating in two phases: the first phase is the stimulation of discovery through the small competitive grants program; the second, or product development phase, is the advancement of promising technologies with product potential through directed contract research or intramural development activities. These two phases have overlapped in time. The research, or discovery, phase occupied the majority of the resources and effort for the first four years. The time frame for individual projects is variable since the outcome of research is by nature uncertain. Product development, the latter phase, is emerging as the predominant activity in the fifth year of the project.

Over this same five-year period, the biotechnology industry, both in the United States and overseas, has been involved in a similar struggle to transform the promise of biotechnology into the reality of valuable products. Many companies have failed, exhausting their risk capital before they were able to bring products to market. Others have found the road to marketing of products difficult on their own and have merged with larger, cash-rich pharmaceutical corporations. DiaTech may be able to draw more product possibilities from this commercial sector, in addition to the 21 projects from DiaTech's own investment in discovery.

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The above, in broadest terms, describes the shape and scope of the DiaTech project as it has evolved. In the following sections, each element of the project is discussed in more detail. The information in this briefing document has been arranged to describe and emphasize the strategies and processes used to manage DiaTech as well as the external and internal factors which have influenced the direction of the project.

II. THE GOALS AND OBJECTIVES OF DIATECH

The goal of DiaTech as defined in PATH's proposal to A.I.D. is to improve the health status of developing country populations. The objectives are:

- To develop diagnostic techniques for selected infectious diseases suitable for field use in developing countries.
- To incorporate these technologies into standard diagnostic practice by carrying out field trials, refinement cycles, product engineering, and packaging.
- To introduce these technologies to public-sector health programs through information dissemination including conferences, workshops, publications, and training materials and programs.
- To carry out operational research to ensure that DiaTech technologies reach the target populations.
- To provide direct technical assistance to the programs of developing countries for the diagnosis of infectious diseases.
- To ensure that DiaTech technologies are produced and distributed by qualified manufacturers in developed and developing countries through the granting of licenses to manufacture, market, and distribute.

Key activities to be undertaken to achieve these goals include identification of the needs for new diagnostic technologies for malaria, diarrheal diseases, and ARI, followed by development of an applied research program, provision of funding for field testing, operational research, and product engineering. In addition, assistance was to be provided to A.I.D. for the establishment or improvement of diagnostic strategies and treatment protocols based on the emerging new diagnostic technologies.

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III. THE PATH ENVIRONMENT: Development, Advancement, and Introduction of "Social Technologies"

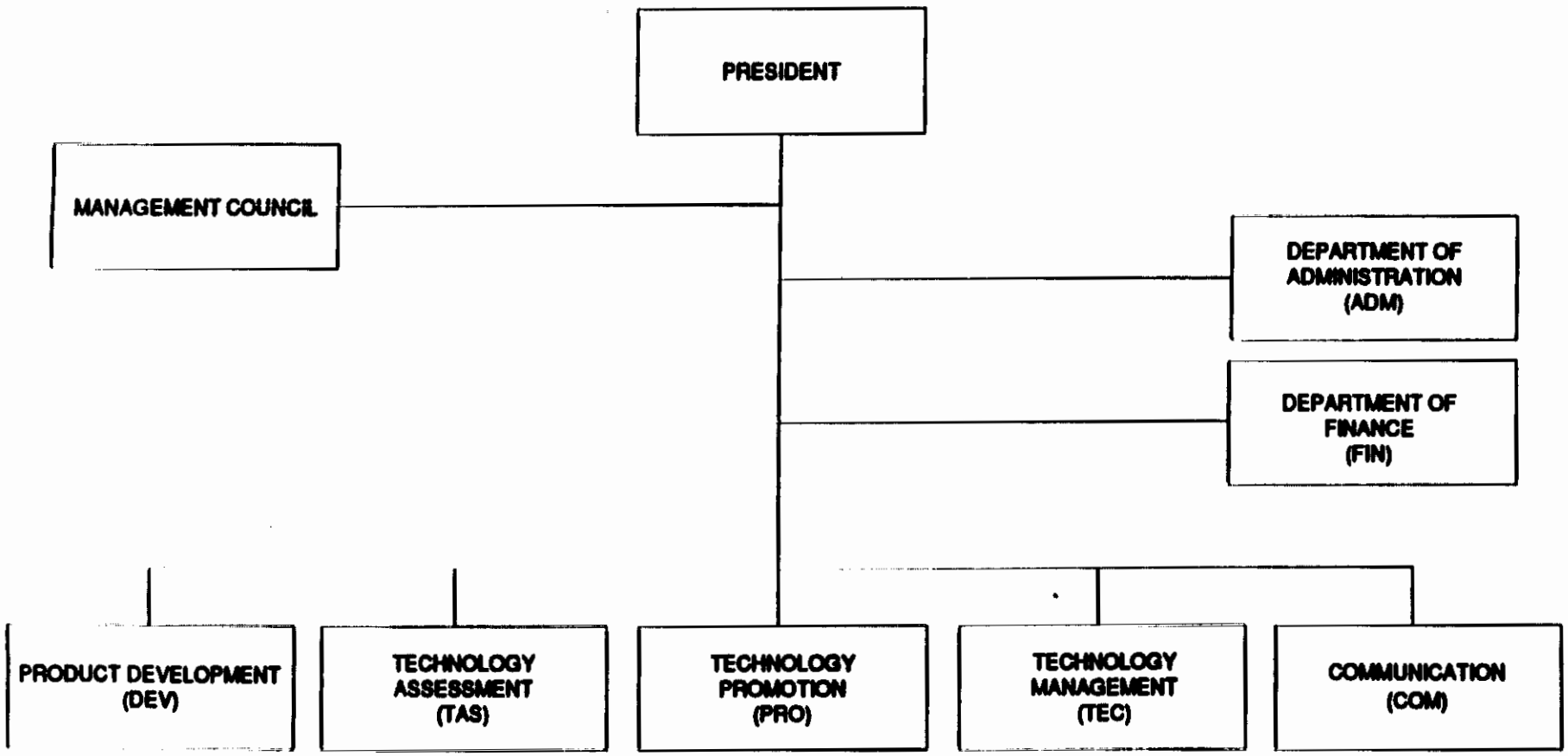
In order to fully understand the approach and strategies that have been used to implement the DiaTech project, it is important to understand how PATH operates. PATH is an international, nonprofit organization dedicated to improving the effectiveness, safety, availability, and acceptance of health products and technologies in developing countries. Its programs strive to bridge the gap between technology and potential users through a wide spectrum of activities including research and development, field assessment, technology transfer, quality assurance, marketing, communication, licensing, and loan financing.

PATH operates on matrix management principles. Its personnel and resources are organized into the following five functional departments (Figure 1): Product Development, Technology Assessment, Technology Promotion, Technology Management, and Communication. Overlapping this function-based line organization is a system of project and product management. For DiaTech, the Technical Director and Project Administrator are responsible to the Vice President and Director of the Product Development Department, who has overall responsibility for program management. However, all of the skills and resources of the other PATH departments are available to the project at the request of the Technical Director, who is empowered to organize task forces, designate product managers or technical resource persons, and assign budgets to mobilize other resources of the organization. For example, when the development of a technology reaches a point at which intellectual property must be documented, licensing agreements written, or business plans developed, key individuals from the Technology Promotion Department are assigned either as full-term product advancement managers for the specific technology or as task managers to accomplish specific tasks. Likewise for field assessment, personnel from the Technology Assessment Department are assigned, and for technology transfer activities, Technology Management personnel are deployed.

Generally, once a technology is sufficiently defined, it is assigned a <u>product management</u> team. This product management team consists of a Technical Product Manager, a Product Advancement Manager, and any variety of technical resource persons as required by the project. Usually the technical product manager has lead responsibility for the product development effort until the effort must be guided principally by the business, field, or market aspects of the project. At this juncture, the product advancement manager takes the lead.

At any point in time PATH has a number of products in the different phases of development: selection and screening, prototyping, field assessment, licensing negotiations, scale-up, or technology transfer to a developing country. Currently, PATH has more than 50 products in the developmental pipeline including those arising from the DiaTech program. These technologies enter the pipeline at any

PATH ORGANIZATION CHART



FUNCTIONS

- *Technology identification
- *Technology development
- *Design Stage Field Trials
- *Product adaptation

- *Field assessment
- *Field introduction (Type I Social Technologies)
- *Medical Affairs

- *Brokering
- *Intellectual property (licensing/trademarks/patents/copyrights)
- *Market research
- *Loan financing
- *Field Offices

- *Local production
- *QA/QC services
- *Procurement
- *Logistics management

- *Materials development and evaluation for:
  - Product introduction
  - Training
  - Counselling
- *Multi-media Communication
- *Methodology Transfer

Figure 1

November 7, 1989

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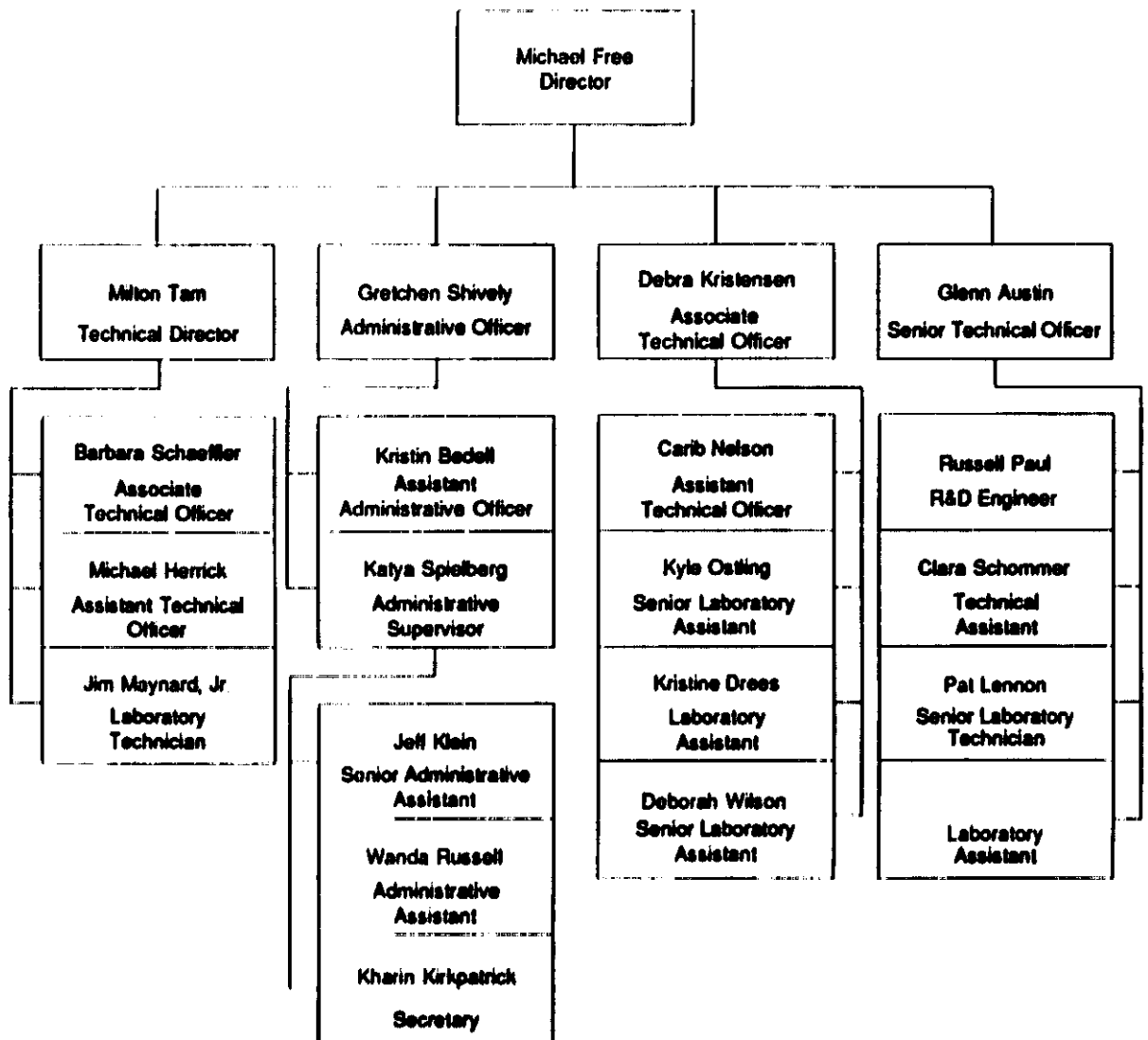
stage of development, i.e., they may be conceived at PATH or licensed in and represented by PATH; they may be adapted from existing or patent-expired products; they may be commercially owned technologies which are evaluated and transferred to developing countries under the auspices of the United Nations or other national/international agencies; or they may be established products (such as contraceptive technologies) which are being promoted under the auspices of public agencies. The contacts, networks, and communications resources for all of these efforts, established over 15 years of continuing PATH activity, are available to DiaTech as needed. Also available to DiaTech are the resources of the three field offices (Thailand, Indonesia, and Kenya) and the Communication Department of PATH, situated in Washington, D.C. Other networks are accessible to PATH through its status as a Collaborating Center to WHO for three areas of activity: Research in Human Reproduction Programs, Hepatitis B Vaccination, and the Global Program on AIDS (GPA). In addition, PATH has status as the secretariat of the International Task Force for Hepatitis B. Organizational charts for the Product Development Department and for the DiaTech program are attached as Figures 2-4 on the following pages.

PATH offers a unique philosophy to all product development and advancement activities. The term "social technologies" is used to differentiate between PATH's products as opposed to products arising from commercial endeavors. Social technologies differ in one important respect from the technologies developed for business: they are driven by a perceived health need rather than a measured market demand. Thus, PATH views public sector interests of acceptability, availability, affordability, and appropriateness as primary objectives while recognizing that sufficient incentives must always be available to attract and sustain manufacturers and distributors in the long-term. PATH employs several strategies to advance technologies from the laboratory stage to actual availability and use. These strategies depend primarily on whether or not a commercial market exists for the product. A detailed discussion of these strategies is given in Section IX.A.3.

The majority of DiaTech technologies have only recently reached the stage of validation where products can be more clearly defined. Thus, product advancement activities, such as securing of representation agreements for subject inventions or intellectual property owned by the subgrantees, or the review and development of business plans, have only recently been initiated. However, the groundwork has been laid over the last two years to ensure the maximum cooperation between subgrantee inventors and DiaTech within the limits of the cooperative agreement and the current law relating to subject inventions. Details of these activities will be given in subsequent sections and elaborated upon by the product advancement team during the evaluation site visit.



Product Development Department
By Reporting Lines
January 31, 1990

Figure 2

DiaTech Management

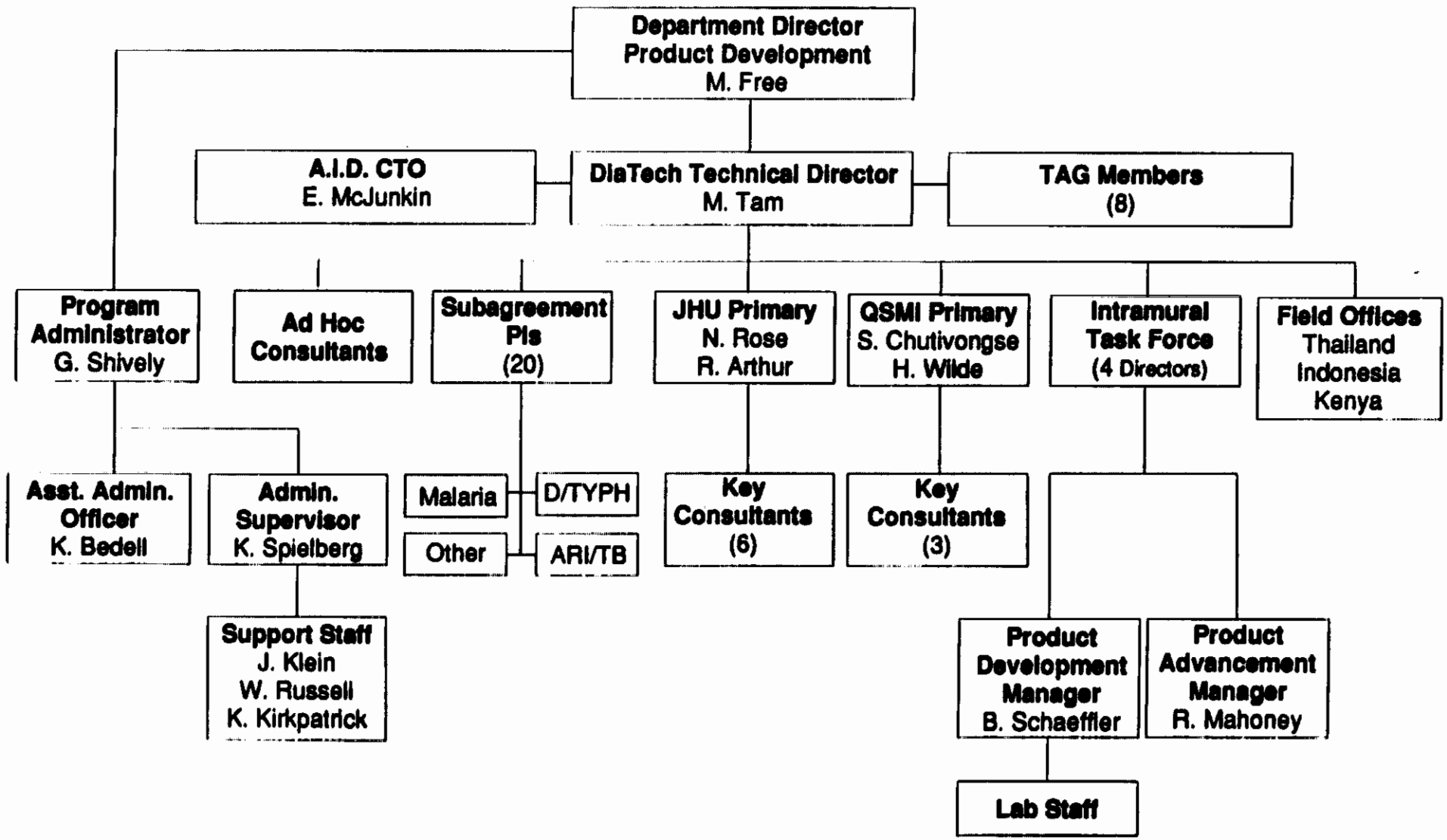


Figure 3

DiaTech Functional Activities

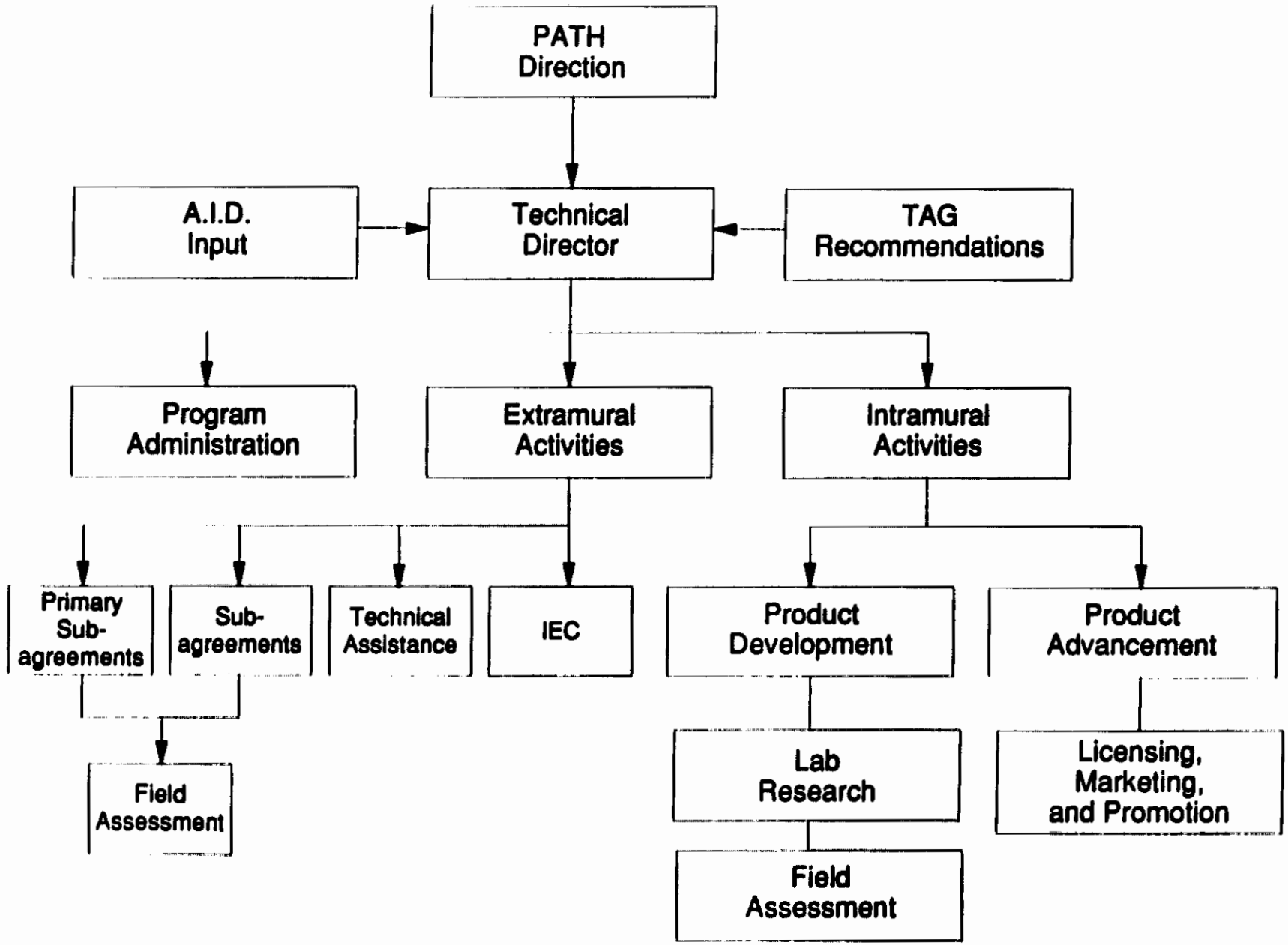


Figure 4

IV. THE BIOTECHNOLOGY ENVIRONMENT: The Promises, Expectations, and Realities of Global Biotechnology Development

The DiaTech program was designed to provide a mechanism for promotion of the development, testing, adaptation, introduction, and local manufacture of state-of-the-art diagnostic technologies. The impetus for this initiative was the advances in cell and gene cloning which occurred in the late 1970s and early 1980s--advances which underpin today's biotechnology industry (see Table 1 for some of the key milestones in the development of the biotechnology industry).

An informal survey conducted at the American Society for Microbiology Annual Meeting in the spring of 1985, six months prior to the initiation of the DiaTech program, showed 227 companies exhibiting approximately 100 products for the diagnosis of infectious diseases. The majority of these products were concerned with isolation and identification of microorganisms by conventional techniques, with proportionately fewer products for culture confirmation and fewer still (less than 25) for direct detection of microbial antigens in patient specimens. Overall, less than 10% of the exhibited products utilized monoclonal antibodies or DNA probes (none of the probe-based assays were FDA-approved at that time). Hence, at the inception of the DiaTech program, the application of leading-edge technology to infectious disease diagnosis could more aptly be described as a promise rather than as a reality.

In the five years which have elapsed since 1985, simple and innovative diagnostic methods have been slow to emerge, despite the considerable resources expended by the biotechnology industry on their development. The flow chart presented as Figure 5 illustrates the evolution of a medical device (diagnostic assays are treated as medical devices by the FDA) from the basic research phase through the FDA approval process. This cycle can consume 8 to 10 years or more, depending upon, among other things, the magnitude of the innovation and its complexity.

Estimating the rate of evolution from product concept to marketed product is not an exact science, as is illustrated in two timely news articles describing the classic product development problems of NeoRx and Genetech, both biotechnology companies (Attachment 1). A recent survey of biotechnology companies conducted by Ernst and Young indicates that most companies significantly underestimate the actual duration of product development, and that the farther along they are in the development process, the less accurate are their estimates. For the average diagnostic product, the development timeline has typically been five years or more.

Development costs for a typical diagnostic are estimated at one million dollars (exclusive of manufacturing start-up costs). In addition, only about 1 in 10 to 1 in 20 initiated projects actually result in a marketed product.

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The Biotech Timeline

1953

Watson & Crick–Double Helix

1970

First Synthesis of a Gene (non-functional)

1971

Restriction (cutting) Enzymes Discovered

1973

Cohen & Boyer Perfect Genetic Engineering Techniques to Cut and Paste DNA (using restriction enzymes and ligases) and Reproduce the New DNA in Bacteria

1975

First Monoclonal Antibodies Produced

1976

DNA Sequencing Discovered

First Working Synthetic Gene

1977

First Expression of Human Gene in Bacteria

Methods for Reading DNA Sequence Using Electrophoresis Discovered

1978

High Level Structure of Virus First Identified

Recombinant Human Insulin First Produced

1979

Human Growth Hormone First Synthesized

1980

Supreme Court Concludes Lifeforms are Patentable–Chakrabarty Patent

1981

Gene Synthesizing Machines Developed

First Monoclonal Antibody Diagnostic Kit Approved

1982

Rat Gene Transferred into Mice

First Synthesized Vaccine

FDA Approves First rDNA Product for Use–Human Insulin (Humulin)

1983

First Artificial Chromosome

First Field Test with Altered Bacteria is Delayed

First Markings for Inherited Diseases Found in Genes

First Sales of rDNA Product–Humulin

1984

Technique for DNA Fingerprinting Discovered

First Genetically Engineered Vaccine

1985

Genetic Markings Found for Kidney Disease and Cystic Fibrosis

FDA Approves Human Growth Hormone–Second Genetically Engineered Drug

1986

First Field Trials of Genetically Engineered Plant–Tobacco

FDA Approves First Genetically Engineered Vaccine (Hepatitis B)

1987

First Field Trials of a Genetically Altered Bacterium

1988

First U.S. Patent for a Vertebrate–a Transgenic Mouse

FDA Enacts Accelerated Regulatory Process for Products Combating Terminal Diseases

1989

First Field Trial of a Recombinant Viral Crop Protectant

FDA Approves EPO

IL-2 Approved in Parts of Europe

Ernst and Young Report, 1989

Table 1

Medical Device Development and Introduction in the United States

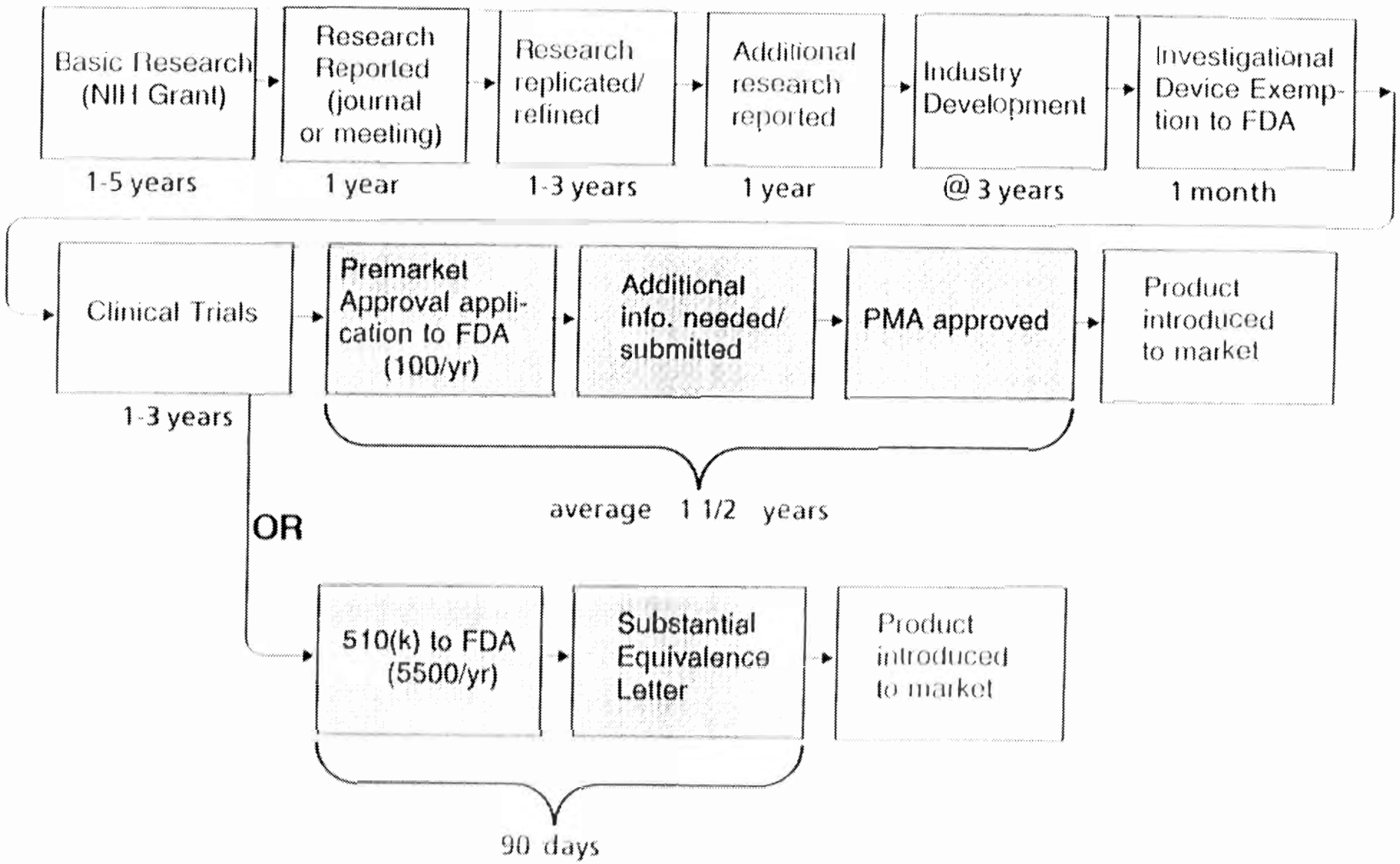

Figure 5

Evaluators Mid-term Report on HealthTech (1989)

V. DIATECH STRATEGIC PLANNING: Transition from Management of Discovery to Directed Product Development

The DiaTech program was initiated, has been influenced by, and has evolved in a dynamic, often uncertain era in which the biotechnology industry first reached a peak of public interest and expectation, then declined to a low point at which the industry collapsed inwardly upon itself, and was followed by the current recovery phase which can be characterized as more rational and realistic in expectations. These events ultimately affected the DiaTech program, since the strategies used and directions followed differed from those originally set at the outset of the project by DiaTech management in cooperation with A.I.D.

To initiate the program, DiaTech staff sought to identify mature technologies that would require a minimum of development and adaptation to produce diagnostic reagents and kits suitable for use in the developing world. As stated in the DiaTech proposal, 35%-40% of available funds were to be obligated for short-term projects, 35%-40% for intermediate-term projects, and only 20%-30% were designated for longer-term, "upstream" research projects. The search for emerging technologies centered on a survey of scientific and clinical literature, as well as a survey of product listings from diagnostic product companies. The conclusion of this extensive survey was that the expected sources of adaptable technologies simply did not exist in any advanced state of development. As a result, DiaTech was then compelled to address the funding of more upstream research to support the early "discovery" of technologies as an alternative to product development of already-proven technologies. DiaTech has spent approximately 85% of its funds on these longer-term efforts.

DiaTech was well prepared to revise its focus on "discovery." This was evident in many elements of the DiaTech program. For example, the first director of DiaTech, appointed after the initial review periods, was chosen for her experience in management of research and development. In addition, the majority of experts originally recruited for the Technical Advisory Group (TAG) were research-oriented academic scientists who were able to lend their expertise, influence, and perspective in identifying and funding research projects which, in their opinions, would provide the best opportunities for diagnostic test development.

In the original DiaTech RFA, A.I.D. and PATH expected that the majority of program funding would be utilized for subagreement support of extramural developers or biotechnology firms for adaptation of their "first world" technologies to developing world needs. Since these opportunities proved to be elusive, most of the funds obligated by DiaTech went to U.S.- or European-based, academically oriented researchers, university professors, and small companies willing to perform upstream research. As an indirect result, only a handful of DiaTech funds was committed to researchers working within the developing world.

As a result, DiaTech has managed a program which, on one hand, has encouraged creativity and innovation at the bench level, and on the other, has tried to retain the goal of serving the developing world. This approach has evolved in late 1989 into a portfolio of goal-oriented, often novel development projects focussed on the basic needs for diagnostics in the developing world. A total of 42 investigators have participated in DiaTech, of which 21 are still active. These investigators have been self-motivated or encouraged by DiaTech management to conduct their research with a specific goal or product-orientation. Often, results have come at a faster pace than normally expected of basic research-oriented laboratories. There have been constant reminders from DiaTech regarding the ultimate goals of the research, the urgent need to develop the technologies to the point of field testing, and the special requirements of appropriate technologies for developing world use.

As outlined in the introduction, the "discovery" phase consumed most of the first four years of the program. In the fifth year, this investment has started to return dividends for the program. Promising technologies with product potential have emerged from this initial phase. In the last year, DiaTech has purposefully and strategically moved towards a program of directed product development effort. To address this change in strategy, the current DiaTech Technical Director is experienced in product development, and the current TAG and roster of key consultants reflect more product and overseas field experience and include several representatives of private industry and developing world health.

The current DiaTech management has been proactive in fostering this shift in focus. Funding was not continued for projects which were not leading to useful diagnostics. The Technical Director actively sought new investigators who were interested and experienced in applied research, field testing, or product development. Collaborations between individual principal investigators have been encouraged, with bench researchers working with product development experts to bring technologies into the next phase. For some projects, DiaTech intramural staff has taken over or expects to take over the responsibilities for product development. Laboratory and field testing of technologies is being conducted by primary subcontractors and other DiaTech subgrantees. Business and technology transfer plans for several of the most developed technologies are being devised. In order to more efficiently control and accelerate this process, DiaTech proposed to A.I.D. funding such activities through a direct contract or purchase order mechanism as opposed to the proposal solicitation approach.

The plan has been to accelerate the product development cycle at a pace equal to or faster than the industry standard. Industry regards one successful product introduction for every 10 or more research projects, a normal rate of attrition. DiaTech will potentially have a much higher rate of success, perhaps at a ratio between 1:2 to 1:3. Having initiated research on basic technologies by making available a pool of research funding, DiaTech has

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now progressed to the stage at which product development strategies must be carefully considered to maximize the variety and availability of simple, rapid technologies, the plan originally envisioned by A.I.D.

VI. IMPLEMENTATION OF DISCOVERY PHASE

This section describes in more detail how the DiaTech program has been implemented based on the strategies and philosophies described above.

A. Team Building

1. The DiaTech Management Team. The key to effective management is a closely knit team which monitors, evaluates, and implements the many strategic and tactical demands of the project and adapts to its changing conditions. Dr. Milton R. Tam, current Technical Director, is primarily responsible for daily operations, management, and implementation, serves as a liaison between A.I.D. and PATH, coordinates the Technical Advisory Group (TAG), and interacts with subgrantees on technical matters. In addition to the technical input to the program, he coordinates the financial, clinical, legal, and other perspectives required to reduce each technology to practice. He consults regularly with Dr. Michael J. Free, Vice President and Director of the Product Development Department, on all major programmatic issues. Dr. Tam is assisted by Ms. Barbara A. Schaeffler, Associate Technical Officer, who is responsible for coordination of biological resources, logistical support of clinical and field trials, and provision of laboratory support to DiaTech subgrantees. Administrative, contractual, and financial issues and responsibilities are handled by Ms. Gretchen O. Shively, Program Administrator, and Ms. Kristin Bedell, Assistant Administrative Officer. These staff members are supported by a team of administrative, clerical, and laboratory personnel.

2. The DiaTech Technical Advisory Group (TAG). DiaTech is assisted in its activities by a TAG whose purpose is to provide informed advice to project staff in identifying and developing projects through their expertise and contacts in their respective fields of knowledge. Current members have experience in infectious diseases, immunology, international health, and diagnostic product development. The TAG has been and will continue to be called upon to provide a valuable source of technical assistance in monitoring of projects through review of progress reports and site visits. The TAG meets, on average, once a year. In addition, subcommittees of the TAG have taken the opportunity to meet at workshops to review progress of subgrantees. These meetings have served in building an effective rapport between the TAG members, key project consultants, representatives of A.I.D., and DiaTech management. Project staff feel that through these meetings, a substantial level of understanding and commitment of the TAG to DiaTech goals

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has been established. During the most recent TAG meeting in January-February 1990, the TAG unanimously expressed their recommendation that the DiaTech project continue beyond the September 1990 end date. The members of the TAG, their institutional affiliations, and their dates of service to the DiaTech program are presented as Attachment 2.

3. DiaTech Key Consultants. Since the TAG members are prominent scientists or physicians with constant demands on their time, they are not always available for DiaTech. Therefore, the project has called upon a number of consultants to serve on TAG subcommittees, as temporary advisors at workshops, or in review of submitted proposals. The majority have been supplied by the two DiaTech primary subcontractors described below. A list of these consultants is included as Table 2.

4. DiaTech Primary Subgrantees. DiaTech has identified two institutions as "Primary Subgrantees" whose assistance to the project goes beyond the development of diagnostic technologies. These are The Johns Hopkins University School of Hygiene and Public Health (JHU) and the Queen Saovabha Memorial Institute of the Thai Red Cross Society (QSMI). At JHU, Drs. Noel R. Rose and Ray R. Arthur serve as Consultant Coordinators. They are assisted by Drs. David Sack, R. Bradley Sack, Mark C. Steinhoff, G. Thomas Strickland, and Robert H. Yolken, key consultants in infectious disease focus areas, and Susan Medgalchi, laboratory technician. At QSMI, the Consultant Coordinator is Dr. Supawat Chutivongse. He is assisted by Physician/Consultants Drs. Henry Wilde, Thiravat Hemachuda, and Vithayuth Namsiripongpun. Mrs. Duangchand Siwasontiwat serves as Administrative Officer, and Mr. Jaturaporn Pornsilapatip is the Project Scientist. Both organizations have provided critical inputs in identifying priorities for diagnostic test development, reviewing applications for DiaTech funding, conducting workshops, assessing the progress of individual subgrantees through review of periodic written reports and site visits, conducting field trials of technologies and products, and procuring and maintaining biological resources for the project.

5. Coordination with A.I.D. DiaTech staff has developed and maintains a close and efficient working relationship with A.I.D. This has been done with three objectives in mind. First, timely communication ensures that the technologies emerging from the DiaTech program conform as closely as possible to the needs and objectives of A.I.D.'s health sector priorities. Second, DiaTech staff take advantage of the field experience and perspectives of the Office of Health, as well as the facilitative powers of A.I.D. in

Table 2

KEY CONSULTANTS

ARI/TB

C. Broome	Centers for Disease Control
T. Buchanan	**University of Washington**
G. Curlin	National Institutes of Health
R. Facklam	**Centers for Disease Control**
R. Good	Centers for Disease Control
M. Hendry	California State Department of Health
F. Luelmo	**World Health Organization**
M. Oxman	**University of California, San Diego**
M. Steinhoff	The Johns Hopkins University
R. Yolken	The Johns Hopkins University

Diarrhea/Typhoid

P. Charache	The Johns Hopkins University
S. Falkow	**Stanford University**
E. Gotuzzo	Universidad Peruana Cayetano Heredia
W. Greenough	**The Johns Hopkins University**
R. Guerrant	University of Virginia
P. Imperato	**State University of New York**
G. Keutsch	**Tufts University**
M. Levine	**University of Maryland**
J. Richards	**Gene-Trak Systems**
J. Robbins	National Institute on Child Health and Human Development
N. Rose	**The Johns Hopkins University**
R.B. Sack	The Johns Hopkins University
D. Sack	The Johns Hopkins University
G.T. Strickland	University of Maryland
I.K. Wachsmuth	Centers for Disease Control
B. Ungar	Uniformed Services University of the Health Sciences

Malaria

R. Arthur	The Johns Hopkins University
K. Campbell	Centers for Disease Control
D. Clyde	**The Johns Hopkins University**
M. Hanvanich	The Thai Red Cross Society
C. Nevill	African Medical and Research Foundation
R. Reese	Agouron Institute
A. Voller	**The Zoological Society of London**
H. Wilde	The Thai Red Cross Society

Other

F. Chapman	Si-med Corporation
S. Charache	The Johns Hopkins University
S. Chutivongse	**The Thai Red Cross Society**
M. Hatch	Centers for Disease Control
J. Koehler	University of Washington
A. Lucas	**Carnegie Corporation of New York**
J. Modlin	The Johns Hopkins University
T. Quinn	The Johns Hopkins University
D. Yelton	Genetic Systems Corporation

Note: Current or past TAG members are indicated in bold-face type.

general, to ensure a successful project outcome. Third, frequent communication between PATH and the A.I.D. CTO minimizes the time required for the A.I.D. review and approval process. Coordination is achieved through frequent reports, management meetings, consultations by telephone, and submission of work plans, subagreements, and other documents for approval.

These mechanisms have resulted in dynamic project management through which A.I.D. is provided continuous and timely information on the project. At the same time, project staff at PATH are able to focus primarily on the development, adaptation, and introduction of diagnostic technologies.

B. Identification of Diagnostic Needs and Priorities

The first steps in implementation of the DiaTech project were the identification of diagnostic needs and priorities in the developing world. To accomplish this, the DiaTech program sponsored two projects. The first was a questionnaire entitled "Survey of Diagnostic Needs for Patient Care" which was included with a mailing of *Health Technology DIRECTIONS* in May 1986. Four hundred respondents returned these questionnaires, which indicated their current diagnostic practices and problems, their priorities for development of diagnostic tools, and other concerns. The results of the survey confirmed that health workers were getting along as well as possible either with imperfect diagnostic tools or with no means of diagnosis at all. Additional concerns which were voiced but are beyond the present scope of DiaTech included adequate staff training to perform diagnostic testing and better patient education. The second project included DiaTech sponsorship of workshops for identification of important pathogens and appropriate diagnostic technologies for: (1) typhoid fever and diarrheal diseases, (2) malaria, (3) acute respiratory infections and tuberculosis, and (4) an overview of state-of-the-art technologies for rapid diagnosis of infectious diseases. More detail about the results and findings are included in Section VIII.

C. Selection of Target Diseases

The priority diseases identified early in the DiaTech program included malaria, diarrheal diseases, and acute respiratory infections--the primary infectious disease groups responsible for the majority of pediatric morbidity and mortality in the developing world. The rationale for these choices in 1985 continues to be valid in 1990.

1. Malaria. More than 1.5 billion people live in malarious areas of the world. Worldwide, an estimated 250-300 million cases of malaria occur each year. In tropical

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Africa alone, an estimated 160-200 million people are infected annually, with more than 1 million mortalities predominantly among infants and small children. Malaria-induced morbidity has been implicated by some for retarding the economic development of entire nations.

With regard to simple diagnostic methods, malaria is traditionally identified with microscopic examination of a Giemsa-stained blood film. This is a sensitive and specific method of diagnosis whereby one or more slides taken from a patient are carefully examined by a skilled microscopist. However, it is a time-consuming, resource-intensive method when large numbers of specimens must be examined, since smears must be scanned carefully and thoroughly before being declared as negative. Furthermore, the level of parasitemia often does not correlate well with the severity of clinical disease, since in cerebral malaria, a potentially dangerous form of the disease, the parasites are sequestered, or in an endemic population, where 30%-90% of individuals will be parasitemic without manifestation of symptoms.

2. Diarrheal Diseases. Diarrheal diseases are a major cause of childhood morbidity and mortality in developing nations. Worldwide, there are between 2 and 12 episodes of diarrheal illness per person per year and approximately 4.6 million deaths annually in children under age five. Prevention of dehydration and, when necessary, rehydration through the administration of oral rehydration solution (ORS) are the major interventions emphasized in A.I.D.'s health sector strategy for promoting child survival.

   Stool culture and direct stool examination are classic methods for detection of enteric pathogens. These techniques have many limitations, however, including the high cost of specialized media, plasticware, and laboratory equipment needed for selective isolation; the delay in time to obtain a diagnosis when culture is required; reduction of parasite viability associated with transport of the specimen; the high level of technical skill required to perform routine clinical laboratory techniques; and the potential for missing an enteropathogen due to bacterial overgrowth or sampling error. Despite recent advances in the diagnosis of viral, bacterial, and parasitic diarrheal illnesses, an agent cannot be identified in nearly one-half of all episodes. Inadequate specimen collection, technical problems with laboratory methods, and as yet unidentified viruses may be responsible for this diagnostic gap.

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3. Typhoid Fever. Development of improved tests and testing methods for typhoid fever is essential because current procedures are inadequate even at the central laboratory level. If typhoid fever is left untreated, there is a resulting mortality of 10%-20% in afflicted children. In some regions, the incidence of typhoid fever is 250-300/ 100,000 school-aged children per year. In Indonesia, where the incidence of typhoid fever is exceptionally high, it may be confused with cerebral malaria. Similar symptoms include headache, malaise, abdominal cramps, and bronchial cough. For these patients, a rapid assay in which results could be obtained in less than an hour would be a valuable diagnostic tool, since the improper administration of antibiotics could have fatal consequences.

   Presently, there are three approaches to the diagnosis of typhoid fever: bacterial culture, direct antigen detection, and serology. Culture cannot be performed adequately outside reference laboratories, and even where done, lacks in true sensitivity since the standard method of bone marrow culture is rarely performed due to ethical or procedural reasons. When compared against bone marrow, blood culture is at most 70% sensitive. Serologic determination of specific antibodies is helpful only if an early serum specimen is obtained so that a rise in titer can be detected by comparison with a specimen taken several days later. Unfortunately, by the time most patients present clinically with acute typhoid fever, they have been infected for one to two weeks and already have elevated antibody titers. An increase in specific antibodies correlates well with infection in young children, but less so in adults since prior infection must always be considered. Antigen detection methods have not been adequately developed. There is a clear need for antigen detection immunoassays or nucleic acid probe methods to be developed and optimized. High volume methods would also be of great value in screening larger numbers of specimens for baseline and vaccine efficacy studies and in identification of chronic carriers. These methods should prove to be much more cost-effective and practical than repeated culture.

4. Acute Respiratory Infections. WHO statistics indicate that 4-6 million children under the age of five die of pneumonia and its complications annually. Due in large part to the A.I.D.-assisted BOSTID program, the identity of the major etiologic agents of ARI is now known for a number of regions in the developing world. Armed with this knowledge, it becomes possible to develop strategies for long-term control of ARI. An integral part of this effort is the development of diagnostic tools to support

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and monitor intervention efforts and to enable prompt initiation of antibiotic therapy, where appropriate.

Streptococcus pneumoniae has been widely implicated as the major etiologic agent of pneumococcal pneumonia in the developing world. Antigen detection assays for the capsular polysaccharide of this organism have been commercially available for more than 15 years; unfortunately, the sensitivity of these assays in serum or urine is poor (26%-50%, compared with culture).

5. Tuberculosis. Tuberculosis (TB) remains a worldwide health problem. In the developing world, it has been estimated that up to 50% of the population in some countries is infected with *Mycobacterium tuberculosis*, about half of whom will develop active, communicable disease. The disease and its complications continue to account for 2 million to 3 million mortalities annually, despite the availability of safe, effective, affordable drugs. Culture methods of diagnosis are still too slow, complex, and costly to be of use in developing countries. Diagnosis by X-ray is still a limited procedure because of the availability of equipment. Microscopy is still the most widely used method for diagnosis but is limited in sensitivity, 40%-50% in comparison to culture, is labor intensive, and depends on the adequacy or quality of the sample obtained for examination. The development of rapid, sensitive, economical diagnostics by the biotechnology industry has not occurred despite an urgent and obvious demand.

   Diagnostics for other disease groups were to be considered on a case-by-case basis. DiaTech involvement in these areas would depend primarily on the strength of technological opportunity, on the importance of the disease in terms of mortality and ubiquity, and on the availability of effective therapy.

D. The Competitive Small Grants Program

Prepared with state-of-the-art information about current diagnostic methods and disease priorities, the DiaTech program began its efforts to fill these unmet needs.

The principal mechanism by which the DiaTech program has fostered the discovery of novel diagnostic technologies is a competitive small subagreements program. As originally proposed, DiaTech resources were to have been allocated so that 70%-80% were committed to later-stage technologies and 20%-30% to longer-range research offering the promise of significant breakthroughs in disease diagnosis. However, relatively few technological opportunities were found in the first category: the vast majority of assays which were

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sufficiently advanced to warrant DiaTech involvement were either technologically inappropriate, i.e., direct detection of microbial antigens by fluorescence microscopy, or did not address infectious agents identified as high priority for diagnosis in the developing world, i.e., Group A Streptococcus, Herpes simplex virus. Consequently, the actual distribution of subagreement funds at DiaTech's midpoint was nearer to 85% for long- and medium-range projects and 15% for late-stage, near-term projects.

In consultation with A.I.D. and the TAG, DiaTech management decided to emphasize development of those products which would most benefit individual patient care, i.e., methods for the identification of infectious agents for which therapy is already available. Receiving lower priority for new funding were promising technologies which addressed diagnostic methods useful primarily for epidemiological purposes, or diagnostics for infectious agents asymptomatic carriage of which is common, or diagnostics for infectious agents for which therapy does not exist, is not generally available, or is not affordable. Thus, projects for development of assays for respiratory and enteric viruses were given lower priority for funding, since antiviral therapy, virtually unavailable in developing countries, is prohibitively expensive. Similarly, methods of detection for non-typhoidal *Salmonella* were de-emphasized since disease due to these organisms is largely self-limiting and therapy is primarily supportive in nature. The development of *Giardia* diagnostics was also de-emphasized since there is a high frequency of asymptomatic carriage of this organism in developing country populations.

E. Soliciting, Screening, and Funding Small Grants

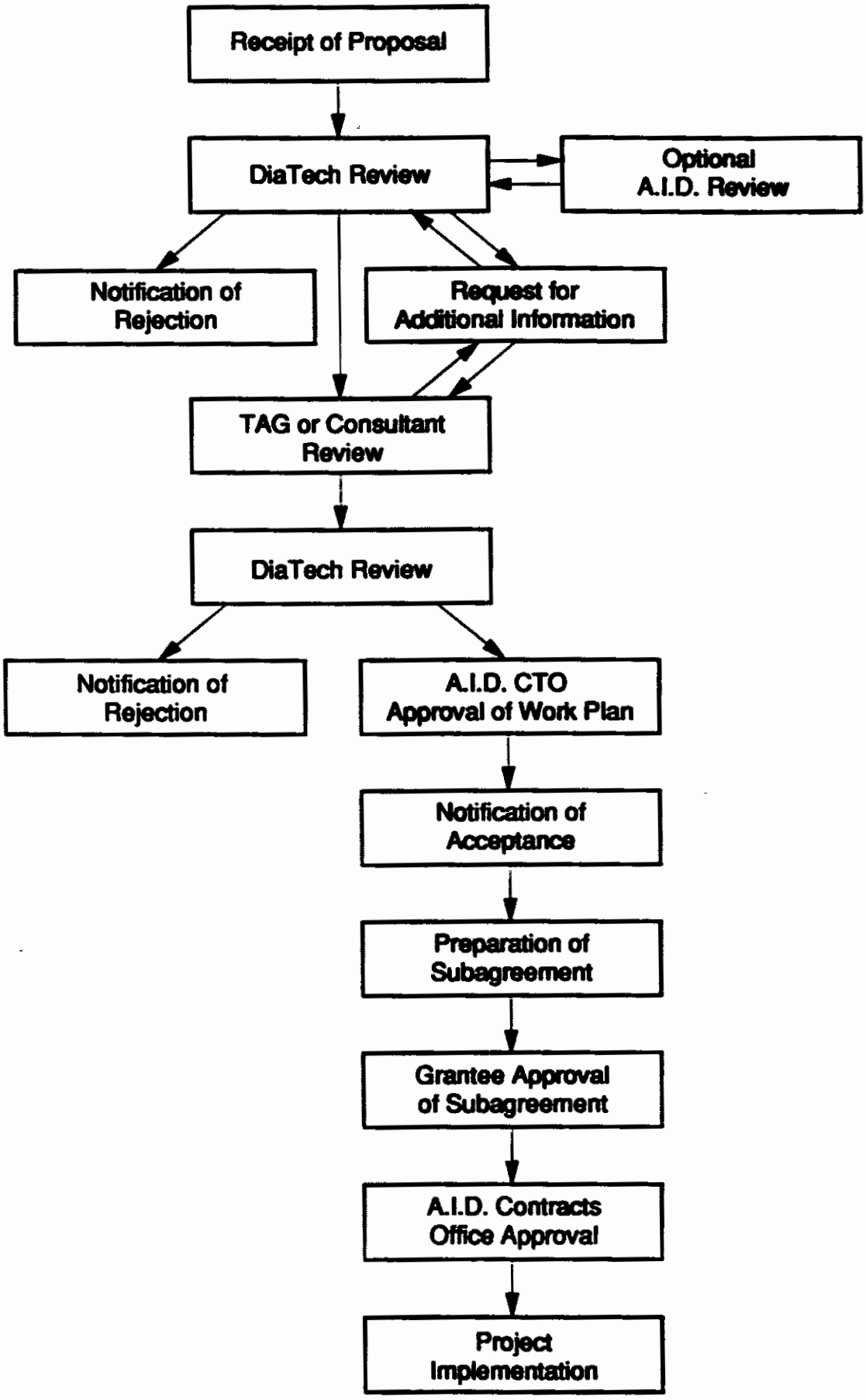
In 1986, DiaTech launched the effort to fund research of diagnostic technologies in each of the disease areas described above. Almost $4 million of the $7.25 million award budget was designated for funding of small grants, or subagreements. Guidelines for submission of proposals were written and procedures and criteria developed for scientific review. On the next page is a flow chart depicting the original procedure (Figure 6) for proposal review and rejection or approval.

Requests for funding were solicited through advertisements in scientific and trade journals including *Science*, *Nature*, *New England Journal of Medicine*, *Dialogue on Diarrhea*, and *Commerce Business Daily*. In addition, there has been direct contact with institutions and investigators known to be conducting research on relevant diagnostic technologies. Unsolicited proposals have also been received and reviewed.

For proposals to be considered and subsequently approved, they must first further the goals of DiaTech (listed on the original instructions for proposal submission). Additional

Figure 6

DiaTech Proposal Review Process



considerations include the soundness of the scientific approach, the competency of the researchers, the adequacy of their facilities and institution, clear presentation and description of methods to be developed, and the likelihood of project completion within the time frame and budget proposed. Development of diagnostic technology for appropriate use both in individual patient treatment decisions and in identification of pathogens in populations is considered.

During the first years of the DiaTech program, when many proposals were received, meetings of the TAG were held at which the proposals were discussed and prioritized for funding.

As shown in Figure 6, a proposal received at PATH is first reviewed by the DiaTech management team. If deemed worthy of further review, management selects 2-3 external reviewers to judge the merit of the proposal. Reviewers can be TAG members or other consultants with specialties in diagnostics of the respective disease area. Each reviewer is asked to comment on the proposal and then to rate the proposal on a points scale. (See Attachment 3 for Guidelines for Proposal Review).

DiaTech management then reconsiders the proposal in light of reviewers' comments and recommendations. If appropriate, more information or revisions to the proposal are requested of the applicant. If these changes are acceptable, the subagreement or grant is awarded. A work scope for A.I.D. CTO approval is prepared. The actual contract is drafted and sent to the institution of the principal investigator for approvals. Finally, the contract is sent to the A.I.D. Contracts Office for approval and work is begun.

Two types of applications are accepted under the competitive small grants program: feasibility studies and formal proposals. These differ in several respects including scope of work, proposal length, maximum funding available, duration of funding, and nature of review. Copies of these applications are included in the DiaTech Mid-Project Report. Although not originally contemplated in PATH's proposal to A.I.D., feasibility studies were instituted approximately 18 months into the DiaTech program to provide a vehicle for exploring higher-risk, higher-potential technologies at minimal expense. These are limited to a six-month time frame, a maximum funding level of $15,000, and generally receive more expeditious review by DiaTech staff. Formal proposals have been solicited to fund programmatic development of diagnostic systems or core technologies; these awards are for one initial year, extendable for a second year, with a total funding limit of $300,000.

As of January 1990, DiaTech has received a total of 239 proposals during the course of the program. Currently, 21 subagreements are active and 5 more proposals are being

considered. The proposals submitted and the ones funded are listed as Attachments 4 and 5.

F. Monitoring the Small Grants Program

To monitor technical progress achieved by the DiaTech researchers, management uses a number of different procedures. Contractually, each subcontractor is required to submit quarterly progress and financial reports. Each progress report includes statement of goals, performance/procedures, timetables, problems encountered, and a summary of progress to date. These are reviewed by core staff at PATH and also sent to JHU for review by key consultants in each disease category. Comments and suggestions for future actions or changes in direction are communicated to the researchers by the Technical Director.

Final reports are submitted for all projects following the year of work. These reports are sent to external reviewers (including JHU consultants and TAG members) for advice if continued funding is requested. For a variety of reasons, e.g., a change in A.I.D. priorities or low level of project success, renewal funding may not be extended. For each completed project, the reagents produced are transferred to the project for future application or reference.

If renewal of funding is requested, the second-year proposal can substitute for a final report. The proposal is reviewed by DiaTech staff and external reviewers, i.e., JHU consultants or TAG members. The reviewers are asked to evaluate the proposal in the context of the goals of DiaTech. They provide a narrative describing the degree of progress compared with the original goals, adherence to those original goals, appropriateness of the budget, the probability of completion within the proposed time frame, and the probability of a useful assay resulting from the project which may be then field tested. If a proposal is favorably reviewed, a new work scope is written and submitted to A.I.D. for approval.

During the course of each subagreement, a site visit is conducted by the DiaTech Technical Director or other technical staff, often assisted by consultants from JHU. Progress is reviewed and evaluated and questions answered that are not always covered in written reports. A summary report of the visit is written which includes recommendations for future activity and suggestions for changes.

Workshops, especially those in the past year which concentrated on progress in specific disease areas and brought together subgrantees, have been more results oriented. These forums provided an opportunity to review progress and share ideas in a collaborative, collegial manner. The most recent TAG meetings have also been used to present results on each

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project, to evaluate the portfolio of DiaTech projects, to set overall direction for each disease area, and to rank each project within that framework. In many cases, this has meant the recognition of lack of progress and the recommendation that projects be dropped or not renewed.

Each subcontractor is also required by contract to acknowledge the source of funding in any professional publication of research data. A notebook of all DiaTech articles and refereed publications is kept at PATH. A bibliography of these publications is included as Attachment 6. As of January 1990, DiaTech researchers had published an impressive number of articles that have involved research funded by DiaTech. These articles supplement the progress reports presented to DiaTech.

VII. IMPLEMENTATION OF THE PRODUCT DEVELOPMENT PHASE: Cooperative Approaches to Product Development and Directed Research Activities

One of the most significant achievements of the DiaTech program has been to initiate and maintain effective collaborations among researchers dedicated to similar goals. DiaTech management has encouraged this cooperative approach to product development, since the scientists who are able to make the greatest contribution to the discovery phases of research are not always the same individuals capable of making the greatest contribution to product development. Moreover, depending on the commercial potential of a reagent or assay system, there are alternative pathways to complete product development which depend on the degree to which private industry is willing to contribute.

A. Augmentation of the DiaTech Team

As the program focus of DiaTech has shifted from primarily oversight of a discovery-oriented phase to one which is more product-oriented, it draws upon the combined technical resources of PATH (see Figures 1-4) in a variety of ways. To advance potential products, the Technical Director is assisted by legal, business, manufacturing, and marketing advisors from the Technology Promotion Department in developing and managing licensing activities. Dr. Richard T. Mahoney, Director of the Technology Promotion Department, supervises the field-based project officers and staff in Thailand, Indonesia, and Kenya. These staff members, Mr. Don Douglas in Bangkok, Ms. Leona D'Agnes in Jakarta, and Ms. Kathleen Sebastian in Nairobi, provide local and regional coordination as products are introduced into the field. Personnel from the Technology Assessment Department, directed by Dr. James E. Maynard, are assigned for field assessment, and for technology transfer activities, the assistance of personnel in the Technology Management Department under the direction of Ms. Margaret Morrow is tapped. As the technologies are sufficiently defined, a product management team can be assigned to each project. As stated in the introduction, the product management team consists of a Technical Product Manager, a Product Advancement Manager, and any variety of technical resource persons as required by the project. The project passes from the Technical Product Manager, who has lead responsibility for the product development effort, to the Product Advancement Manager as the business planning, field assessment, technology transfer, or marketing aspects of the project unfold. This transfer of responsibility is by mutual agreement between the two managers and their department directors.

B. Promoting Interactions

DiaTech has sponsored a series of nine workshops to promote interactions between DiaTech subgrantees, key consultants, TAG members, representatives of A.I.D., PATH, and private industry

(Table 3). Early workshops were useful in assessing the state-of-the-art of relevant technologies and establishing project priorities. Later workshops, starting with the workshop entitled "Progress in Malaria Diagnosis" in August 1987, were forums in which A.I.D. and DiaTech could assess the progress made by individual participants, assess the research of potential participants, and facilitate strategic planning. These workshops also provided the participants with the opportunity to develop collaborative relationships and to exchange ideas and materials.

A number of important concepts have arisen directly from these workshops including the idea that two or more DiaTech participants could collaborate in different stages of product development. The first collaborator participates in the discovery, or research, phase of a technology to the point at which it is ready to leave the research bench. A second collaborator conducts further development, assay optimization, and verification with clinical samples. A third collaborator can then arrange and conduct design phase or validation stage field trials. This concept is designed to accelerate the pace at which products are developed, as they are in commercial companies, using groups of biotechnical subgrantees with the most appropriate knowledge, experience, and expertise in each phase of product development.

C. Directed Research

To fast-track a product, however, it is essential to streamline certain approval processes so that the process is not delayed by bureaucratic procedures. The "directed research" concept, therefore, was conceived, proposed to A.I.D., and implemented.

Those investigators with promising technologies, who are currently funded or who are to be assigned projects by the TAG or DiaTech management to take diagnostic tests from the earlier discovery or research stage and through the later development stage, are funded by contract rather than through the subagreement mechanism. This fast-track method of funding projects, illustrated in Figure 7, is crucial since some investigators are more interested in providing this essential component of development expertise than others.

In contrast to the normal proposal and subagreement process, the directed research procedure has been implemented with the following guidelines: DiaTech staff composes a detailed scope of work and budget with the identified collaborator and submits the work plan to A.I.D. in the name of the assigned subcontractor for approval. Once approved, a purchase or service order is used to finance the work directly with the researcher. External review by consultants or by the TAG is at DiaTech management's option. Thus, the tasks for assay

Figure 7

DiaTech
Directed Research Review Process

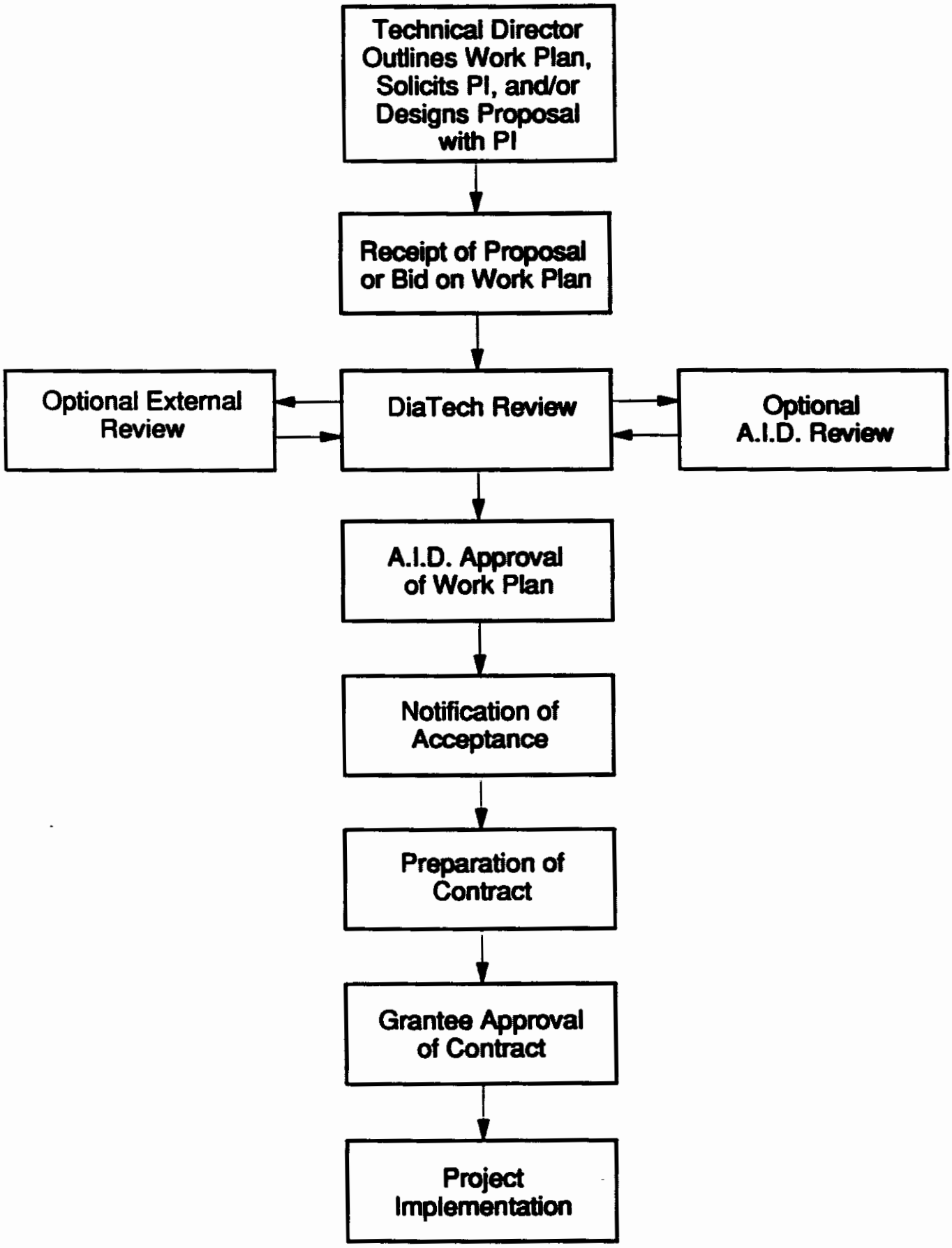

Table 3

DiaTech Workshops

Diagnostics for Use in Developing Countries: Typhoid Fever and Diarrhea Diseases	December 1985
Diagnostics for Use in Developing Countries: Malaria	January 1986
Diagnostics for Use in Developing Countries: Technologies for the Rapid Diagnosis of Infectious Diseases	April 1986
Diagnostics for Use in Developing Countries: Acute Respiratory Infections/Tuberculosis	March 1987
Acquired Immunodeficiency Syndrome in the Developing World	June 1987
Diagnosis of Malaria by Immunoassay and DNA Hybridization	August 1987
Progress in the Development of Acute Respiratory Infections/Tuberculosis Diagnostics	June 1989
Progress in the Development of Malaria Diagnostics	June 1989
Progress in the Development of Diarrheal Diseases/Typhoid Diagnostics	November 1989

development are identified, expedited, and controlled directly by DiaTech management. Since the work plans are not required to pass through the A.I.D. Office of Procurement, a potentially lengthy review and approval process is significantly reduced. The first DiaTech project to follow this track is the provision of funding for Dr. Alister Voller who has been assigned the responsibility for optimization and development of Dr. Diane Taylor's ELISA assay for serum and urine detection of malarial antigen. Several more contracts for services or purchase orders are currently being negotiated.

The directed research and development approach to project funding is used for assay development which has been recommended or declared to be essential by A.I.D. or the TAG in discussion with DiaTech management. The work scope for the assignments is by necessity concise and well-defined, as is typical of contractual arrangements rather than standard subagreements, and the end dates of the assignments are or will be on or before September 26, 1990.

D. Role of the Primary Subgrantees

DiaTech has also employed the two primary subgrantees, JHU and QSMI, in the cooperative implementation of the product development phase. JHU maintained one part of the DiaTech Biological Resources Bank, through the key consultants, and has provided a reservoir of biological resources to validate the accuracy of technologies in identification of specific pathogens. JHU can also provide standardized reagents for development of immunoassays. JHU has the capacity to evaluate and test technologies in the prototype development phase or in the field through their network of collaborators. Meanwhile, QSMI can provide a field testing component of DiaTech, for diagnostic test kits as well as devices, and valuable panels of specimens for use in development of assays for malaria and tubeculosis.

E. Interactions with the Private Industrial Sector

DiaTech management realizes that the more complex technologies cannot complete a timely development cycle without the participation of industry. The private sector is expert at essential development tasks such as reagent scale-up, documentation, manufacturing, quality control, quality assurance, and marketing. From its inception, the DiaTech program has interacted with private sector development experts with regard to proposal review, participation in subcontract research, and representation at workshops or TAG meetings. DiaTech, through its frequent, interactive relationships with management-level development personnel at companies such as Abbott Laboratories, Becton Dickinson and Company, E-Y Laboratories, Fairleigh Dickinson Laboratories, Inc., Gene-Trak Systems, Genetic Systems Corporation, and Syva Company, will continue to

receive valuable advice from this reservoir of expertise for the downstream phases of product development.

VIII. RESULTS

A. Results of the Discovery Phase

1. Establishing Needs and Priorities for Diagnosis of Infectious Diseases

In 1985 and 1986, the DiaTech program sponsored a series of workshops (Table 2) to discuss the needs and priorities for diagnostics which could be applied to the developing world. The topic of the first workshop concerned diarrheal diseases. Typhoid fever, caused by *Salmonella typhi*, was of most concern because it is life-threatening, responsive to antibiotic therapy, and can be easily misdiagnosed as malaria in regions where the diseases are co-endemic. *Shigella* and enteroinvasive *E. coli* were considered second in need of improved diagnosis since both cause serious, potentially fatal bacillary dysentery and have similar patterns of antibiotic sensitivity. Diagnosis of enteropathogenic and enterotoxigenic *E. coli* were of lesser importance since these organisms produce a watery diarrhea which is generally self-limiting and, when recognized, responds to supportive therapy with oral rehydration solution. Amebiasis, produced by *Entamoeba histolytica* also received a high priority for improved diagnosis. The symptoms of amebic dysentery are similar to *Shigella* dysentery, but antibiotic therapy is different. Since it produces unpleasant side effects, unnecessary treatment needs to be avoided. Cholera, produced by *Vibrio cholerae*, received an intermediate ranking. The diarrhea produced is usually severe but can be treated with ORS alone; however, some patients require administration of i.v. fluids, which may be avoided if the organism is diagnosed without delay and treated with appropriate antibiotic therapy. *Campylobacter*, rotavirus, and *Giardia* ranked lower because of limited applicability of diagnostic testing when applied to developing country settings. With regard to types of tests, the expert group agreed that tests for individual patient care would need to be different than those developed for epidemiologic studies. Both nucleic acid hybridization assays and immunoassays were judged as appropriate for diagnostic test development; however, no novel assays were identified which could be immediately adapted and applied to developing world diagnostic needs.

Subsequent workshops addressed other DiaTech disease groups, i.e., acute respiratory infections (ARI), malaria, and technologies for the rapid diagnosis of infectious diseases. Similar discussions were held to prioritize diagnostic development of technologies. For ARI, the greatest need for new technology was found to be for diagnosis of pneumococcal pneumonia. Tuberculosis,

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although not strictly categorized as an ARI, was targeted for diagnostics development. Organisms receiving lower priorities included *Bordetella pertussis*, the agent of whooping cough, and respiratory viruses.

For malaria, the focus was upon what types of diagnostic needs existed and how to address those needs by development of diagnostic methods. Microscopic diagnosis of blood films was recognized to be the de facto gold standard, since for the majority of laboratories with microscopy, it is the established method for identification of malarial infection. However, since microscopy is labor intensive, limited in sensitivity, and dependent on microscope availability and trained technical assistants, the system of microscopic diagnosis needed to be reassessed. Thus, the expert group recommended facilitation of microscopic diagnosis by evaluation of economical field microscopes and adaptation of the staining formulation to produce clearer results of staining. With regard to assay development, the emphasis was more on applications for epidemiological settings, rather than on primary health care. Immunoassays for antigen capture, as well as DNA probe assays, were discussed for application.

The last in the series of discovery-oriented workshops considered the application of novel technology for diagnosis of infectious disease in the developing world. With regard to identification of ideal tests, i.e., those which are rapid, cost-effective, instrument-independent, simple, and stable, it was no surprise that tests were identified which could meet all of the necessary criteria. Participants emphasized the importance of carefully matching technologies to actual needs, accounting for both the application in which the technology would be utilized and the setting in which it would be applied. Multiple types of technologies were recommended for development since the developing world is heterogeneous, and technology appropriate for one purpose and location may not necessarily be appropriate for another.

2. Funding of Research Projects with the DiaTech Small Grants Program. As the result of the information presented at preliminary workshops and meetings with key consultants, the DiaTech program funded a number of grants, in addition to the two primary subagreements. As stated earlier, the majority of funding consisted of research-oriented projects, since there were few opportunities to fund projects in which the preliminary research phase had already been completed. For more detail, a technical summary of each of the technologies supported is submitted as Section X.

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The smaller number of subagreements at the start of 1990 compared to earlier in the program represents a process of natural attrition as grants were not renewed due to lack of progress, a narrowing of project focus, and at the recommendation of either the TAG or A.I.D. The ratio of total number of projects funded to those which are promising is about 2:1. The remaining subagreements all represent promising technologies and are mainly the result of funding upstream research.

B. Status of the Product Development Phase

The active subagreements are listed below by disease group and the progress of each is summarized. More detailed technical summaries for all active subagreements, including timelines for product development, are presented in Section X.A.

1. Tuberculosis (TB). The emphasis of DiaTech's TB portfolio has been to identify specific epitopes which can distinguish *Mycobacterium tuberculosis* from other mycobacteria. Both Dr. Thomas Buchanan and Dr. Arend Kolk have prepared large numbers of monoclonal antibodies to screen for this purpose. While Dr. Buchanan has discontinued his efforts due to a lack of ELISA assay sensitivity using clinical specimens, Dr. Kolk has continued his efforts in development of immunoassays for direct detection of TB, attempting to improve the antigen extraction from sputum. Both immunoblot and immunofluorescence methods are in development. In addition, a rapid culture method using ELISA as signal has been shown to work for the speciation of *M. tuberculosis* in a field trial performed in late 1989. Dr. Kolk will now convert his ELISA to a simple agglutination method and is planning a field test in 1990.

   In an attempt to provide a serological screening assay for identification of active TB infections in populations, Dr. Thomas Daniel is optimizing a test for TB using a defined antigen system ("Antigen 6"). Current field testing results in Mexico indicate that the sensitivity of his method has improved and was reported at 70% for his latest study. Specificity was 100%. Further assay development will employ epitope scanning for definition of specific peptide sequences which can then be synthesized cheaply. This field test will be repeated in Uganda in early 1990. DiaTech has also supported a molecular approach to diagnosis of TB by supporting efforts which identify specific genomic sequences that can be used in molecular probe assays.

   In 1989, Dr. Buchanan requested to shift his research emphasis from an immunological approach to one which would identify specific PCR primers for TB diagnosis.

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Several primers have been identified and synthesized which are TB-specific. Optimizations in the method and confirmation of primer specificity with a proficiency panel are proceeding, and a field trial is planned for 1990. Dr. Jack Crawford has developed DNA probes as well as specific PCR primers for diagnosis of TB in clinical specimens; he expects to field test his methods in 1990.

2. Acute Respiratory Infections. Early DiaTech workshops concluded that the need in this area was for development of assays which could be used in epidemiological studies to discover the relative importance of the many organisms to produce disease in developing world countries. Since epidemiological assessment has been effectively addressed by the BOSTID study, implemented by the National Academy of Sciences and in part financed by A.I.D., DiaTech has in addition funded development of tests for individual diagnosis of ARI organisms for use in primary health care settings. Immunoassay detection methods have been funded since viable molecular probe approaches have been lacking.

   Dr. Larry Anderson has produced immunofluorescence and immunoblot tests for viral ARI pathogens RSV, adenovirus, and parainfluenza. Third-year support will be for the field testing of reagents. Dr. Anderson has already supplied his monoclonal antibodies to interested companies for their evaluation. Using three approaches with different antigens, Dr. Alan Parkinson is in the process of developing ELISA methods for diagnosis of pneumococcal pneumonia. He will first test his assays on the Native American population in Alaska. He will be collaborating with Dr. Richard Facklam (CDC, Atlanta) in development of assays.

3. Diarrheal Diseases. DiaTech has concentrated on development of tests for detection of organisms producing inflammatory or invasive diarrhea, since lives can be saved by identifying and properly treating them with antibiotic therapy. For *Shigella*, an important cause of bacillary dysentery, DiaTech has funded two efforts. Dr. Nils Carlin's feasibility study will assess the practicality of an immunoassay using a unique monoclonal antibody he developed which is specific for *Shigella* outer membrane protein antigen. Antigens will be concentrated on magnetic beads prior to and after rapid culture, which may improve sensitivity.

   Dr. Richard Oberhelman is currently field testing a non-isotopic DNA probe method for detection of *Shigella* in Peru. The method is potentially more accurate than culture since it allows for more rapid results and does not require viable organisms. DiaTech is funding

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Dr. Richard Guerrant for the development of an immunoassay which will detect the presence of fecal leukocytes. This nonspecific test using an agglutination method is very inexpensive, potentially cost-effective used in a clinical setting, and would be especially appropriate for development of a diagnostic algorithm. The result of the test would determine whether patients with diarrhea should be treated either with antibiotics or merely with supportive therapy (ORS) and would result in cost savings of bacterial culture. An additional application could be for diagnosis of nosocomial diarrhea caused by *C. difficile*, which can lyse fecal leukocytes, resulting in false reading of watery diarrhea upon microscopic evaluation of stools. The assay will be ready for field testing in Brazil in 1990.

Dr. Jonathan Ravdin has developed a rapid assay for discrimination of invasive amebiasis. A present diagnostic difficulty is to accurately distinguish between invasive and noninvasive strains (zymodemes) in countries where amebiasis is endemic. Identification of pathogenic strains in patients with inflammatory diarrhea indicates a necessity to treat with antibiotics. Both a serological test and an antigen detection method using stool as specimen are in development and will be field tested in South Africa in 1990.

DiaTech is also supporting Dr. David Sack's production and field trial of an agglutination test to identify *V. cholerae* antigen in direct stool specimens, since the course of disease can be shortened and complications reduced by the timely diagnosis and treatment with antibiotic therapy. A field trial is planned for India in early 1990.

4. Typhoid Fever. DiaTech is currently supporting two active efforts for immunodiagnosis. Dr. Suttipant Sarasombath has developed monoclonal antibodies to the Salmonella Vi antigen in Year 1, and development of a simple co-agglutination immunoassay is underway. She will then field test the diagnostic methods in Indonesia, a region where typhoid fever is endemic. This will be especially valuable if urine can be used as specimen. In addition, DiaTech has recently funded Dr. Felipe Cabello for development of a test method for the detection of the 36 KDal protein in serum of typhoid patients.
Dr. Cabello has produced monoclonal antibodies specific for the protein with which he will develop his assay. In addition, the feasibility of a serological assay will also be considered since cloned 36 KDal antigens also exist.

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5. Malaria. DiaTech has supported a multidimensional approach to diagnosis of malaria, i.e., microscopy, immunoassays, and nucleic acid probe tests, which may be more suited either to individual diagnostic testing or to screening of populations. This is because the disease is complex by nature, and a range of technologies is needed for application to different conditions encountered in the developing world. With regard to microscopy, DiaTech has sponsored the field testing of basic field microscopes in Thailand, since identification of a low-cost, high-quality instrument would contribute to overall availability of malarial diagnosis. As a refinement of the microscopic method, Dr. Henry Wilde at QSMI has and will continue to field test the Quantitative Buffy Coat (QBC) device manufactured by Becton, Dickinson and Company for detection of malaria, in which organisms are stained with a fluorescent tag and identified with a specialized microscope detection system. Proof-of-concept has already been established in a rural (provincial) hospital in Thailand where the method is slightly more sensitive than thick smear and is much more rapid. Future studies will include testing a portable, low-cost fluorescence attachment for a light microscope and the feasibility and cost effectiveness of combining differential blood cell count (CBC) to justify the cost of the method in developing countries.

Dr. Savanat Tharavanij is developing an inhibition ELISA for *P. falciparum* using polyclonal rabbit serum. The test has demonstrated relatively good accuracy, although the P.I. thinks it can be improved. This can be a benchmark test against which other experimental diagnostic methods can be measured. Dr. Savanat's laboratory has access to clinical specimens and will test other DiaTech-supported technologies as directed. Dr. Diane Taylor is also developing monoclonal antibody-based ELISA tests which appear to be specific for soluble malarial antigens in serum and urine. If development of the urine-based assay is successful, it would be a significant advance in screening technology since noninvasive specimens can be obtained for testing. Since the commercial market for malaria ELISA tests are at best uncertain, Dr. Alister Voller is serving as a development expert to optimize and scale-up these assays in preparation for clinical testing and manufacturing.

Dr. John Martin has been funded to develop PCR approach for development of molecular probes which will be useful for malaria diagnosis. Primer sequences have been identified for vivax and falciparum malaria to allow identification to be simultaneous with speciation. In addition a primer for drug resistance factors encoded on a plasmid has also been developed. While PCR will not be

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directly applicable for individual diagnoses in the near future, it is potentially valuable as an epidemiologic monitoring tool. Dr. Gerald McLaughlin has received funds to develop a dot-blot assay for falciparum malaria using nonisotopic DNA probes. A successful field study in late 1989 was performed in The Gambia which confirmed the practicality of the dot-blot assay in favor of an alternative *in situ* slide method. Based on the outstanding performance of the dot-blot method, an additional study is planned for 1990 in the laboratory of Dr. Savanat Tharavanij and in rural locations in Thailand. With regard to therapeutic drug monitoring, DiaTech has supported Dr. Alister Voller for development of an ELISA method for the determination of serum quinine levels. The availability of assays which will meet the need for therapeutic monitoring is especially timely, since quinine is being reevaluated as a cost-effective therapy for malaria with the emergence of parasite resistance to other drugs such as chloroquine, pyramethamine, and fansidar. Quinine has been reformulated for use as a therapeutic drug with much of its bitterness removed; its bad taste has traditionally been a serious objection, which has resulted in poor patient compliance.

6. Other Projects. The DiaTech program continues to support development of selected appropriate laboratory equipment and devices where the priority for such devices to assist in the diagnosis of an infectious disease-related condition is the greatest. As an example, Dr. Richard Curtis is being supported to develop a portable field hemoglobinometer which will be capable of objectively determining anemia using finger-stick blood as specimen. A prototype device has been successfully tested in the field by Khymer medics at a Cambodian refugee camp, a project coordinated by QSMI. An in-house effort includes the evaluation of simple centrifuges for determination of hematocrits in the field using modified flashlights.

C. Technical Assistance

Early in the project, the DiaTech program received a variety of technical assistance requests. Many were from developing country scientists wishing to finance their sabbatical period in the United States. Since there was no formal procedure devised for dealing with these requests, none were granted. In Year 4 and Year 5 of the DiaTech program, the technical assistance component has been deemphasized in favor of development of extramural subagreements. However, two requests for assistance were implemented earlier in the project. The first was a request from Rector Natth Bhamarapravati of Mahidol University, Bangkok, Thailand, for technical assistance in the area of protection of international intellectual property rights. The second request was from Projet SIDA for

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assistance in assessing rapid methods for the screening of donated blood in Kinshasa, Zaïre.

In Year 2, a three-day seminar on the International Protection of Intellectual Property Rights was held at Mahidol University. This seminar allowed faculty and staff of Bangkok-area universities access to information on patent, trademark, and copyright law in the U.S., E.E.C., Japan, and ASEAN countries. Staff members of the Thailand A.I.D. mission and of the White House Office of Science and Technology Policy were in attendance.

In Year 3, a study was initiated in conjunction with Dr. Thomas Quinn of JHU and Dr. Robin Ryder of Projet SIDA and the CDC. Projet SIDA is an international AIDS research consortium based at Mama Yemo and Ngaliema hospitals in Kinshasa. Approximately 4,000 samples of blood were evaluated prospectively for antibodies to HIV-1 using five rapid, qualitative assays, the results of which were compared with ELISA and Western blot as reference methods. In addition to such considerations as sensitivity, specificity, and reproducibility, other parameters addressed test adaptability to developing country conditions and ease of use by minimally trained technicians. Apart from its immediate reference value for safe blood programs, this activity was also considered to be a prototype mechanism for testing of DiaTech diagnostics under appropriate operational conditions.

The conclusions reached were that two of the five tests, one from Du Pont and the other from Fujirebio, Inc., were more applicable to the trial conditions. Both tests demonstrated a high degree of accuracy, a low level of intra-reader variability, and appeared to be more robust under the conditions of testing. The ultimate decision to use one test method in preference to another, of course, is based on the combination of test factors including cost, volume of specimens, time necessary to obtain a result, the need for confirmatory testing, and an uninterrupted availability of the assay. An important observation was that if two rapid tests were used in combination for screening and confirmatory testing, the results were as accurate as ELISA and Western blot, indicating that HIV-1 testing in developing countries using rapid tests could be as accurate as that found in industrialized countries using more complex, instrument-dependent methods. Abstracts from this study have been presented at the fourth and fifth International Conference on AIDS, and manuscripts describing results of the study have been published in *The Lancet* and *The Journal of Clinical Microbiology*. These publications are included as Attachment 7.

D. Information, Education, and Communication

1. Technical Monographs Database (DiaCat). DiaTech maintains a database of approximately 2,000 technical publications relating to diagnosis, treatment, prevention, and epidemiology of selected infectious disease groups. Project and PATH library staff regularly scan more than 60 journals for articles of relevance to the project. In addition, computerized reference searches of the current international literature are conducted by PATH library staff for other relevant publications.

2. Dissemination of Applied Research Results. DiaTech actively encourages the dissemination of results through publications and abstracts. To date, DiaTech-funded research has resulted in a large volume of work submitted or published in refereed journals, including two publications listed above which resulted from the Kinshasa study (Attachment 7).

3. Workshops. During the first four years of the project, DiaTech has sponsored nine workshops (Table 2). Early workshops were useful for assessing disease priorities and appropriate technologies for the project. Later workshops concentrated on progress reported by DiaTech subgrantees. The workshops also facilitated strategic planning for the subject areas since TAG members and key consultants have the opportunity to exchange ideas and materials with the participants.

4. Biological Resources Bank (BRB). The BRB was created to provide a source of clinical and other materials which could be utilized for development and evaluation of diagnostic tests. The bank is administered by a TAG subcommittee headed by Dr. Michael Oxman, with representatives from JHU, project staff, and other experts in the infectious disease community. For reasons of geographic convenience, the bank is physically divided between JHU and PATH. To facilitate management of this bank, DiaTech staff have devised a centralized database, called Biostore, whereby each site can access the samples stored at the other site. The BRB is supplemented by additional sources of samples stored at sites worldwide to which DiaTech can gain access to provide specimens in response to requests from subgrantees.

5. Several versions of a brochure publicizing the DiaTech program were distributed. The earliest versions included a solicitation for proposals; the latest version (Attachment 8) is purely informational and descriptive.

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IX. FUTURE PLANS

A. Identification of the Fast Track Portfolio

The DiaTech program's present portfolio of projects has addressed the development of diagnostics for the majority of organisms which have been emphasized at the various workshops and TAG meetings. To date DiaTech has funded over 40 projects and has expended about 85% of its resources for subagreements or "discovery," which addresses upstream research projects for both assay and reagent development. There has been a normal attrition and a sorting out of the less-promising projects over time.

Resulting from these research commitments, DiaTech has identified and continues to support 21 of the most promising projects. In addition, there are other potentially useful intramural projects which are in development within the two primary subagreements programs at JHU and QSMI. As a result of assessing current progress, demonstrated assay proficiency, potential for impact on diagnosis of infectious disease in the developing world, and feasibility for a rapid cycle of development, DiaTech management is now positioned to prioritize activities during DiaTech's final year and beyond.

1. The Technologies, Timelines, and Budgets. As the most successful projects are not necessarily the ones with the highest priority for product development, DiaTech is currently evaluating and prioritizing each project. DiaTech management has concluded that with a limited budget for personnel, intramural research, clinical studies, business and market planning, and with the considerable shortfall in resources to conduct lengthy and expensive product development cycles, there are not enough resources to develop all projects at once. Therefore, projects must be prioritized for advancement. Recently, development timeline milestones were identified by the subgrantees of all current projects and modified by DiaTech management. These timelines are included for active subagreements in Section XI. Project progress was also assessed at the workshops held in 1990, at site visits, or through informal interactions with individual investigators.

   In this process, many factors were considered, including the relative need for a simple diagnostic method and its impact on recognition and treatment of disease in the developing world, cost-effectiveness of the technology, higher priority use for individual diagnosis *vs.* detection of disease in a population, commercial *vs.* public sector market potential including feasibility of cost-sharing with private industry. With that information and other considerations, projects were prioritized and

ranked with regard to their potential for immediate advancement in 1990, advancement in the near future, or advancement at a later date. This information is presented as Table 4.

Priority 1A projects have already or are about to undergo preliminary field testing and may prove to have considerable impact on the diagnosis of infectious disease in developing world; much of DiaTech's efforts for the remainder of Year 5 will be to prepare these projects for fast-track development by creating business and marketing plans, interfacing with private industry, and exploring the possibility of technology transfer of manufacturing in the developing world.

Priority 1B projects are those which may have similar potential, but which lag slightly behind the first-rank projects and need to undergo additional refinement of concept or clinical testing. If breakthroughs occur in technology, preliminary development, or field testing, or if a strong demand is identified, these projects could easily move up and even displace higher-ranked projects.

Priority 2 projects include those with excellent potential, but which have only recently been funded by DiaTech; these technologies must be given time to mature and develop by undergoing clinical proficiency testing and proof-of-concept by specimen testing.

Priority 3 projects are the more upstream, core technologies--applications must catch up with the technologies or reagents which have been already developed. These projects do not presently need as much input and direction as others, but rather, should be monitored for progress in development of more simplified or cost-effective delivery systems so that the technologies can be adapted to the rigorous conditions of testing found in the developing world.

2. Validation Procedures. Technology validation will continue to be accomplished by two methods. First, a test panel of standardized specimens is assembled from the BRB and distributed by the JHU Biological Resources group. The same specimens are distributed among DiaTech subgrantees working within the same disease area with differing assay technologies, i.e., probe technology *vs.* ELISA. Thus, accuracy and sensitivity of the differing technologies are compared among the respective investigators.

Second, field evaluations of candidate technologies have been and will continue to be sponsored by DiaTech. These evaluations provide essential information from which

Table 4

PRIORITIZATION OF DIATECH PROJECTS FOR DEVELOPMENT OF BUSINESS AND MARKETING PLANS

Priority	Investigator
1A	Kolk Anderson Taylor (malaria assay) Ravdin Guerrant Curtis
1B	Suttipant Daniel Voller (quinine assay) McLaughlin Parkinson/Facklam
2	Sack Savanat Cabello Carlin Oberhelman
3	Martin Crawford Buchanan Perrone (QBC)

decisions can be made regarding the performance of the technology outside the research laboratory, including how easily the assay method can be performed by different laboratories and under varying conditions, the identification of appropriate users of the technology, assessment of the need for further development or adaptation of the technology, and recognition of other factors which can be used to successfully introduce the technology into routine health care procedures. This assessment and validation process will be applicable not only to products directly emerging from the DiaTech research and development program, but also to new technologies developed in the public or private sector and identified by PATH, its collaborators, or A.I.D.

Field assessment provides valuable feedback when the research prototype kit or device is under evaluation. First, these trials are intended to provide basic qualitative and quantitative feedback on technical performance with regard to function (sensitivity, specificity, reproducibility) and acceptability to users (ease of use, level of training required, cultural concerns). Trials at this stage are carried out by the subgrantees themselves or others with early prototypes that have previously been evaluated in a laboratory to ensure basic performance and safety. For example, Dr. Gerald McLaughlin recently reported on a successful design-stage field trial in The Gambia. Second, the information collected during design field trials is then used to plan modifications before large-scale production or introduction of the technology is begun. Third, field assessment is used to screen out technologies which do not meet the claimed performance criteria before DiaTech invests in further development. At this point, the technology may be ready for more widespread testing if there are no major modifications needed. A list of subgrantees who have already evaluated their technologies in the field or plan to in the near future appears as Table 5.

The comparative trial of HIV-1 screening assays in Zaïre sponsored by DiaTech is illustrative of DiaTech's capability to supervise a validation-phase field trial. The experience gained in this trial will assist in DiaTech efforts to validate the performance of other products. Of the five assays evaluated, three were already on the market, and two were in the final prototype stage. Performance was compared to the manufacturers' claims as well as to each other and to the ELISA and Western blot reference methods. A proficiency panel was developed for use in training technicians to perform and interpret each of the assays. Cost per HIV-positive unit of blood was determined for each of the five rapid assays evaluated as well as for the Western blot and ELISA reference methods.

Table 5

FIELD TESTING OF DIATECH TECHNOLOGIES

1989-1990

(17 of 20 Projects)

Investigator	Site(s)
Anderson	Brazil, India?
Buchanan	TBD
Crawford	TBD
Curtis	Thailand
Daniel	Mexico, Uganda*
Guerrant	Brazil
Kolk	Thailand, Vietnam*
Martin	Guatemala
McLaughlin	The Gambia*, Madagascar
Oberhelman	Peru
Parkinson	Alaska
Ravdin	South Africa
D. Sack	India
Savanat	Thailand
Supawat	Thailand
Taylor	Thailand
Voller	Malawi

*Testing not funded by DiaTech

Ease of use, ease of interpretation, and ease of presentation were scored for each of the assays evaluated, and user-to-user variability was assessed. Performance validation is designed more for external evaluation than for feedback to the developers. Ideally, performance validation trials are conducted in multiple, geographically separated sites. In choosing sites, consideration is given to the level of technical skill of the end-user, the disease prevalence in the population studied, and the prevalence of other diseases in the community which may impact performance.

3. Product Advancement Plans: Licensing, Product Engineering, Technology Transfer, and Scale-up

   a. Incentives for Commercial Involvement. Technologies designed and developed expressly for the purpose of meeting a public health need range from simple products suitable for home or cottage industry to highly sophisticated technologies requiring state-of-the-art industrial processes and materials. All of these social technologies differ in one important respect from the technologies developed for business: they are produced to meet a perceived health need rather than a measured market demand. Since need does not equate with ability to pay, the economic viability of social technologies is always in question. Because of this, DiaTech must attend to the commercial-sector interest in the products or find substitute arrangements that assure availability, quality, and low cost.

      The most important factor determining the strategy used to gain commercial-sector interest is whether or not a commercial market exists for the products. Some social technologies have no significant private market potential--these are labelled "Type A" in PATH nomenclature. An example of such a technology among DiaTech products is the co-agglutination test for detection of *V. cholerae*. This simple technology for determining the presence of the pathogen causing cholera after rapid stool culture has no obvious utility other than in developing world health care settings. For this type of technology, there are no opportunities for gaining economies of scale or for covering the capital risks of development with profits from commercial-sector sales. The public health sector must therefore bear the entire cost of these technologies by underwriting the development costs, purchasing the capital goods, and paying a price that covers the remaining cost as well as margins.

It follows that a Type A technology must always involve simple, inexpensive materials and processes so that the risks are reasonable and so that manufacturing can be carried out locally on a small or medium scale within the countries which are proximal to product use. Generally, it is useful to provide some business and marketing assistance to the local manufacturing units, as well as quality assurance auditing, up to the point where production and sale of the product to responsible agencies are well established.

When a social technology has an identifiable commercial market potential ("Type B"), the strategies for product development can be quite different, depending upon the extent to which those private markets are marginal or exist only in the developing world rather than the developed world. Examples of a Type B technology among DiaTech projects are the rapid, quantitative immunoassays for detection of *Mycobacterium tuberculosis* or *Entamoeba histolytica*. Several multinational companies have expressed interest in test development since the demand for a TB diagnostic is clearly worldwide. PATH's ability to demonstrate a sizeable market for tests such as these in the developing world, coupled with DiaTech assistance, both financial and technical, in conducting field trials, and input into the development of simplified test formats, can result in a technologically appropriate diagnostic, simultaneously allowing parallel commercial development of the technology to serve the disparate needs and requirements of the developed world.

Other technologies may or may not have an immediately apparent commercial market, but because of their inherent complexity, absolutely require a commercial collaborator in order to be scaled-up, manufactured, or distributed ("Type C"). An example of a Type C technology among DiaTech projects is the quantitative buffy coat (QBC) analyzer. This technology for the histochemical identification of malarial parasites in a finger-stick blood specimen requires glass capillary tubes, the internal diameter of which must fall within narrow limits of tolerance, and a specially manufactured centrifuge. Other examples of diagnostic technologies which are potentially Type C include those which utilize recombinant proteins, where scale-up requires fermentation capability, or serological assays, where large quantities of an infectious agent are needed for use as target antigens.

b. Management of Intellectual Properties: Protection of Public Sector Interests. In the product advancement and technology transfer of diagnostic technologies, DiaTech will seek to preserve, as much as possible, the proprietary nature of the technology through pursuit of patents, licenses, and confidentiality to encourage commercial collaborators to view the technologies to be developed as protected and therefore of value.

Private, for-profit companies are brought into the development cycle either by the subgrantee or by interaction with DiaTech management, since they are essential in the majority of projects for product development. Letters have been sent to several subgrantees to introduce PATH policy regarding the development and commercialization of intellectual property and to ensure consideration of public sector access to the property developed.

Despite PATH's stated position concerning development of diagnostic products, DiaTech cannot assume that the commercial-sector collaborator will always be motivated by the public health objectives for which the technology was developed. Rather, companies may be motivated to recover the costs of the project as quickly as possible and, in addition, generate a substantial profit to justify overhead and the risks of investment. Consequently, protecting the public-sector interest in terms of universal product availability, lowest possible cost, and responsiveness to local needs, becomes the full responsibility of DiaTech and other interested parties. The broad strategies that must be adopted include (1) maintaining sufficient control of the product as it moves into the commercial sector, and (2) achieving the greatest possible efficiencies and economies of scale.

Under the first strategy, maintenance of control, protection of the technology at the outset with patents, licenses, copyrights, design registrations, or even trademarks, is essential so that the title to the invention is clear, and business negotiations are not delayed by questions of ownership. Licensing strategies which seek to avoid monopolies and maintain as much as possible the nonexclusivity of public-sector sales to promote price competition must be implemented. If possible, price ceilings are mandated for public-sector sales.

For the second strategy, achieving efficiencies and economies of scale, efficiency is rewarded, since reduction in costs can have a considerable impact on the price of the product. Manufacturers in the developing world should be employed whenever possible to encourage the building of local institutions and capabilities, to take advantage of lower costs, and to reduce the length of the supply pipeline. The latter is particularly important when dealing with biologicals which typically have a limited shelf life and a marked sensitivity to temperature. This must be done, however, with full consideration of developing world conditions and must not give the impression of being second rate because of local manufacturing.

Unlike many "social" technologies with which PATH has had product development experience, however, production costs generally have little bearing on the final price of diagnostic products. In the diagnostics industry, product pricing is often determined by what the market will bear, rather than by a formula for cost recovery. High profit margins are realized where diagnostic products enjoy high value in use or replace a much more expensive gold standard method for diagnosis.

Pricing can also be high relative to production costs because the average biotechnology company possesses relatively few marketed products over which to spread its initial capital costs. Dual pricing systems, which allow the commercial sector to carry the burden of cost recovery, have been effective in reducing the cost of many social products to a level commensurate with public-sector needs. For example, the vaccine industry produces many mature products in which the capacity for production significantly exceeds the current demand. However, dual pricing cannot always provide a solution for advanced biotechnology-derived products, many of which are manufactured by small, start-up companies with limited production capability and which are often attempting to become profitable and provide a return on investment to their investors. Unless there is an (unlikely) successful appeal to altruism in such companies, diversion of limited product from high-margin to low-margin markets is not an economically viable proposition.

At least seven DiaTech subgrantees are currently involved in negotiations with private-sector companies, and two have sought assistance from PATH. Business plans have been initiated to

identify the steps required to accomplish a complete technology transfer. Management hopes to implement more of these steps in the remaining months of the DiaTech program.

c. Development of Strategies for Introduction. In product advancement, DiaTech must carefully assess and design the best methods for introducing new diagnostic technologies into developing world health care settings. Over the next seven months, DiaTech will develop a strategic plan for product introduction and distribution of selected technologies. Implementation would occur during a follow-on program.

Strategic planning will include operational research, i.e., in-house and on-site surveys to assess the methods for and problems likely to be encountered in the introduction of specific technologies. Considerations will include diagnostics distribution, procurement, logistical limitations of distribution systems, characteristics of end users, qualifications of health care personnel currently using diagnostics, capabilities for learning new techniques, and sophistication of facilities at different levels of the health care system. These introduction strategies for specific technologies will then be implemented. DiaTech will offer field-oriented diagnostic assistance programs, ranging from distribution of diagnostics to training and instructions on their use. DiaTech will draw on PATH's considerable experiences in introduction of other health technologies.

d. Implementation of Strategies for Introduction. Once a diagnostic kit has been field-tested and information on performance and acceptability has been generated, DiaTech will proceed with technology introduction. Since the best application of the diagnostic depends on both country and product factors, a range of possibilities for technology introduction will be considered. This could range from simple distribution, if local production is not economically or technically justifiable, to full local production, in cases where the market is of adequate size and there are no commercial or technical reasons that make local production undesirable. In this instance, a complete transfer of the technology will be attempted.

Quality control and quality assurance measures and procedures will be put into place, regardless of local production, since this is a key to product

acceptance. In many cases, the source of a technology continues to manufacture a key ingredient of the product, then arranges for local assembly, e.g., bottling, labeling, packaging. Alternatives for local production will always be evaluated to determine if this approach can contribute to reducing the price or otherwise increasing the availability of the product.

DiaTech staff will, in the formulation of advancement plans, examine the country and product variations and attempt to establish the economically appropriate level of use. PATH has often served as a broker, introducing the sources of a technology to local partners in developing countries. The local firms and the technology sources must, in each case, negotiate and set the level of local production.

In deciding where to locate initial product introduction efforts, PATH will take several factors, including geographic considerations, into account. First, a significant need for the diagnostic must be present because of the existence of endemic disease. Second, the test must be shown to be appropriate for the diagnostic condition through a prior successful experience with the technology, i.e., through participation in field trials. Infrastructure elements, such as trained personnel, necessary facilities or equipment, transportation and distribution systems, and sufficient financial resources will be evaluated to increase the likelihood of correct use of the diagnostic, for its adequate distribution, for solving unanticipated problems in its use, and for availability of necessary maintenance and/or repair services.

DiaTech will assess these manufacturing and distribution considerations to ensure that the limited resources available for product introduction activities are targeted to those countries where successful use is probable, where the technology can have a valuable impact, and where lessons can be learned that will be useful in broader introduction efforts. Throughout the stage of product advancement, DiaTech staff will therefore continue to manage the process and serve as the coordinator of PATH resources, A.I.D. input and requirements, and participation by public and private sector collaborating organizations.

B. Other Promising Technologies

As of January 1990, DiaTech has a number of new opportunities for funding projects to round out the portfolio of technologies supported. DiaTech is considering only the feasibility studies of considerable promise which can, in the opinion of the proposers and DiaTech management, finish their projects by the end of September 1990. As a result, only focussed projects which address clinical assessment and field testing of well-developed technologies are being considered for funding. Examples are (1) clinical and field testing of an ELISA method for the epidemiologic assessment of five enteric viruses; (2) field assessment of a plate colony-blot test for the identification of *Shigella* and shigella-like toxins; (3) a serological method for detection of pneumococcal pneumonia which detects antibodies to a recently discovered internal protein antigen common to all serotypes of *Streptococcus pneumoniae*; and (4) a laboratory and field assessment of a method for the rapid identification of *Bordetella pertussis*, the etiologic agent of whooping cough, in clinical specimens.

C. Continued Screening for New Downstream Product Opportunities

DiaTech will continue, as time and resources permit, to tap and encourage development of the results of the biotechnology revolution. The TAG, the key consultants, subgrantees, and established collaborators are all sensitized to the imperative for new product development opportunities for DiaTech which are beyond the research stage and which may have been tested for proof-of-concept with clinical specimens. New proposals will be invited by referral from persons within the DiaTech network or as a result of scanning the current scientific and medical literature. Unsolicited proposals will be considered as well. To fund these efforts, DiaTech management may use the "directed research" approach to take immediate advantage of the opportunities identified and to accelerate the pace of development. These activities will necessarily be on a scale commensurate with available funding and will most likely be considered as feasibility studies.

In addition, DiaTech intends to explore the possibility of licensing products which have already been fully developed by the commercial sector. DiaTech management has been invited to and will visit companies such as Abbott Laboratories, Becton Dickinson and Company, and ADI Diagnostics, Inc., to discuss products appropriate for developing country use and technology transfer.

D. Summary

In conclusion, the DiaTech program is currently well on the way towards its goals of delivering appropriate diagnostic tools for use in the developing world. The core of the

program has been the support of basic and applied research for the development of technologies for diagnosis of malaria, acute respiratory diseases, tuberculosis, diarrheal diseases, and typhoid fever. In the fifth year of the program, DiaTech management has identified promising projects in all five of the disease areas. Successful introduction and use of these assay methods will likely have a considerable, positive impact on the diagnosis of infectious disease in the developing world. These projects have been designated as high priority for product development and will receive considerable attention from now to the end of September 1990.

To accelerate product development, the cooperative interaction of DiaTech management with the subgrantees will be required to guide the technologies through product development activities. Handoffs of projects from more research-oriented laboratories to those which are more development-oriented will be implemented where necessary. DiaTech management will also ensure that adequate clinical and field testing is performed to validate assay methods. Finally, DiaTech will coordinate the development of necessary business and marketing plans, including plans for introduction and technology sharing.

A considerable amount of work remains to be accomplished in the next seven months of the program to successfully lay the framework for the next product development phase of DiaTech. DiaTech and PATH management are fully committed and prepared to devote the necessary resources and efforts to bring these technologies to fruition.

TABLE OF CONTENTS
ACTIVE SUBAGREEMENTS

Principal Investigator	Institution	Title
Acute Respiratory Infections/Tuberculosis		
Anderson, L.J.	Centers for Disease Control (Year II)	Antigen and Antibody Immunoassays for Respiratory Viruses
Buchanan, T.M.	Univ of Washington (Year II)	Development and testing of diagnostic reagents for *M. tuberculosis*
Crawford, J.T.	Biomedical Research Foundation/McClellan Memorial Veterans Hospital	Genetic probes for the detection of *M. tuberculosis*
Daniel, T.M.	Case Western Reserve Univ (Year II)	Practical tuberculosis ELISA
Kolk, A.H.J.	Koninklijk Instituut voor de Tropen (Year III)	Development of a field assay for the detection of *M. tuberculosis* in sputum
Parkinson, A.J.	Centers for Disease Control/Anchorage (Year II)	Development and evaluation of immunodiagnostics for detection of pneumococcal infections
Diarrheal Diseases/Typhoid Fever		
Cabello, F.	New York Medical College	Development of diagnostic assays for typhoid fever
Carlin, N.	National Bacteriological Laboratory	Development of a rapid and sensitive detection assay for *Shigella flexneri* antigen in stool samples
Guerrant, R.L.	Univ of Virginia/Dept of Geographic Medicine	Studies of potentially simpler methods for detection of fecal leukocytes as a guide to inflammatory diarrhea
Oberhelman, R.	The Johns Hopkins Univ/Dept of International Health	A clinical field trial of a nucleic acid probe for the detection of *Shigella* infections
Ravdin, J.I.	Univ of Virginia School of Medicine (Year III)	Diagnosis of pathogenic *E. histolytica* infection by detection of anti-lectin antibodies in serum and adherence lectin antigen in feces
Sarasombath, S.	Mahidol Univ/Faculty of Medicine (Year II)	Development of Vi monoclonal antibodies for the diagnosis of typhoid fever
Malaria		
Martin, W.J.	Professional Staff Assn/Univ of Southern Calif (Year II)	Nonradioactive ultrasensitive detection of amplified *P. vivax* and *P. falciparum* genes
McLaughlin, G.	Univ of Illinois (Year II)	Detection of malaria parasites using enzyme-linked synthetic DNA probes
Tharavanij, S.	Mahidol Univ/Faculty of Tropical Medicine (Year II)	Two-site ELISA for the detection of *P. falciparum* antigen
Taylor, D.W.	Georgetown Univ (Year III)	Development of monoclonal antibody-based assay for malaria
Voller, A.	The Zoological Society of London	Development of malaria antigen ELISA
Voller, A.	The Zoological Society of London	Development of an ELISA to monitor quinine levels in malarious patients
Other		
Curtis, R.	Artel, Inc. (Year II)	Development of a direct-reading hemoglobinometer
Rose, N.R.	The Johns Hopkins Univ/ Primary I	Provision of assistance to management and laboratory resources
Chutivongse, S.	Queen Saovabha Memorial Institute/Primary II	Provision of assistance to management and sample collection

Acute Respiratory Infections/Tuberculosis

laboratory resources are available to DiaTech for the stages of the program when technologies need to be tested and evaluated.

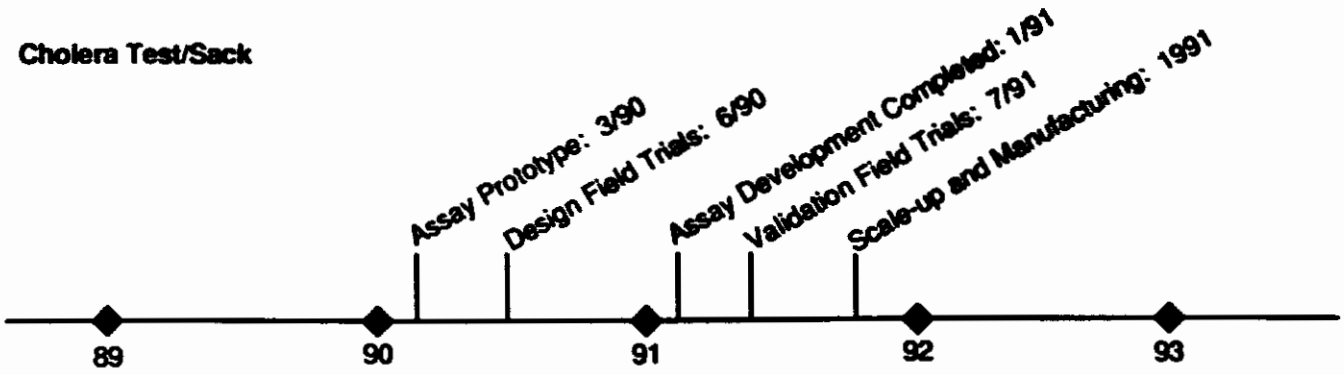
Fourth and fifth year activities in addition to those described above include or included: (1) evaluation of a test for *E. hystolytica* in Egypt, (2) laboratory evaluation of the Artel hemoglobinometer, (3) development of a co-agglutination method for culture-accelerated detection of *Vibrio cholerae* and planning for validation-stage field testing of the method, (4) review of existing commercial tests for ARI and selection of the most robust tests for field testing, (5) assembly of a proficiency panel of mycobacterial and malarial specimens for testing by DiaTech subgrantees, and (6) provision of a standardized, high-quality source of *Staphylococcus aureus* Cowan strain A for development of co-agglutination tests for projects within the DiaTech program.

The DiaTech program has also approved subagreements submitted by collaborators of JHU consultants. For example, a collaborator of Brad Sack, Richard Oberhelman, is assessing the utility of an oligonucleotide probe for detection of *Shigella* in a field test in Peru; Nils Carlin, a collaborator of David Sack, has recently been approved to develop a rapid immunoassay for detection of *Shigella* antigen, which, if successful, he intends to field test at a Native American reservation in Arizona.

Funding Amount	Year 1-3: $232,086 Year 4-5: $149,498 Total: $381,576
Period	October 1, 1986 - September 26, 1990

Timeline for Development

Cholera Test/Sack



February 19, 1990
HS01305V

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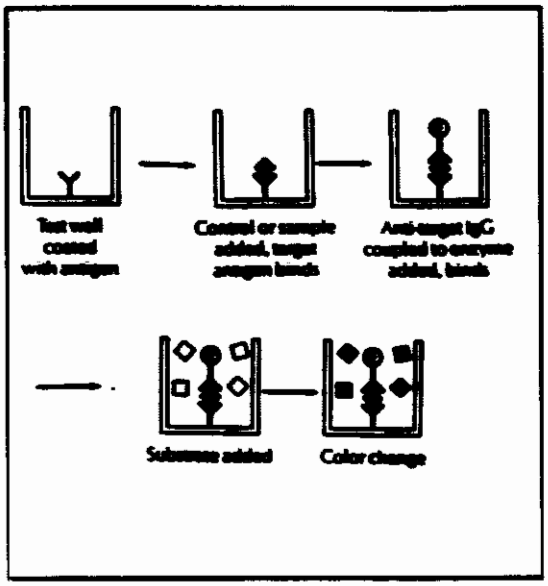
ACTIVE SUBAGREEMENTS

Principal Investigator	Institution	Title
Acute Respiratory Infections/Tuberculosis		
Anderson, L.J.	Centers for Disease Control (Year II)	Antigen and Antibody Immunoassays for Respiratory Viruses
Buchanan, T.M.	Univ of Washington (Year II)	Development and testing of diagnostic reagents for *M. tuberculosis*
Crawford, J.T.	Biomedical Research Foundation/McClellan Memorial Veterans Hospital	Genetic probes for the detection of *M. tuberculosis*
Daniel, T.M.	Case Western Reserve Univ (Year II)	Practical tuberculosis ELISA
Kolk, A.H.J.	Koninklijk Instituut voor de Tropen (Year III)	Development of a field assay for the detection of *M. tuberculosis* in sputum
Parkinson, A.J.	Centers for Disease Control/Anchorage (Year II)	Development and evaluation of immunodiagnostics for detection of pneumococcal infections
Diarrheal Diseases/Typhoid Fever		
Cabello, F.	New York Medical College	Development of diagnostic assays for typhoid fever
Carlin, N.	National Bacteriological Laboratory	Development of a rapid and sensitive detection assay for *Shigella flexneri* antigen in stool samples
Guerrant, R.L.	Univ of Virginia/Dept of Geographic Medicine	Studies of potentially simpler methods for detection of fecal leukocytes as a guide to inflammatory diarrhea
Oberhelman, R.	The Johns Hopkins Univ/Dept of International Health	A clinical field trial of a nucleic acid probe for the detection of *Shigella* infections
Ravdin, J.I.	Univ of Virginia School of Medicine (Year III)	Diagnosis of pathogenic *E. histolytica* infection by detection of anti-lectin antibodies in serum and adherence lectin antigen in feces
Sarasombath, S.	Mahidol Univ/Faculty of Medicine (Year II)	Development of Vi monoclonal antibodies for the diagnosis of typhoid fever
Malaria		
Martin, W.J.	Professional Staff Assn/Univ of Southern Calif (Year II)	Nonradioactive ultrasensitive detection of amplified *P. vivax* and *P. falciparum* genes
McLaughlin, G.	Univ of Illinois (Year II)	Detection of malaria parasites using enzyme-linked synthetic DNA probes
Tharavanij, S.	Mahidol Univ/Faculty of Tropical Medicine (Year II)	Two-site ELISA for the detection of *P. falciparum* antigen
Taylor, D.W.	Georgetown Univ (Year III)	Development of monoclonal antibody-based assay for malaria
Voller, A.	The Zoological Society of London	Development of malaria antigen ELISA
Voller, A.	The Zoological Society of London	Development of an ELISA to monitor quinine levels in malarious patients
Other		
Curtis, R.	Artel, Inc. (Year II)	Development of a direct-reading hemoglobinometer
Rose, N.R.	The Johns Hopkins Univ/ Primary I	Provision of assistance to management and laboratory resources
Chutivongse, S.	Queen Saovabha Memorial Institute/Primary II	Provision of assistance to management and sample collection

Acute Respiratory Infections/Tuberculosis

Title	Development of Antigen and Antibody Immunoassays for Adenovirus, Parainfluenza Viruses, and Respiratory Syncytial Virus
Number	880200210
P.I.	Larry J. Anderson, M.D.
Institution	Centers for Disease Control/Atlanta
Status	Active



Objectives

Using existing monoclonals, polyclonal antisera, or antigen sources to develop EIAs and IFAs for detection of adenovirus, parainfluenza, and RSV antigens or antibodies. To evaluate the sensitivity and specificity of the assays developed on laboratory and clinical specimens. To select those assays with best accuracy, accompanied by instructions and guidelines, for distribution in developing countries (through A.I.D., WHO, PAHO, etc.).

Significance

Prompt and specific diagnosis of viral infections is increasingly important as advances in viral therapy are made. Conventional methods for isolation of many viruses are time-consuming and require skills and facilities that are not routinely available in developing countries.

Progress to Date

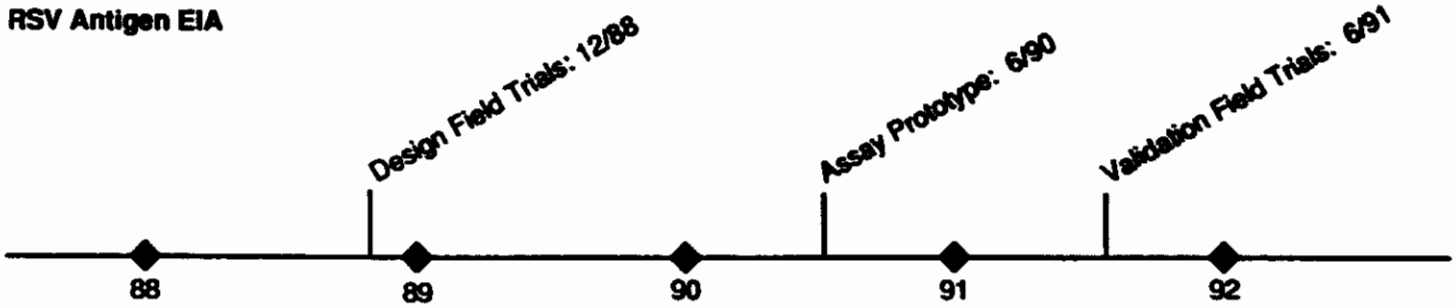
This project is derived from the P.I.'s earlier work which concentrated on production of monoclonal antibodies. The work plan as approved was both extensive and ambitious. MAbs were initially evaluated by immunofluorescence and pools prepared which were tested for brightness of fluorescence, specificity, and sensitivity on multiple strains. Three antigen detection assays were evaluated: time-resolved fluoroimmunoassay with MAbs (TR-FIA), indirect ELISA using MAb biotin-avidin conjugates, and an ELISA using polyclonal antisera. TR-FIA gave good results for all five virus systems (adeno, RSV, paraflu 1, 2, 3). The P.I. realizes, however, that TR-FIA is inappropriate for developing country use and will concentrate on development of MAb-ELISA. Preliminary results using clinical samples with

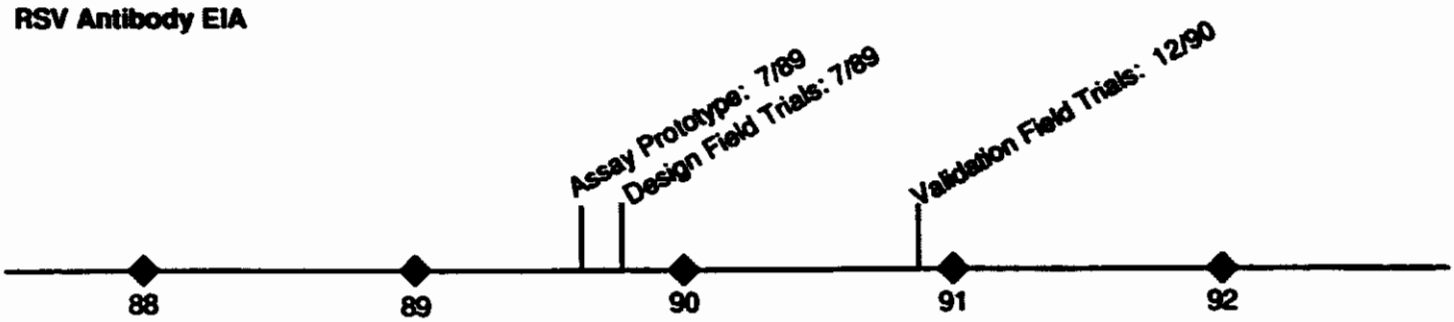
MAb-ELISA indicate that acceptable to good results can be obtained for RSV, adeno, and paraflu 1 and 3, but that more work must be done for paraflu 2. The P.I. will concentrate on stability testing of reagents and direct HRP conjugation of MAb reagents, developing guidelines for use of the reagents. DiaTech has supplied a protocol for direct FITC conjugation of MAbs to the P.I., since he has indicated that there is a use for the reagents for direct FA diagnosis of clinical samples. The P.I. indicated that he has already transferred a few of his monoclonal antibodies to commercial companies, which have provided extensive help in development, scale-up, and kit or reagent preparation using his reagents.

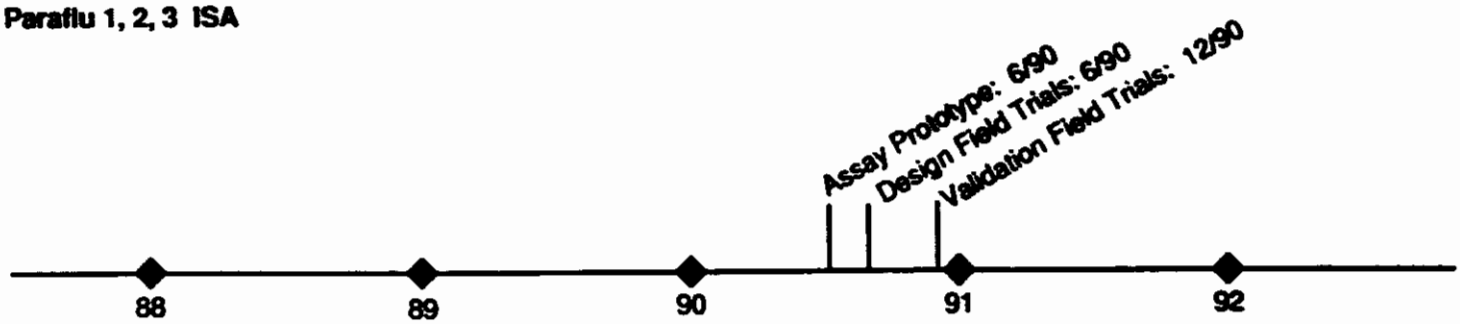
Funding Amount Year 1: $59,743
Year 2: $79,715
Total: $139,458 (funded by PASA)

Period January 5, 1989 - September 26, 1990

Timeline for Development



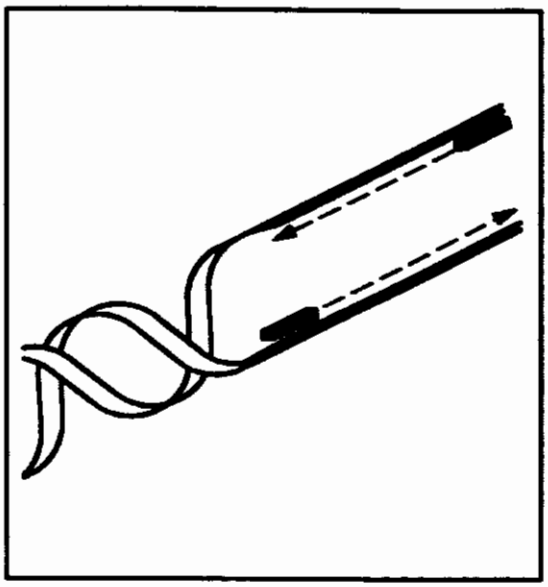




DiaTech
Subagreement Technical Summary
January 1990

Disease Area: ARI/TB
Diagnostic Type: DNA Amplification

Title	Development and Testing of Reagents for *M. tuberculosis*
Number	861200163
P.I.	Thomas M. Buchanan, M.D.
Institution	University of Washington
Status	Active



Objectives

To generate monoclonal antibodies reactive with protein epitopes of *M. tuberculosis* and to determine which reagents will be useful for the diagnosis of tuberculosis. To develop primer pairs specific for *M. tuberculosis* which will be useful in PCR diagnosis.

Significance

It has been estimated that 50% of people in the developing world are infected with tubercle bacilli, about half of whom will develop active disease. There are perhaps as many as 3 million to 4 million deaths from tuberculosis worldwide per year. And yet, tuberculosis is a disease which can be treated with safe, effective, and relatively inexpensive drugs. Current techniques for its diagnosis suffer from a number of serious limitations, including that they are slow, resource-intensive, and less than ideally sensitive and specific. It has recently been determined that there are at least 7 epitopes which can be used to distinguish *M. tuberculosis* from 23 other species of mycobacteria, opening the door to development of rapid and simple immunoassays for the diagnosis of tuberculosis.

Progress to Date

Peptides analogous to a species-specific epitope on the 65 kDa protein of *M. leprae* were synthesized. The *M. leprae* sequence of this peptide is APALDKLKLTGDEATG whereas the *M. tuberculosis* sequence is APTLDELKLEGDEATG. These peptides were used to develop 3-4 monoclonal antibodies (MAbs), reacting both with the whole protein molecule and with the peptide used for immunization. Evaluation of two MAbs by dot blots showed a limited range of reactivity as predicted. Whereas MAbs to an adjacent portion of the molecule

recognized most species of mycobacteria, the site-directed MAbs strongly recognized only *M. tuberculosis*, *M. ulcerans*, and *M. flavescens*, and weak reactivity was also seen with *M. gastri*. The pathogens *M. chelonei*, *M. fortuitum*, *M. kansasii*, *M. marinum*, and *M. scrofulaceum* were not recognized, nor did the MAbs react with nonpathogens *M. diernhoferi*, *M. duvali*, *M. non-chromogenicum*, *M. perigrinum*, *M. phlei*, *M. terrae*, and *M. vaccae*. This approach therefore was promising for the development of species-specific antibodies to *M. tuberculosis*.

The maximum sensitivity of the antigen capture immunoassay using selected MAbs as reported by the P.I. is approximately 2-4 x 10^5 as a best effort. The P.I. feels that other reports appearing in the literature claiming lower sensitivity are likely to be the result of assaying aggregates of bacteria; that his current sensitivity is about maximum for the immunoassay technique; and that this immunoassay approach will not prove sufficiently sensitive to replace culture.

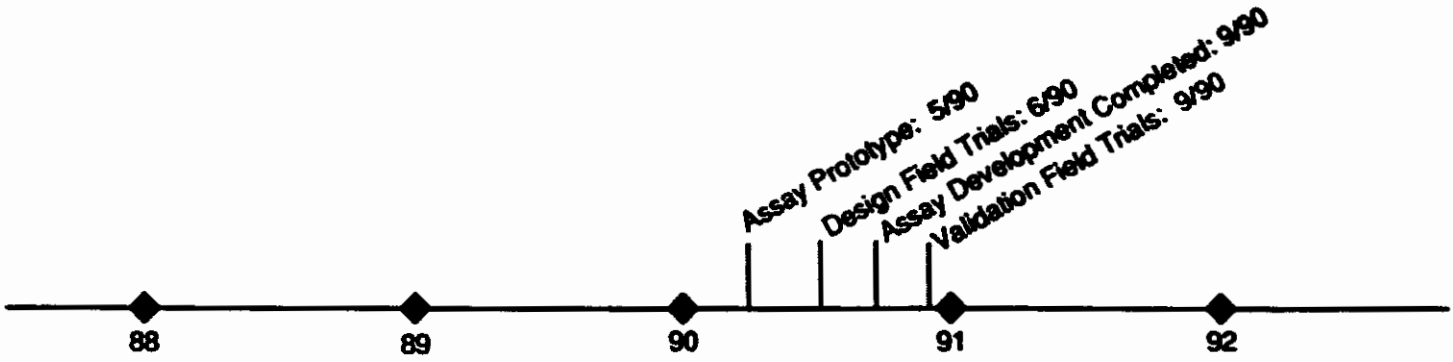
Therefore, in late 1988, investigations into the use of the polymerase chain reaction (PCR) were begun for development of a rapid test that is equal to or more sensitive than culture. Primers from the previously sequenced 65 kD heat shock protein were synthesized for use as probes. Of these, several probes have been found to exhibit specificity for the Mtb group necessary for assay development. For positive strand, 13T, 15T, and 4T were found specific, while two negative strand primers were found with specificity, MT and NC31.

Comparison of the PCR with immunoassay using the same suspensions of bacteria indicate that the PCR is 3 logs more sensitive (10^{2-3} vs. 10^{5-6}). The limitation on sensitivity currently is the efficiency of DNA extraction, which is difficult when dealing with a robust organism such as the mycobacteria, and is estimated at only 5%. A digestion procedure for mycobacteria is under development which appears to be an improvement over standard techniques. The P.I. has been recently site-visited by DiaTech management. He has collected a number of clinical samples and will start testing these samples when the assays are completely developed.

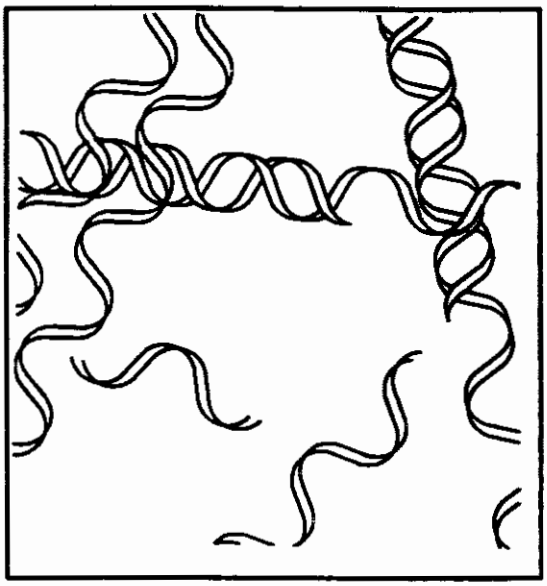
The P.I. is willing to work with DiaTech in field testing the technique, in development of a rapid readout system, and in reagent development and commercialization of the appropriate probes. Questions about the cost and accessibility of the technology to the developing world remain with the PCR technique, although rapid progress is being made on assay simplification, specificity, and general technology.

Funding Amount	Year 1:	**$ 89,242**
	Year 2:	**$ 89,000**
	Total:	**$178,242**
Period	December 1, 1987 - March 31, 1990	

Timeline for Development



Title	Genetic Probes for the Detection of *Mycobacterium tuberculosis*
Number	860700124
P.I.	Jack T. Crawford, Ph.D.
Institution	Biomedical Research Foundation/McClellan Memorial Veterans Hospital
Status	Active



Objectives

To develop a simple technique for lysis of *M. tuberculosis* and application of the sample to a membrane in a form suitable for hybridization; to develop a simple procedure for processing sputum and determination of the effect of sputum components on the lysis and hybridization techniques; to test various specific probes for sensitivity and specificity and to compare these probes to the use of total *M. tuberculosis* DNA as a probe; and to test the procedure using actual clinical samples and compare with results of routine microscopy and culture methods.

Significance

The only simple procedure currently available for rapid diagnosis of tuberculosis is microscopy, i.e., acid fast or auramine-rhodamine stains. Nucleic acid hybridization is a very promising technique for large-scale screening of clinical samples. A simple, reliable, non-isotopic procedure which is usable in the field will be very difficult to develop because of resistance of the bacillus to lysis.

Progress to Date

Funding for this subagreement is only for materials and supplies. The P.I.'s main grant is derived from the Veterans Administration Hospital system. Using a simple NaOH method for extraction of mycobacterial DNA, a standard dot blot ^{32}P isotopic methodology was developed, which was followed by a nonisotopic probe method. Only small amounts of extract were used, however, since the filter was easily clogged. This limited sensitivity,

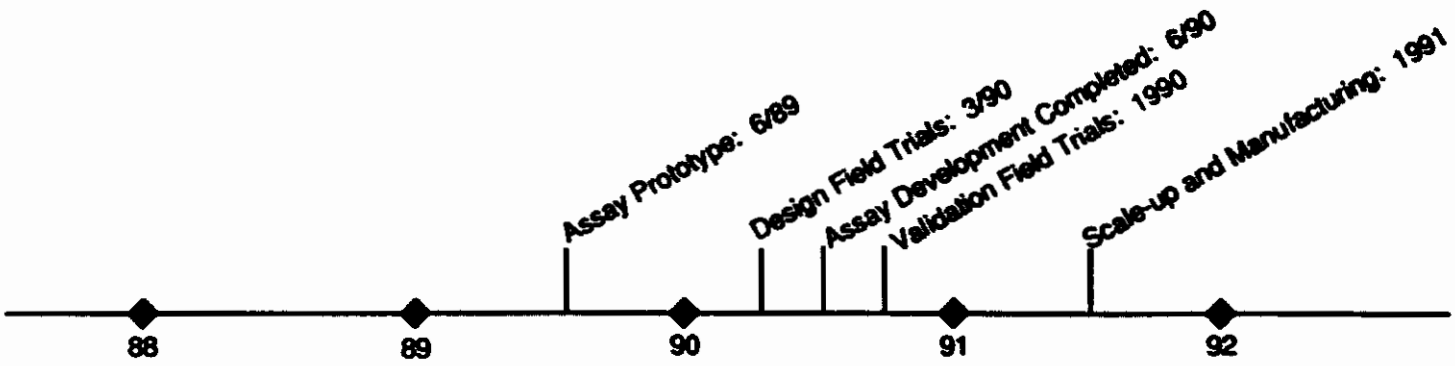
currently at about 10^6 organisms per assay with specific probes or similar to microscopy, is still fairly low. Using total Mtb DNA as probe, sensitivity is 4-16 times higher. The P.I. continues to work closely with the Arkansas State Health Association which maintains a TB control program. All samples from patients in the statewide area are cultured in the lab next door, so there is no shortage of specimens.

At the DiaTech-sponsored workshop on ARI/TB held in June 1989, the P.I. indicated that he had started work into the PCR technique for diagnosis of TB. He is extracting DNA with a similar NaOH and NALC or DTT method, using a probe which results in a 123 base pair product. Application of PCR to sputum samples requires clean sample extracts, i.e., liquidation of sputum, centrifugation, treatment of sediments with lysozyme, boiling in NaOH-SDS, and concentrated with a commercial kit which takes advantage of DNA binding to powdered glass. The P.I. is also investigating a simpler extraction technique using NP-40 followed by digestion with proteinase K. Many of the variables have been worked out to produce a relatively specific reaction. For example, when a high annealing temperature (68°C) was used, the elevated temperature eliminated the nonspecificity and resulted in increased amplification of the specific product. The laboratory has now found what they believe to be a Mtb species-specific oligo primer pair from a nonstructural protein, which was co-discovered with the Pasteur Institute. The P.I. will continue to pursue research into the PCR method in collaboration with Pasteur, will continue improving the sensitivity of the DNA probe assay, and would like to compare the two methods in a field test sometime in 1990.

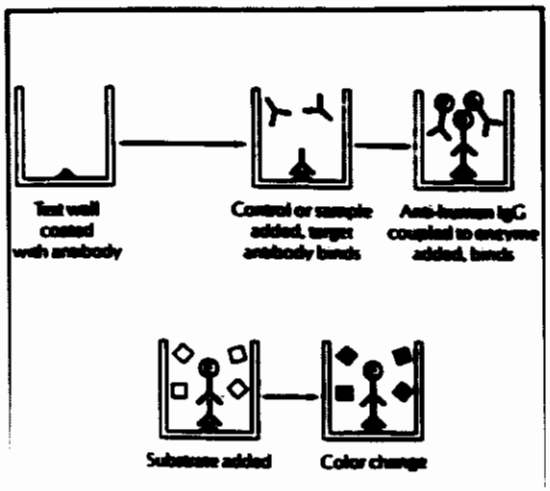
Funding Amount Total: $ 6,000

Period January 1, 1988 - September 26, 1990

Timeline for Development



Title	Practical Tuberculosis ELISA
Number	870500177
P.I.	Thomas M. Daniel, M.D.
Institution	Case Western Reserve University
Status	Active



Objectives

To improve the specificity of ELISA tests for the serologic diagnosis of tuberculosis while retaining sensitivity, simplicity, low cost, and widespread applicability. To accomplish this, the P.I. will develop a mycobacterial antigen preparation suitable for ELISA which can be prepared at low cost in continuing supply; he will develop simple methods for modifying mycobacterial ELISA antigens to improve specificity by exposing specific, or destroying nonspecific, epitopes; and will develop simple yet specific ELISA techniques for field use.

Significance

Tuberculosis remains an enormous cause of morbidity and mortality despite adequate methods for treatment. It is estimated that 30 million people have tuberculosis in the world, with 10 million new cases and 3 million deaths annually. This makes tuberculosis one of the most important infectious disease problems on earth today, approximately equal as a cause of death to childhood acute respiratory infections. Because tuberculosis in developing countries afflicts primarily young adults in the wage earning and child rearing years, its economic impact is great. There is now general agreement that the main thrust of tuberculosis control efforts should be directed at case detection among high risk groups and treatment of all infectious cases both in low and high prevalence areas. An expert group convened by WHO to consider tuberculosis research priorities emphasized the need for improved methods of diagnosis and specifically cited the need for improved serodiagnosis.

Progress to Date

Work has concentrated initially on testing of *M. tuberculosis* antigens 5 and 6 for use in serological assays. Serodiagnosis with Ag5 was studied in 3 prospective trials in adults: Argentina (sensitivity 64%, specificity 100%), China (Beijing; sensitivity 87%, specificity 100%), and the United States (Cleveland; sensitivity 49%, specificity 98%). Unfortunately, Ag5 work has been deferred due to stability and production problems.

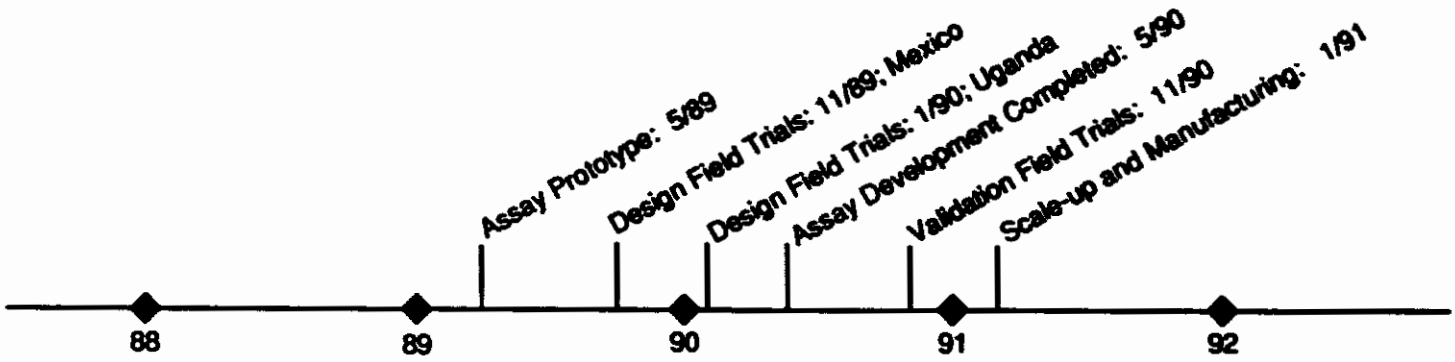
Therefore, Ag6, a fibronectin-binding protein, was considered the best candidate, since previous work had indicated that this antigen was easier to produce and more stable. However, using a panel of 44 sera tested by ELISA on Ag6, overall sensitivity of positives was low, and one normal serum included in the calculations continued to give an exceptionally high false positive signal. Chloroform-methanol treatment of Ag6 has reduced the activity of this false positive serum, and the P.I. thinks he now has a promising method. A collaboration with Tom Schinnick (CDC) to discover an Ag6 fusion protein containing a *M. tuberculosis*-specific epitope was not successful. The P.I. tested his Ag6 ELISA in a field study in Mexico City at the Instituto Nacional de Enfermedades Respiratorias in late 1989. He reported a sensitivity of 69.5% in 59 patients with pulmonary TB, and a specificity of 100% in 125 control subjects. Positive predictive values are calculated as 93%.

DiaTech management will work with the P.I. in 1990 to improve the serological assay for TB. DiaTech will arrange to have peptides synthesized by the Geysen technique (epitope mapping) for Ag5 to discover the immunologically reactive peptide sequences in seropositive patients. The appropriate peptides can be then be synthesized in quantity and used for ELISA, dot blot, or agglutination methods.

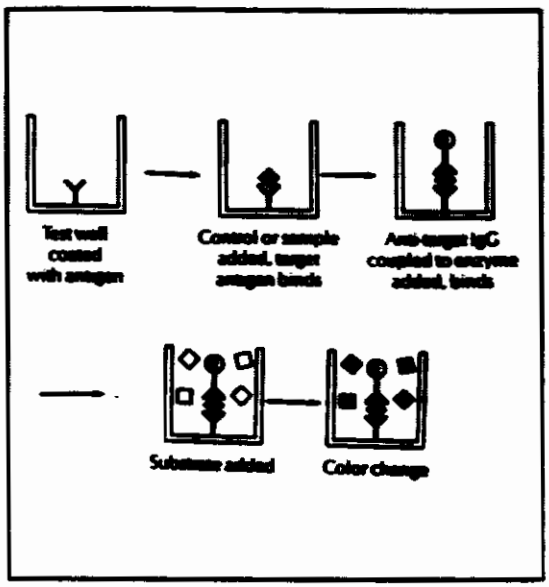
There is still controversy over whether serology will become a standard indicator of active TB. This needs to be resolved.

Funding Amount	Year 1: $ 85,804 Year 2: $ 69,078 Total: $154,882
Period	May 1, 1988 - May 31, 1990

Timeline for Development



Title	Development of a Field Assay for the Detection of *M. tuberculosis*
Number	860700087
P.I.	Arend Kolk, Ph.D.
Institution	Koninklijk Instituut voor de Tropen, Amsterdam
Status	Active



Objectives

To develop monoclonal antibodies to *M. tuberculosis* and apply these in immunoassay procedures for the detection of antigens in body fluids.

Significance

It has been estimated that 50% of people in the developing world are infected with tubercle bacilli, about half of whom will develop active disease. There are perhaps as many as 3 million to 4 million deaths from tuberculosis worldwide per year. And yet, tuberculosis is a disease which can be treated with safe, effective and relatively inexpensive drugs. Current diagnostic techniques suffer from a number of serious limitations, including that they are slow, resource-intensive and less than ideally sensitive and specific. It has recently been determined that there are at least seven epitopes which can be used to distinguish from 23 other species of mycobacteria, opening the door to development of rapid and simple immunoassays for the diagnosis of tuberculosis.

Progress to Date

Monoclonal antibodies have been produced which are reactive with and specific to *M. tuberculosis* (Mtb), *M. kansasii* (Mka), and *M. avium-intracellulare* (Mai) by the following techniques:

Western blot. Three Mabs were found to be specific and react with discrete bands. When assayed by WB on cultured bacteria, 58/59 strains of Mtb were found to be reactive

(sensitivity 98%). The WB technique may serve as a rapid confirmatory method for identification of mycobacterial species.

Immunofluorescence. All reference strains of Mtb reacted with F24-2. However, when identifying Mtb from clinical isolates, only 61/68 strains reacted (sensitivity 90%). Therefore, IFA was not as sensitive as hoped. Reasons may be that there is antigenic diversity among strains of Mtb, or that the appropriate surface antigens are sometimes buried in the cell wall and often not accessible to the monoclonal antibody probes. Pools of specific MAbs will be used to raise assay sensitivity.

Antigen capture ELISA. For ELISA, MAb F26-6 was used as capture with a polyclonal rabbit anti-Mtb used as signal. Results are still preliminary, but it is clear that working with sputum as sample is very difficult and interferes with Ag-Ab reactions. The P.I. has, therefore, been developing three possible methods to treat sputum prior to assay:

1. Treat with DTT and PCA, neutralize with NaOH, and pass through a Sephadex G-5 column. Sensitivity is still low with this method, about 5×10^5 bacteria per assay.

2. Treat with zwitterionic detergent CHAPS, 15 mM in PBS, pH 7.4.

3. Treat with DTE.

The P.I. indicates that CHAPS alone and in combination with DTE are promising. The limit of detection is at present quite low, at 10^6 organisms/test, but work is continuing on other monoclonal-polyclonal Ab combinations. The use of sputum as specimen, however, has traditionally been a major problem. Other specimens such as urine, serum, and pleural fluid will be investigated as alternatives.

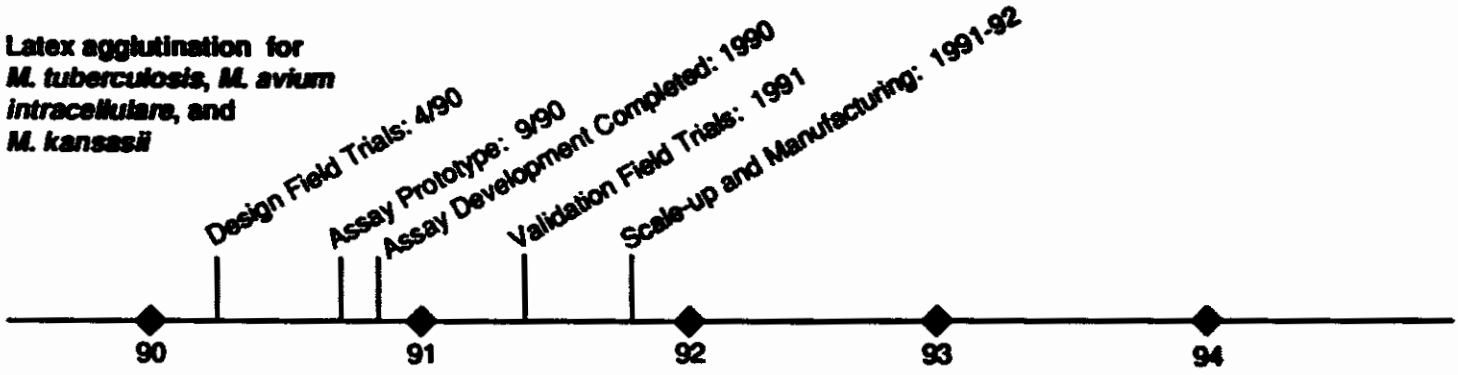
The P.I. has, however, used this plate ELISA to advantage for confirmation of isolation after rapid culture in liquid Middlebrook medium. Assays for Mtb, Mka, and Mai were developed. An inoculum of 10,000 Mtb was detected by ELISA within 7 days. These studies will be repeated with clinical specimens. A test of mycobacterial strains isolated from several laboratories indicated that sensitivity and specificity for Mtb and Mka were both 100%, whereas the sensitivity was found to be only 69% for Mai, indicating that antibodies of additional strain-specificity will be necessary for complete identification.

Agglutination methods. In Year III the P.I. will complete optimization of agglutination methods for Mtb, Mka, and Mai detection, which will greatly improve the convenience and the cost of the assay. The method is currently slightly less sensitive, as to be expected, when compared with ELISA. The culture time will be standardized at 7 days, which the P.I. hopes will be sufficient for optimal detection of Mtb, and will be made possible by further improvements in growth medium and vigorous aeration of the cultures.

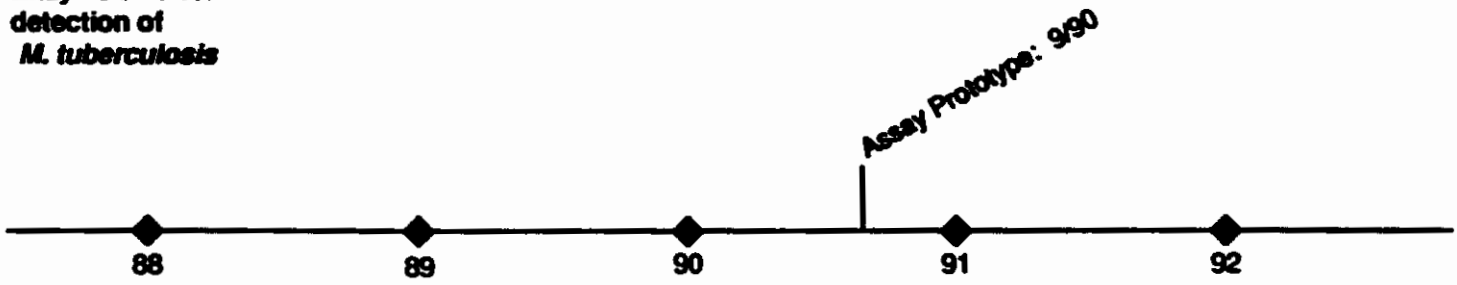
Funding Amount	Year 1: $103,978 Year 2: $ 88,703 Year 3: $ 50,000 Total: $242,681
Period	December 1, 1987 - September 26, 1990

Timeline for Development

Latex agglutination for *M. tuberculosis*, *M. avium intracellulare*, and *M. kansasii*



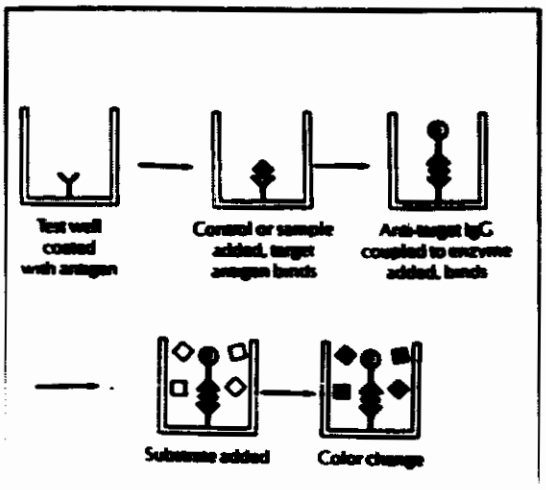
Enzyme EIA for detection of *M. tuberculosis*



February 19, 1990
HS01282V

18

Title	Development and Evaluation of Immunodiagnostics for Detection of Pneumococcal Infections
Number	880400214
P.I.	Alan J. Parkinson, Ph.D.
Institution	Centers for Disease Control/Arctic Investigations Program
Status	Active



Objectives

To develop an antigen capture ELISA assay for detection of pneumococcal C polysaccharide in sputum, serum, or urine from patients with pneumococcal pneumonia.

Significance

Acute respiratory disease due to pneumococcus is a major cause of childhood morbidity and mortality in the developing world. For example, in Goroka, Papua New Guinea, *H. influenzae*, *S. pneumoniae*, or both were isolated from blood or lung aspirates in over half the children with pneumonia. Development and use of simple, accurate immunodiagnostics to confirm etiology of infectious agents will allow their more rapid administration in the developing world where culture techniques are difficult or impossible. Interest has been heightened by the potential for prevention of pneumococcal and hemophilus disease with vaccination. To appropriately apply a vaccine, methods must be improved to rapidly identify and/or serologically type the pathogen causing respiratory disease so as to more rapidly and accurately diagnose hemophilus and pneumococcal disease in populations in remote areas. The project outlined in this proposal will establish an antigen capture immunoassay for the detection of pneumococcal C polysaccharide (PnC) in body fluids. PnC, a bacterial phosphocholine, is a common antigen found on all strains of *S. pneumoniae* which can be used to identify the species, unlike the capsular polysaccharides which are individual strain-specific determinants.

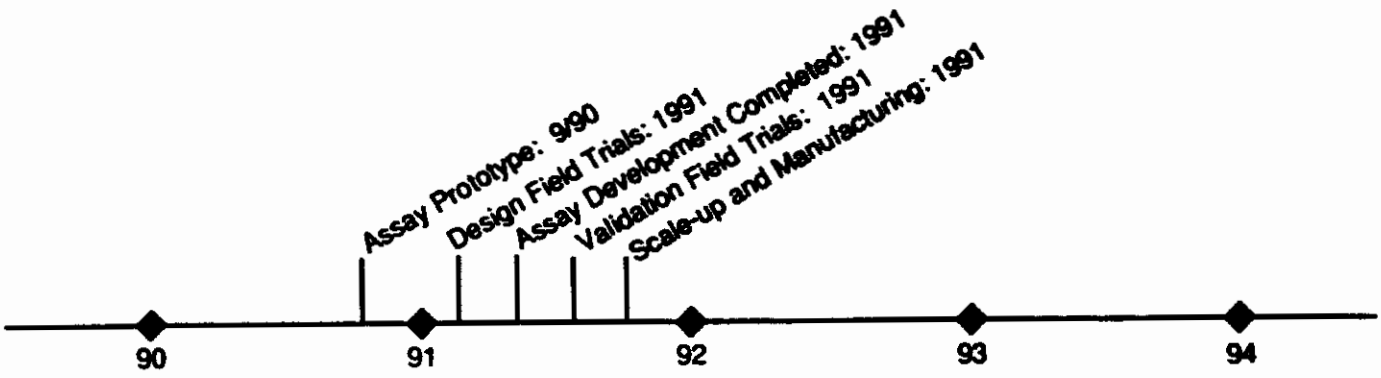
Progress to Date

Sputum samples were collected from Native Americans at a local clinic presenting with pneumonia-like symptoms and tested with the LKB prototype EIA test for detection of pneumococcal PnC. Initial results indicated that the EIA detected 20/24 culture positive specimens (sensitivity 83%) but was also positive in 19/110 specimens from which obvious pneumococci were not cultured (specificity 83%). LKB has since discontinued production of the PnC assay system. Compared with the LKB, the CDC PnC test is more sensitive; however, for detection of PnC in sputum, the diagnostic sensitivities are virtually identical. Since there are preexisting antibodies, the PnC test could not be used in human serum unless serum IgG was precipitated.

If this pretreatment step is applied, PnC can be detected in about 50% of patients with culture-confirmed bacteremia. The P.I. has received second year funding of his project to accomplish the following: (1) to continue to evaluate his PnC assay in sputum and urine of bacteremic patients, (2) to develop and evaluate a test for pneumococcal cell wall protein using monoclonal antibody reagents from David Briles of the University of Albama/Birmingham, (3) to develop an agglutination method for restricted serotypes of pneumococcal capsular polysaccharide to be used as a diagnostic, and (4) to develop and evaluate a test for pneumolysin developed at CDC/Atlanta.

Funding Amount	Year 1: $ 17,908 Year 2: $ 71,330 Total: $ 89,238 (funded by PASA)
Period	October 1, 1988 - September 26, 1990

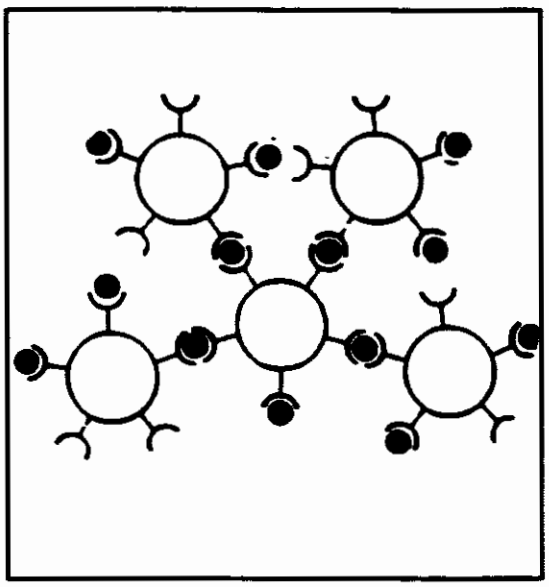
Timeline for Development



February 19, 1990
HS01283V

Diarrheal Diseases/Typhoid Fever

Title	Feasibility Study: Development of Diagnostic Assays for the Diagnosis of Typhoid Fever
Number	890600230
P.I.	Felipe Cabello, M.D.
Institution	New York Medical College
Status	Active



Objectives

To develop a coagglutination assay based on monoclonal antibodies (MAbs) specific to a 36 kD porin antigen of *S. typhi* and to determine the clinical utility of the assay to detect the antigen in clinical specimens. To purify the cloned 36 kD porin in large amounts from a strain of *E. coli* expressing this protein, and to use this antigen for serological detection of specific antibody in patient sera.

Significance

Typhoid fever is endemic and poses serious public health problems in many developing countries. Laboratory diagnosis of this disease by the Widal test is made too late to be of any value in patient care, while blood culture is notoriously insensitive. The P.I. offers an alternative approach to testing for Vi antigen or diagnosis by more complex, invasive culture techniques by detection of porin in direct specimens by an agglutination method or by a serodiagnostic method using a recombinant 36 kD porin protein.

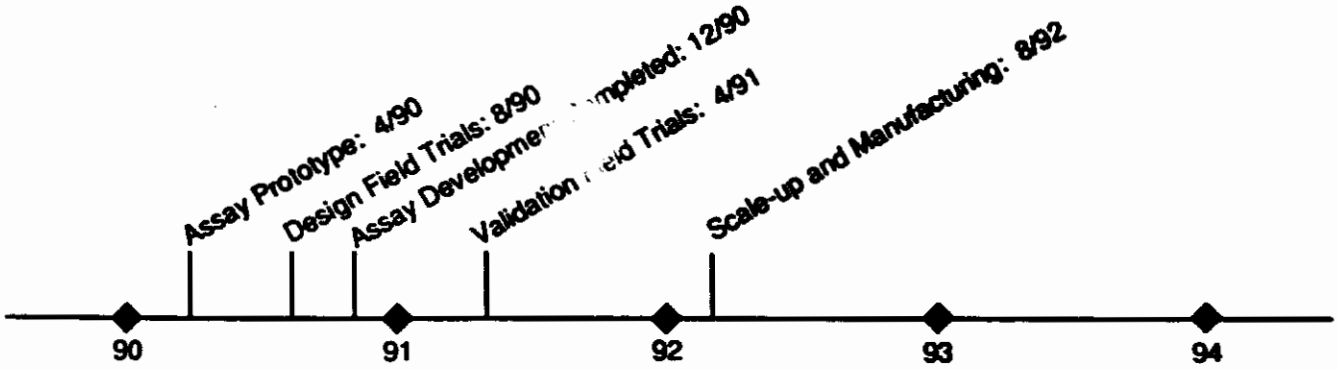
Progress to Date

The feasibility study was approved in late 1989, and the first progress report is due on May 15, 1990. The P.I. presented preliminary data at the DiaTech-sponsored workshop on diarrheal diseases in November 1989 describing the human immune response to *S. typhi* infection in which the 36 kD protein was highly immunogenic. He also presented evidence that monoclonal antibodies specific for the porin will be useful in specimens such as blood, urine, and stool when used in an agglutination method.

Funding Amount Total: $ 16,500

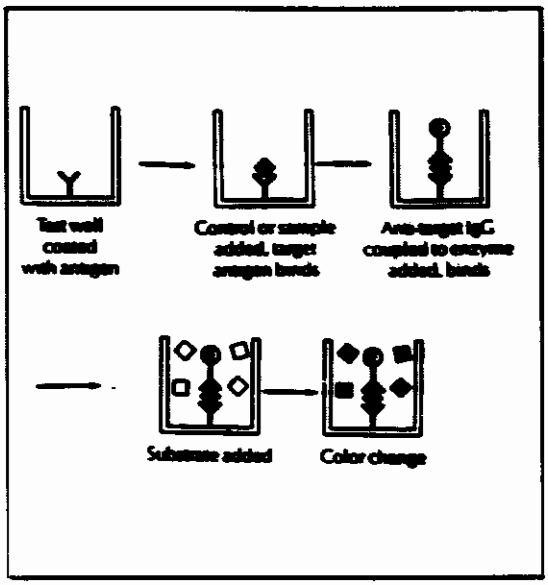
Period December 1, 1989 - August 31, 1990

Timeline for Development



February 19, 1990
NS01272V

Title	Development of a Rapid and Sensitive Detection Assay for *Shigella flexneri* Antigen in Stool Samples
Number	890600229
P.I.	Nils Carlin, Ph.D.
Institution	National Bacteriology Laboratory, Stockholm
Status	Active



Objectives

To establish a useful rapid diagnostic antigen detection assay for *Shigella dysenteriae* 1 and *S. flexneri* using a specific monoclonal antibody in a magnetic bead ELISA assay, either in an enrichment culture or directly from stool specimens. Results of the assay will be compared with results of culture. If the P.I. demonstrates that a sensitive, specific assay has been developed, he will field test his assay in Phase II at a Native American Reservation in the southwest where *Shigella* is endemic.

Significance

Shigella spp. have long been recognized as important enteropathogens causing inflammatory diarrhea in infants and children. While *Shigella* infections in the developed world are characterized as brief and easily treated with antibiotics, the picture is different in the developing world, where the disease is more chronic, associated with malnutrition, and can produce a significant level of mortality. Rapid diagnosis and treatment, where possible, is instrumental in preventing complications. Rapid diagnosis is also essential to distinguish *Shigella* from other enteropathogens such as *Campylobacter* for differential diagnosis. At present the only readily available method for detection of *Shigella* is stool culture, which requires fresh specimens for maximal recovery of organisms. Among the species of *Shigella* which produce significant amounts of disease in developing countries, *S. flexneri* and *S. dysenteriae* 1 account for the vast majority. Hence, although the monoclonal antibody which will be used cannot detect all shigellae, they will be able to detect those serotypes of greatest importance.

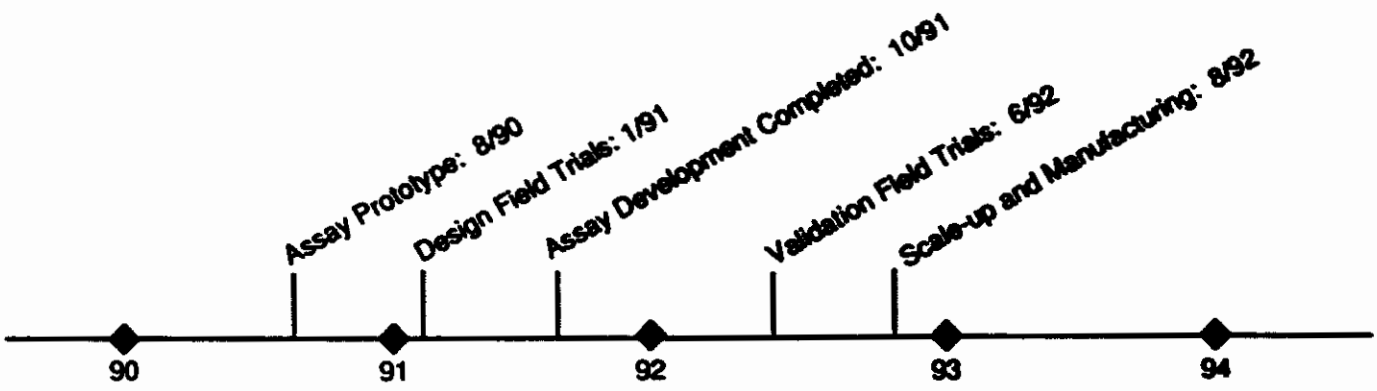
Progress to Date

The P.I. has only been recently funded. The first progress report is due April 15, 1990, although a preliminary report describing the technology and research plan has been presented at the DiaTech-sponsored workshop on diarrheal diseases in November 1989.

Funding Amount Total: $ 34,496

Period January 1, 1990 - September 26, 1990

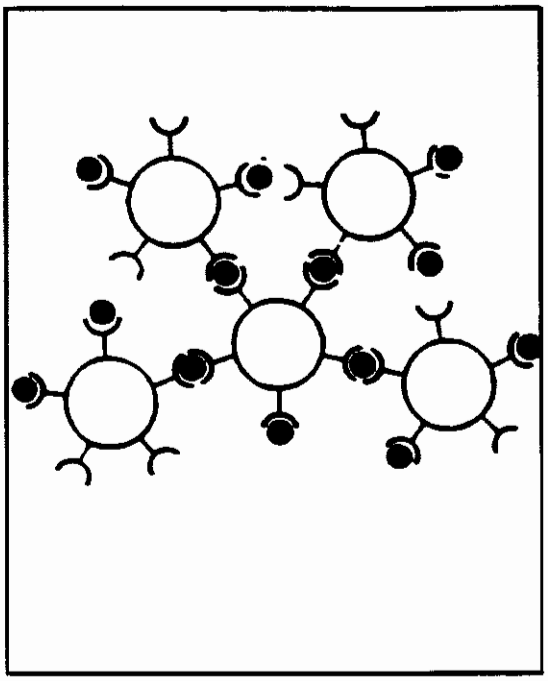
Timeline for Development



February 19, 1990
HS01273V

85

Title	Feasibility Study: Studies of Potentially Simpler Methods for Detection of Fecal Leukocytes as a Guide to Inflammatory Diarrhea
Number	861200164
P.I.	Richard L. Guerrant, M.D.
Institution	University of Virginia School of Medicine/Division of Geographic Medicine
Status	Active



Objectives

The purpose of this feasibility study is to assess the potential of several proteins as markers for the presence of fecal leukocytes. Potential markers include leucocyte esterase, myeloperoxidase and lactoferrin. Initial experiments will utilize a commercially available dipstick, Chemstrip, which detects leukocyte esterase in urine. Fecal specimens lacking leukocytes and to which 5,000 to 10,000 human polymorphonuclear neutrophils per mm^3 have been added will be examined. Leukocyte myeloperoxidase, a second candidate marker, is detected photometrically and may not be easily adaptable to a simpler methodology. A third potential leukocyte marker is lactoferrin, for which a highly specific antibody already is commercially available. Control fecal specimens and suspensions, as well as those to which varying numbers of human neutrophils (between 5,000 and 10,000 PMNs/mm^3, obtained by Ficoll-Hypaque separation) have been added, will be assayed for lactoferrin by radial immunodiffusion. The laboratory will then obtain aliquots of fecal specimens in the clinic for further testing and/or correlation with microscopic and stool culture results.

Significance

On a global scale, diarrheal diseases constitute a major cause of morbidity and mortality, second only to ARI. Most illnesses are of a noninflammatory type and are thus appropriately treated by oral rehydration therapy. However, a subset of illnesses, those of an inflammatory type, may warrant further diagnostic and therapeutic intervention. Indiscriminate culture or treatment is both costly and inappropriate. A simple examination for

fecal leukocytes could greatly help to discriminate inflammatory from noninflammatory diarrheal illnesses. Detection of fecal leukocytes is not difficult, provided one has a light microscope and a trained microscopist. If a simpler method, perhaps analogous to the dipstick method for detecting urinary leukocytes, were available as a screening test for inflammatory diarrhea, much wider application could certainly be envisioned. It is expected that the cost-benefit analysis of selected stool cultures for inflammatory pathogens such as *Shigella, Salmonella*, and *Campylobacter jejuni* would also be improved by coupling to such a screening test.

Progress to Date

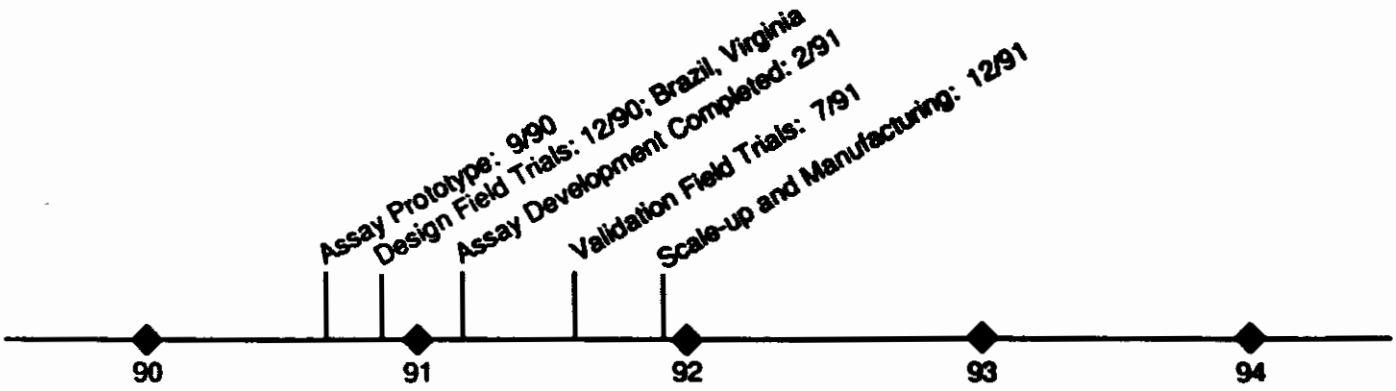
The leukocyte esterase and myeloperoxidase markers are no longer being investigated because they must be used in a calorimetric testing format.

The P.I., however, has successfully developed a latex bead assay with anti-lactoferrin monoclonal antibody. This assay can detect leukocytes even after sample refrigeration and freezing. A handful of specimens (with and without spiked leukocytes) were tested and two cases of salmonellosis were identified. Titers of samples from inflammatory diarrheas were as high as 1/128. Persons infected with *C. difficile* secreting toxin were detected as positive, the ones with the highest toxin titers, interestingly, tended not to have fecal leucocytes present (complete lysis by toxin?). Fecal samples obtained from children with diarrhea in northeastern Brazil were tested by the latex agglutination method. Specimens from 16 of 17 children with inflammatory diarrhea (1-5 or more leukocytes per microscope high power field) were positive. This may be compared with only 3 of 12 specimens from children with diarrhea with less than 1 leukocyte per HPF, and 0 of 7 specimens from normal children. Therefore, results are encouraging for the first test on clinical specimens; however, the 25% positives in leukocyte-poor fecal samples will have to be addressed and resolved. As an additional application, the P.I. will test cervical samples with the assay to determine its utility with specimens obtained from Dr. Michael Rein of the University of Virginia.

Funding Amount Total: $ 14,957

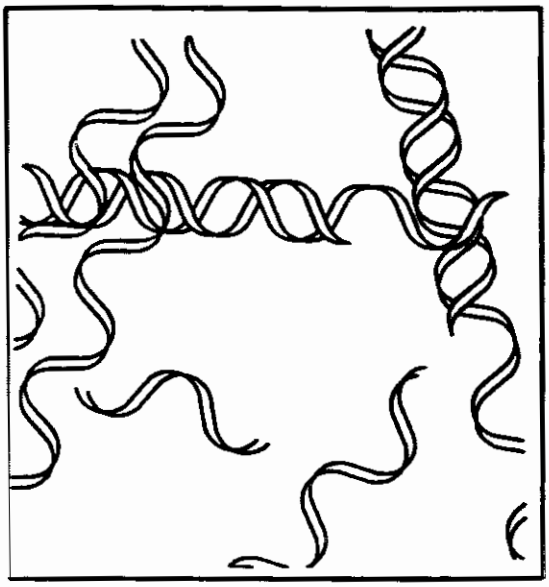
Period May 1, 1988 - April 30, 1990

Timeline for Development



February 19, 1990
HS01281V

Title	A Clinical Field Trial of a Nucleic Acid Probe for the Detection of *Shigella* Infections
Number	89100226
P.I.	Richard Oberhelman, M.D.
Institution	The Johns Hopkins University/Department of International Health
Status	Active



Objectives

To determine the utility of a nonisotopic oligonucleotide probe and to determine specificity of the probe using a blot method to detect *Shigella* in growth-amplified stool specimens. This will take place in a clinical field study in Lima, Peru, a location of an endemic population of adults and children over 12 months of age. Stool culture will be used to compare the accuracy of the probe method. If successful, the blot hybridization method will be used to detect *Shigella* in direct stool specimens.

Significance

Shigella spp. have long been recognized as important enteropathogens causing inflammatory diarrhea in infants and children. While *Shigella* infections in the developed world are characterized as brief and easily treated with antibiotics, the picture is different in the developing world where the disease is more chronic and is associated with malnutrition. *Shigella* can produce a significant level of mortality. Rapid diagnosis and treatment, where possible, is instrumental in preventing complications. Rapid diagnosis is also essential to distinguish *Shigella* from other enteropathogens such as *Campylobacter* for differential diagnosis. At present, the only readily available method for detection of *Shigella* is stool culture, which requires fresh specimens for maximal recovery of organisms. Nucleic acid probes, however, may have an advantage where the interval between sampling and assay is less critical. Nucleic acid probes have recently been developed to detect specific sequences within the invasion plasmid antigens ipa A, B, and C. When used with nonradioactive markers, these probes should facilitate rapid field diagnosis of *Shigella*, and detect nonviable organisms which would be missed by stool culture. The probe method will have use for individual diagnosis of infection and as an epidemiological tool.

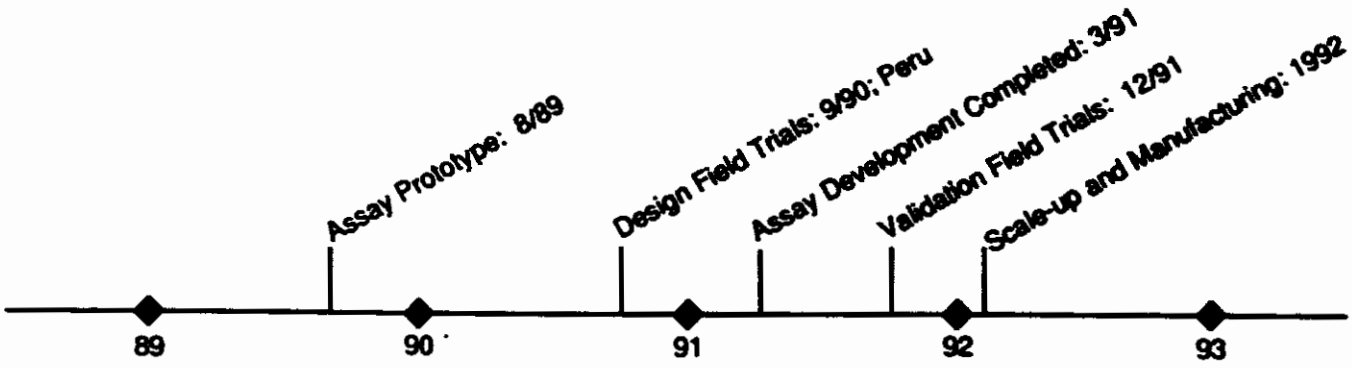
Progress to Date

In the first half of the study, ipa C and ipa H probes will be tested and assay conditions optimized. Growth amplification conditions indicate that 6 hours to overnight is optimal, and that ipa H probe substrate incubation can be as short as 2 hours. Preliminary results indicate that the ipa H is the better of the two probes, demonstrating good specificity and sensitivity and allowing a relatively fast culture amplification and assay development time. Data indicate that the assay may be performed within a 24 hour period. The second half of the study will test the optimized method on a larger population base. The P.I. has travelled to Peru to train personnel and initiate the studies.

Funding Amount Total: $ 50,000

Period July 15, 1989 - September 15, 1990

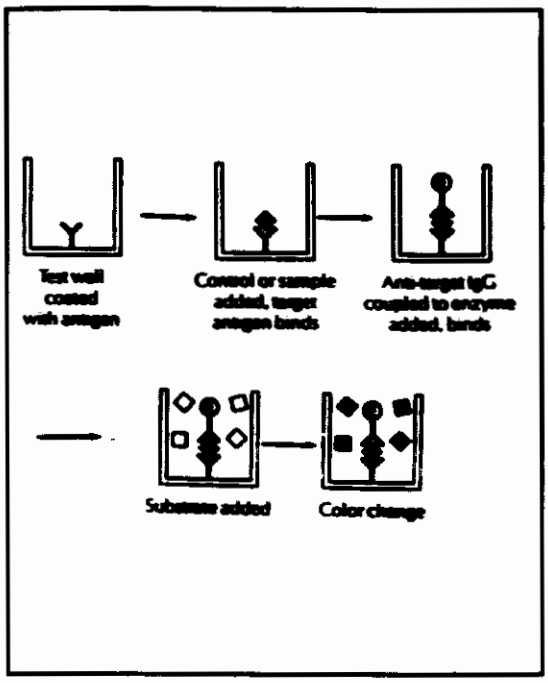
Timeline for Development



February 19, 1990
HS01280V

90

Title	Diagnosis of Pathogenic *E. histolytica* Infection by Detection of Anti-lectin Antibodies in Serum and Adherence Lectin Antigen in Feces
Number	860700143
P.I.	Jonathan I. Ravdin, M.D.
Institution	University of Virginia School of Medicine
Status	Active



Objectives

To develop an ELISA for *E. histolytica* in stool specimens and liver abscess material and to evaluate the utility of the Gal/GalNAc lectin binding protein for distinguishing between virulent and avirulent strains of *E. histolytica*.

Significance

E. histolytica infection is prevalent in the developing world and is the third leading parasitic cause of death worldwide. At present, there is no available rapid diagnostic method for examination of stool which does not require expert microscopy utilizing morphologic criteria. ELISA methods of detection which have been described in the literature have suffered from inability to discriminate virulent from avirulent strains of *E. histolytica* and from poor reactivity toward cyst forms (found in stool) of the organism.

Progress to Date

Monoclonal antibodies and two rabbit antisera to the lectin-binding protein (mw >400,000) were produced. Using a pool of four monoclonals as capture and a rabbit antiserum as the detection reagent, good assay sensitivity was obtained, but cross-reactivity and high nonspecific binding was a significant problem. To solve this problem the P.I. has used two of the monoclonals as capture and the other two as signal. The P.I. first developed a RIA system to optimize the assay and has now labelled his antibodies with alkaline

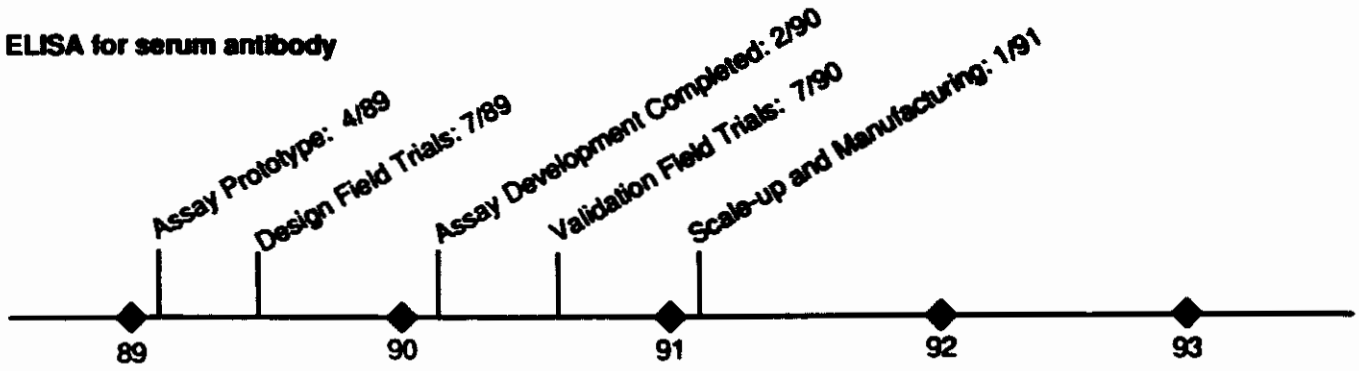
phosphatase to produce an EIA. The EIA version remains very sensitive, and stool does not interfere with the immunoassay. The P.I. will soon start to test clinical specimens and will also test serum samples from patients with hepatic amebiasis. This all-monoclonal antibody approach is very likely to be successful.

Results of the serological assay for anti-adherence lectin antibodies have been encouraging. In a blinded test of 253 specimens from South Africa, sera were diluted 1:1,000 and tested by plate ELISA against purified adherence lectin. The results indicate a high assay sensitivity (99%) in patients with amebic liver abcesses or pathogenic, amebic intestinal infection. However, 25% of sera from patients with nonpathogenic, amebic intestinal infections or which were culture-negative for *E. hystolytica* were also above the cutoff and considered positive. Interestingly, none of 69 sera from North Americans with other parasitic diseases or from healthy control subjects were positive, leading to the hypothesis that the "false positive" group represented those patients with antibody to previous invasive amebic infections. To address this concern, the P.I. will evaluate the use of a salivary IgA test for determination of specific anti-*E. hystolytica* antibody, since there is evidence that the IgA response is of short duration. The P.I. has also optimized a dot immunobinding dipstick immunoassay using protein A-colloidal gold as signal. This assay was originally developed by PATH for an HIV-1 test and will be assessed, first on the 253 South African specimens and subsequently in the field in parallel with the ELISA method.

Funding Amount	Year 1: $ 58,130 Year 2: $ 29,386 Year 3: $ 45,043 Total: $132,559
Period	August 1, 1987 - September 26, 1990

Timeline for Development

ELISA for serum antibody



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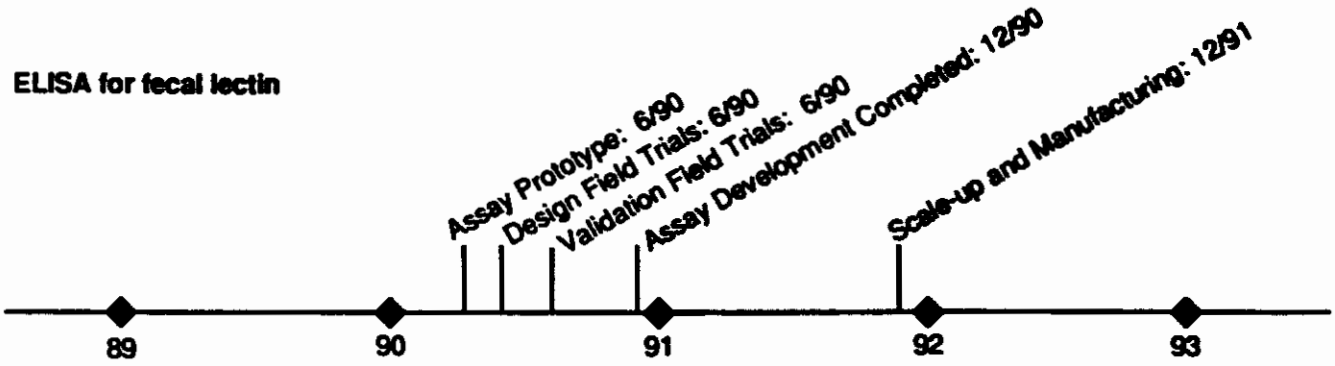
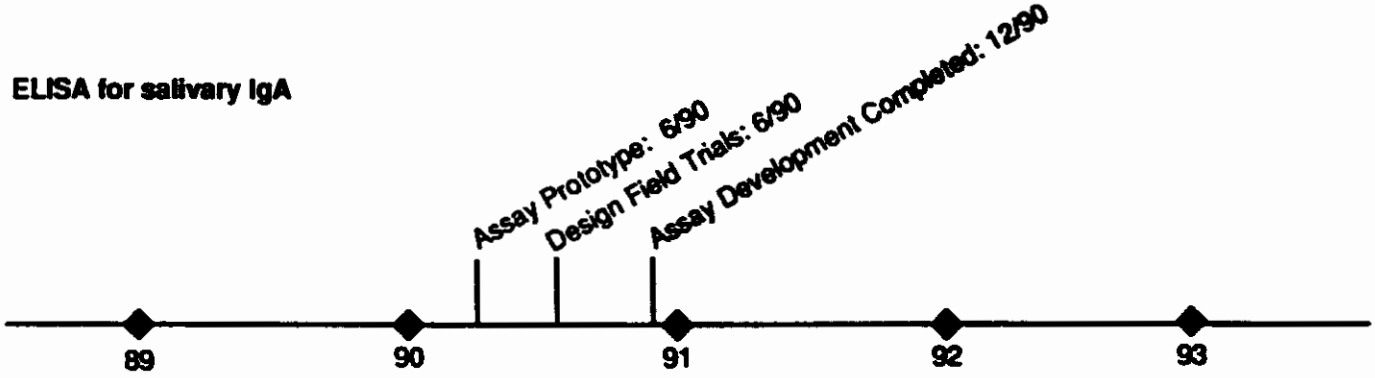
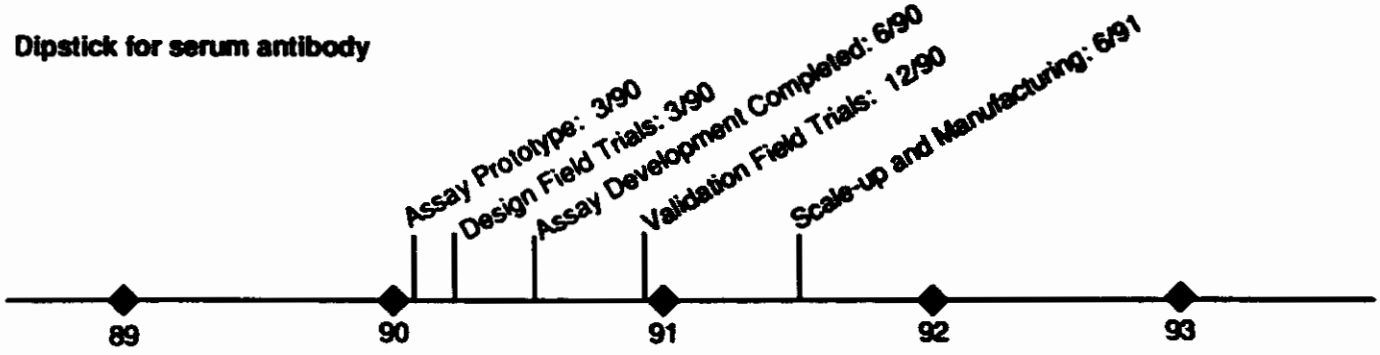
ELISA for fecal lectin



ELISA for salivary IgA

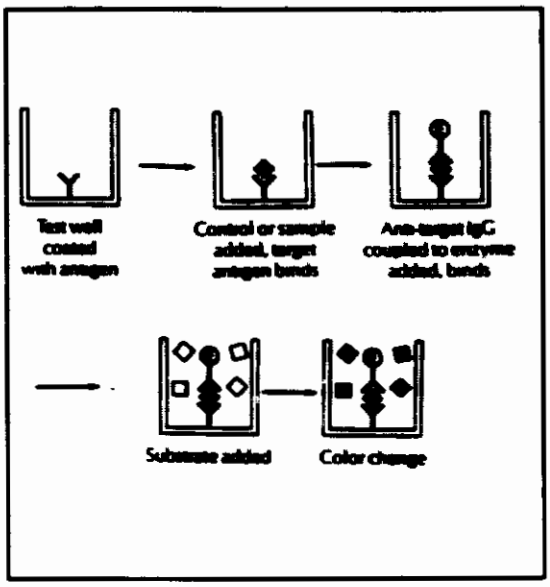


Dipstick for serum antibody



February 19, 1990
HS01279V

Title	Development of Vi Monoclonal Antibodies for the Diagnosis of Typhoid Fever
Number	870600196
P.I.	Suttipant Sarasombath, M.D.
Institution	Mahidol University, Bangkok
Status	Active



Objectives

To develop a panel of mouse monoclonal antibodies (MAbs) specific to Vi capsular polysaccharide antigen of *S. typhi* and to develop an immunoassay(s) capable of detecting the Vi antigen in clinical specimens.

Significance

Typhoid fever is endemic and poses serious public health problems in many developing countries. Laboratory diagnosis of this disease by the Widal test is made too late to be of any value in patient care, while blood culture is notoriously insensitive. Urine represents an attractive fluid to assay because it can be obtained in large quantity through noninvasive methods. Vi antigen is known to be excreted in urine.

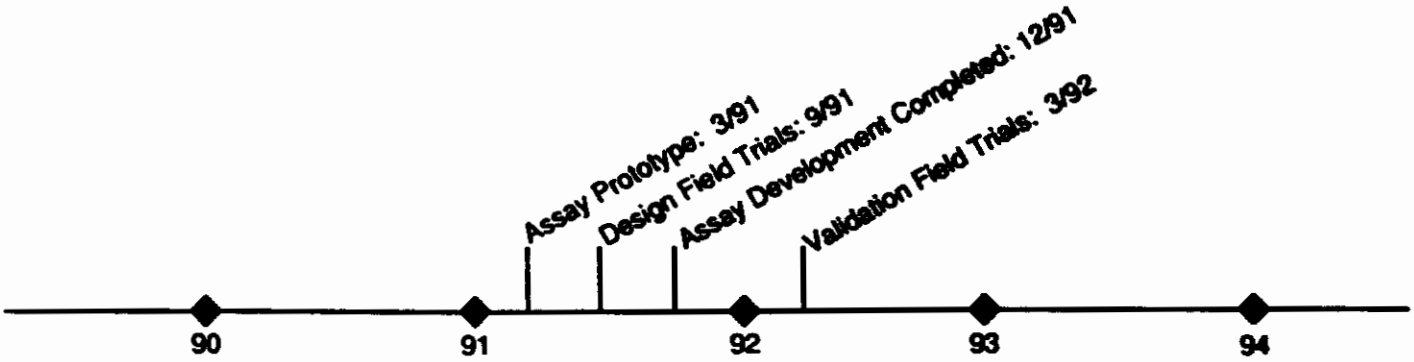
Progress to Date

Acetone-killed, dried bacteria were extracted with 0.85% NaCl, and the supernatant extracted with ethanol and Cetavlon. The yield of Vi prepared in this way from *S. typhi* was 60 ug/3 g dry weight of bacteria and 1 mg/4 g from *C. freundii*. These preps were used to immunize mice. After several fusions, the P.I. has cloned and stabilized several monoclonal antibodies which react with *S. typhi* and *C. freundii* Vi antigen. With use of selected MAbs, the P.I. has developed a double-sandwich ELISA for Vi antigen, which was capable of detecting as little as 0.5 microgm/ml. In addition to the ELISA method, the sensitivity was increased to 0.05 microgm/ml by means of a co-agglutination method using a single MAb, 8A2H9. The agglutination method was tested on five frozen urine specimens from which *S. typhi* was recovered by hemoculture. Three of the urines were positive;

however, frozen urines are not the best to use, as freeze-thaw cycles have been shown to result in a decrease in sensitivity in spiked specimens. The initial effort to produce a co-agglutination reagent is therefore promising. The P.I. would like to continue her studies to evaluate the agglutination method to detect Vi with fresh specimens, using hemoculture and the Widal tests for comparative purposes. The P.I. has recently received Year II support.

Funding Amount	Year 1: $ 64,703 Year 2: $ 30,054 Total: $ 94,757
Period	January 1, 1988 - September 26, 1990

Timeline for Development

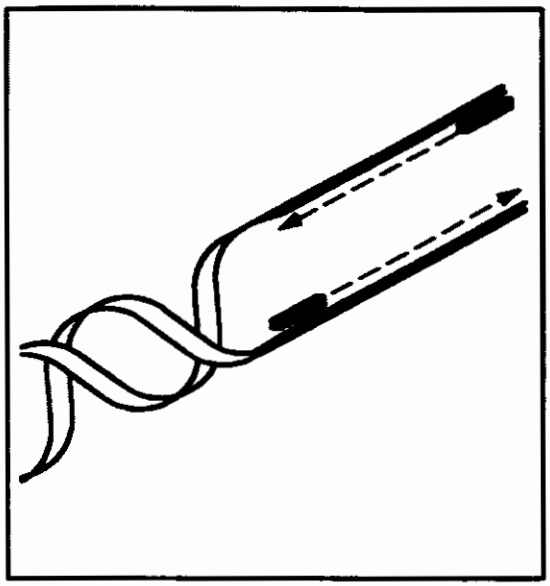


February 19, 1990
HS01288V

95

Malaria

Title	Non-Radioactive Ultrasensitive Detection of Amplified *P. vivax* and *P. falciparum* Genes
Number	860500061
P.I.	W. John Martin, M.D., Ph.D.
Institution	Professional Staff Association, University of Southern California
Status	Active



Objectives

To apply a recently developed technique (selective gene amplification via polymerase chain reaction) for selectively amplifying a specific nucleotide sequence to the detection of the circumsporozoite (CS) genes of *P. vivax* and *P. falciparum*. In this method, synthetic oligonucleotide primers are annealed upstream and downstream of the region to be amplified. Multiple cycles of primer annealing, synthesis, and melting are conducted, resulting in the selective expansion of the sequence flanked by the primers. If biotinylated dNTPs are used, the expanded sequences will be detectable using avidin-biotin/enzyme.

Significance

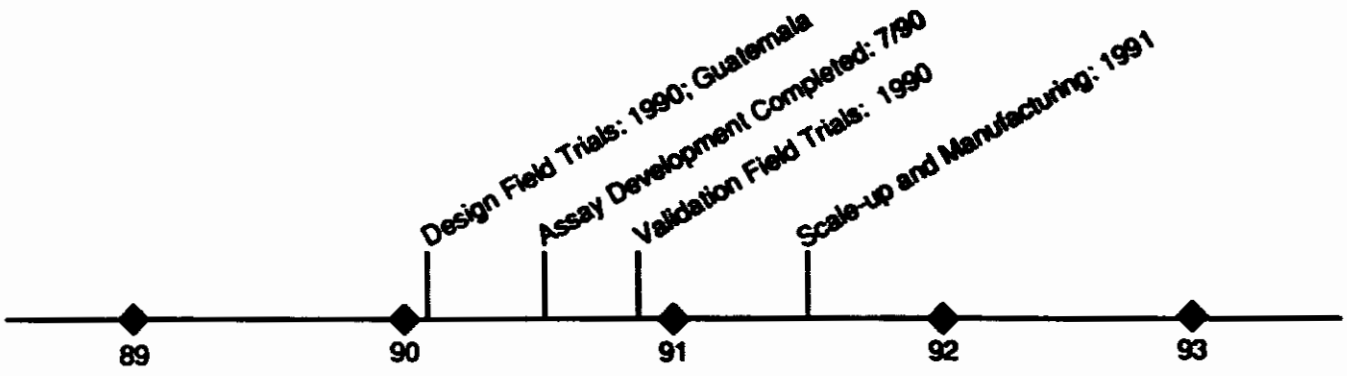
Microscopic diagnosis of malaria is tedious and time-consuming when large numbers of samples must be examined. Existing DNA hybridization methods are insufficiently sensitive to replace microscopy. The above-described method can amplify a selected sequence 200,000-fold in one hour or less. In addition to its potential for malaria diagnosis, this method should be broadly applicable to diagnosis of a variety of pathogens. The method is applicable to epidemiological surveillance and vaccine studies and has the potential to identify pyrimethamine and chloroquine resistance in isolates of falciparum malaria. Because of its extreme sensitivity, the PCR method will have less application for primary diagnosis of malarial infection unless a reliable method is devised for quantitation of organisms, since the majority of individuals in an endemic area will harbor parasites.

Progress to Date

During Year I, the limit of detection for this method was shown to be approximately 20-30 parasites per microliter of infected blood. The feasibility of amplifying both *P. vivax* and *P. falciparum* sequences from the CS gene in the same specimen was also demonstrated since different sized probes can be used, then identified on gels. In Year II, a nonisotopic version of the assay based on the uptake of biotinylated nucleotides was developed for CMV, which can be directly applied for malaria. Further procedural improvements comprised the following: (1) use of a thermostable polymerase, (2) simplified handling and dilution steps for blood specimens, (3) increased accuracy and reliability of the technique to eliminate false-positive reactions, (4) considerable increase in the sensitivity of the PCR assay, and (5) use of an inexpensive instrument for automating the more labor-intensive steps of the assay. Most recently, primer sets have been identified which are specific for the falciparum dihydrofolate reductase gene, the falciparum multidrug resistance-related gene. This finding is exciting, since it will allow the rapid, direct testing for drug-resistant strains of malaria in individual patients. Field studies will be designed for evaluation of PCR technology in Guatemala in mid- to late 1990 with emphasis on use of nonisotopic methods for speciation of isolates and detection of drug-resistant strains.

Funding Amount	Year 1: $ 44,986 Year 2: $145,960 Total: $190,946
Period	November 1, 1986 - April 30, 1990

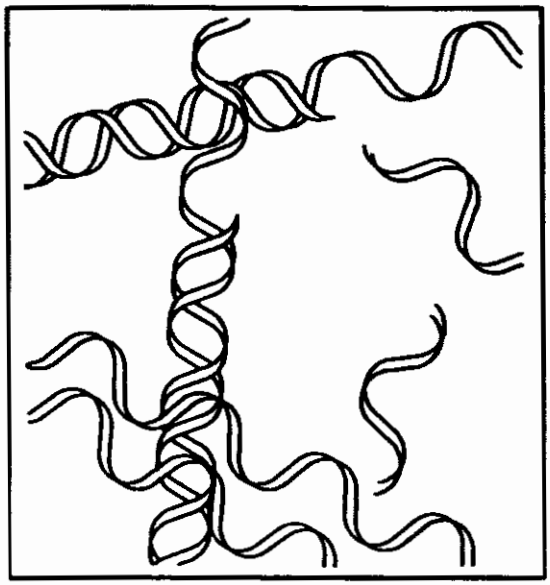
Timeline for Development



February 19, 1990
HS01301V

98

Title	Detection of Malaria Parasites Using Enzyme-Linked Synthetic DNA Probes
Number	861100160
P.I.	Gerald McLaughlin, Ph.D.
Institution	University of Illinois
Status	Active



Objectives

To develop simplified, field-suitable methods of detecting malarial DNA using an enzyme-labeled synthetic oligonucleotide probe to repetitive genomic sequences of *P. falciparum*.

Significance

Falciparum malaria is a major cause of morbidity and mortality in the developing world. DNA probes represent an important new technology in the diagnosis of infectious diseases and are particularly attractive whenever a large number of samples must be screened. Previous reports have shown that isotopically labeled DNA probes can detect parasitemias as low as 0.0001% in a 10-microliter blood sample. The present study will attempt to simplify DNA hybridization methodology to render it more appropriate for field use. Improvements include utilization of enzyme labels, use of a proprietary minicolumn developed by Molecular Biosystems Inc. (San Diego, California) for quantitative recovery of parasite DNA from lysed whole blood, and development of sandwich formats for hybridization.

Progress to Date

A synthetic oligonucleotide probe, PFR1, to repeated DNA was directly labeled with alkaline phosphatase. This probe, supplied by Molecular Biosystems, Inc., was used to detect target DNA in two types of assays: (1) an *in situ* hybridization assay using a biotinylated probe, and (2) by dot hybridization on nitrocellulose membranes. The P.I. has decided to concentrate on development of the membrane dot blot hybridization assay, since this method is viewed as most applicable to high-volume testing for epidemiologic surveillance

studies and public health intervention measures in developing countries, while the *in situ* method has not been verified as more time or cost efficient when compared to simple microscopy.

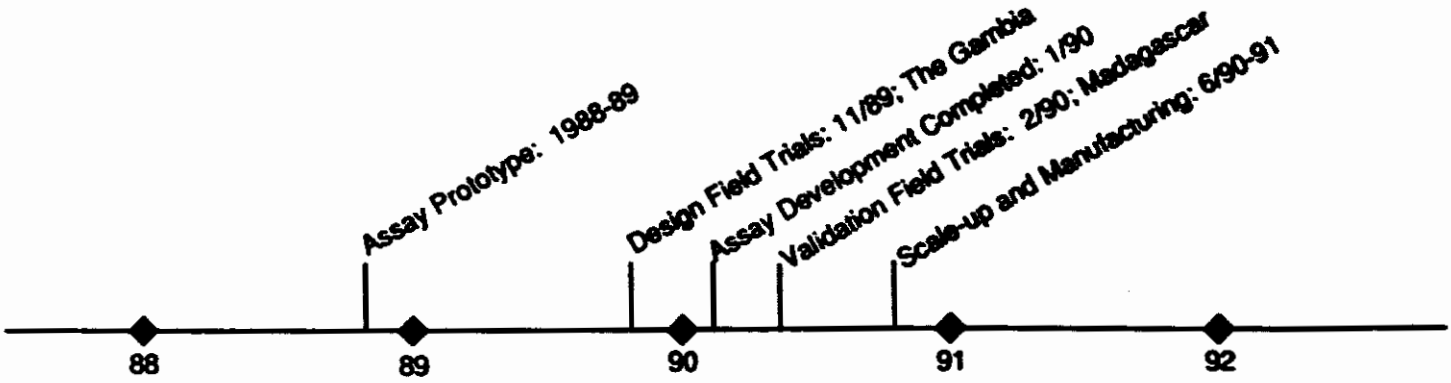
For the *in situ* hybridization method, parasite nuclei and cytoplasm could be clearly stained and are at least as sensitive as Giemsa staining and conventional microscopy. Parasite morphology is surprisingly well conserved with this method. This method was reported at the ASTMH meeting in Washington, D.C., in December 1988.

Sample preparation for the dot-blot assay is straightforward, requiring lysis of 30 microliters of peripheral blood in buffer, followed by transferring the specimen to a 96-well manifold where sample is aspirated through a nitrocellulose membrane. The membrane, containing as many as 96 samples and controls, is then subjected to the nonisotopic probe protocol. The assay method requires three hours or less to perform and could detect a parasitemia as low as 0.05%. A panel of blood smears from Africa, as well as laboratory testing in a WHO biotechnology workshop in Geneva, and a workshop in Beijing, China, helped in validation of the *in situ* method. The P.I. then evaluated his dot-blot assay in the design-phase in The Gambia in late 1989 using funds he obtained from WHO. Results were very good, and the assay proved robust enough to perform under a variety of field conditions. As a result of the field study, P.I. has fine tuned his assay, and assembly of 500 to 1,000-test kits has started in preparation for validation-stage field trials in early 1990.

PCR methods are also being developed using 21-base oligomers from DNA repetitive sequences, as well as control rRNA primers. These studies have been de-emphasized in 1989. The rRNA primers produced a major and several minor bands, while the repetitive sequence primers gave single bands or smears of DNA.

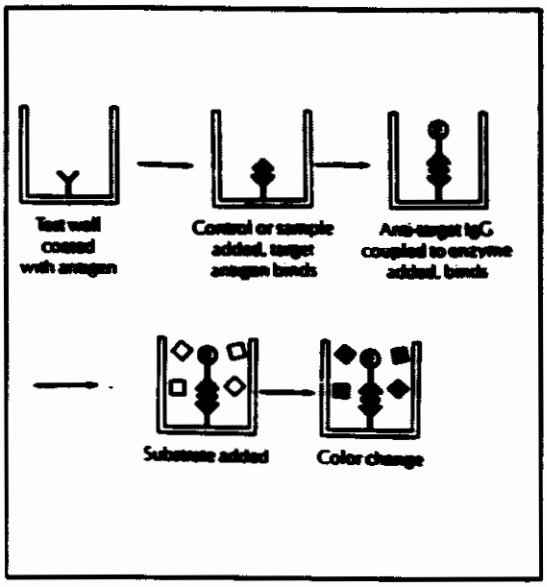
Funding Amount	Year 1: $ 45,660 Year 2: $254,023 Total: $299,683
Period	July 1, 1987 - June 30, 1990

Timeline for Development



February 19, 1990
HS01278V

Title	A Monoclonal Antibody "Antigen Capture" Assay for Diagnosis of Malaria
Number	860700121
P.I.	Diane Wallace Taylor, Ph.D.
Institution	Georgetown University
Status	Active



Objectives

To develop an immunoassay for the detection of blood stages of *P. falciparum*.

Significance

More than 1.5 billion people live in malarious areas of the world. Worldwide, an estimated 250-300 million cases of malaria occur each year. In tropical Africa alone, an estimated 160-200 million people are infected annually and more than 1 million people die, predominantly infants and small children. Microscopic diagnosis requires technical skill and is tedious and time-consuming, especially when large numbers of samples must be examined. An improved method of diagnosis will also facilitate vaccine trials.

Progress to Date

Dr. Taylor has developed a panel of monoclonal antibodies which react with antigens of *P. falciparum*. One such antibody, designated 7H8, is directed at a heat-stable antigen (Pf93) present in blood stages of all species of *Plasmodium* tested to date, including rodent, human, and non-human primates. An antigen-capture EIA developed and optimized currently has a 75%-80% sensitivity and 100% specificity. Diluted serum is used as specimen, an advantage over the use of lysed RBC, which has been shown to be problematic. The 7H8 antigen was shown by the P.I. to be present in the urine of some, but not all, experimentally infected mice. The biotin-labelled 7H8 MAb as well as other candidate capture MAbs were tested in the DiaTech laboratory. The results of the P.I. were essentially confirmed. From there, DiaTech arranged to have the assay developed and optimized further by Alister Voller. A recent progress report indicates that the epitope

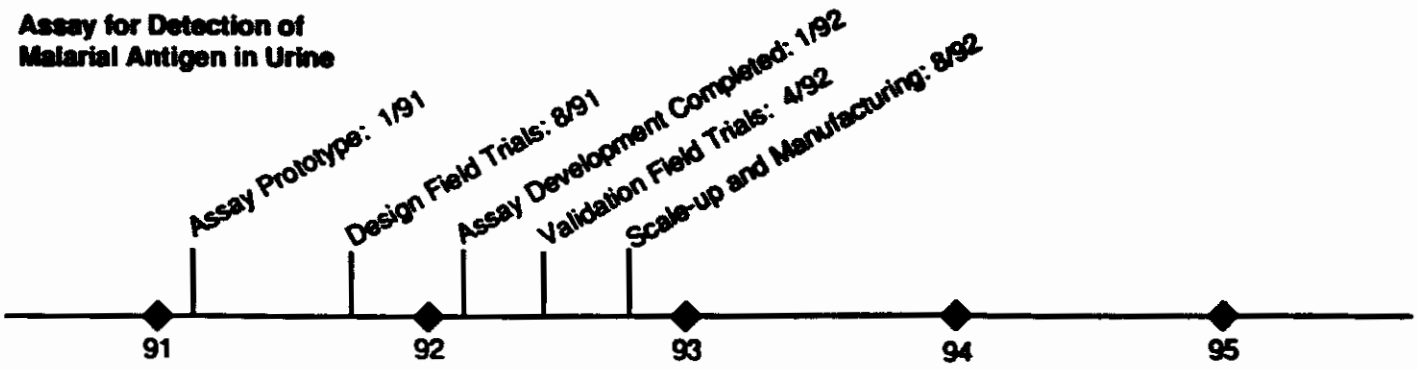
defined by MAb 7H8 is conformational in nature and may not be stable upon prolonged storage of serum.

A second, histidine-rich antigen is designated PfHRP-2 and is restricted in expression to *P. falciparum*. A second EIA developed using anti-PfHRP-2 Mabs has been tested but currently detects *P. vivax*-infected specimens in addition to *P. falciparum*. Further work has revealed that the PfHRP-2 antibodies cross-react with several components in the serum of normal as well as infected individuals. The malaria-specific antigen, however, is identified by Western blot. While sensitivity and specificity of a Western blot assay are excellent, the method is, of course, inappropriate for diagnosis outside the research laboratory.

As a Year III activity, the P.I. has generated polyclonal antisera as well as 28 MAbs which bind high molecular weight (>200 kD) proteins in urine, and has therefore, unequivocally demonstrated the presence of malarial protein antigens in urine. This may well be the most promising assay system, since it will be a noninvasive method of testing. Antigens have been detected in urine specimens from both Ghana and Thailand. More specimens have been requested and will be provided through QSMI. MAbs to at least 7 different epitopes have been produced. The MAbs produced will be further characterized for epitope specificity and analyzed for development of a urine-based immunoassay.

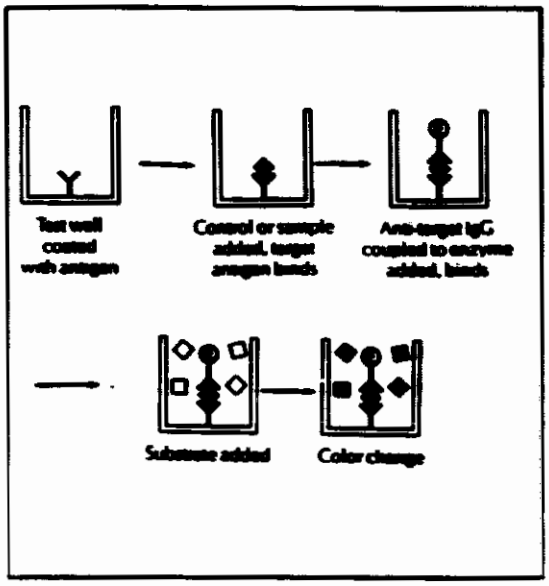
Funding Amount	Year 1: $ 67,599 Year 2: $ 84,858 Year 3: $ 34,496 Total: $186,953
Period	March 1, 1986 - May 31, 1990

Timeline for Development



February 19, 1990
NS01277V

Title	Two-Site ELISA for the Detection of *Plasmodium falciparum*
Number	870500180
P.I.	Savanat Tharavanij, M.D., Ph.D.
Institution	Mahidol University, Bangkok
Status	Active



Objectives

To develop a two-site ELISA for diagnosis of falciparum malaria; to assess sensitivity and specificity of the assay; and to apply this assay to detect low-grade *Plasmodium falciparum* infections in patients with malaria.

Significance

More than 1.5 billion people live in malarious areas of the world. Worldwide, an estimated 250-300 million cases of malaria occur each year. In tropical Africa alone, an estimated 160-200 million people are infected each year and more than 1 million people die, predominantly infants and small children. Microscopic diagnosis requires technical skill and is tedious and time-consuming, especially when large numbers of samples must be examined. An improved method of diagnosis will also facilitate vaccine trials.

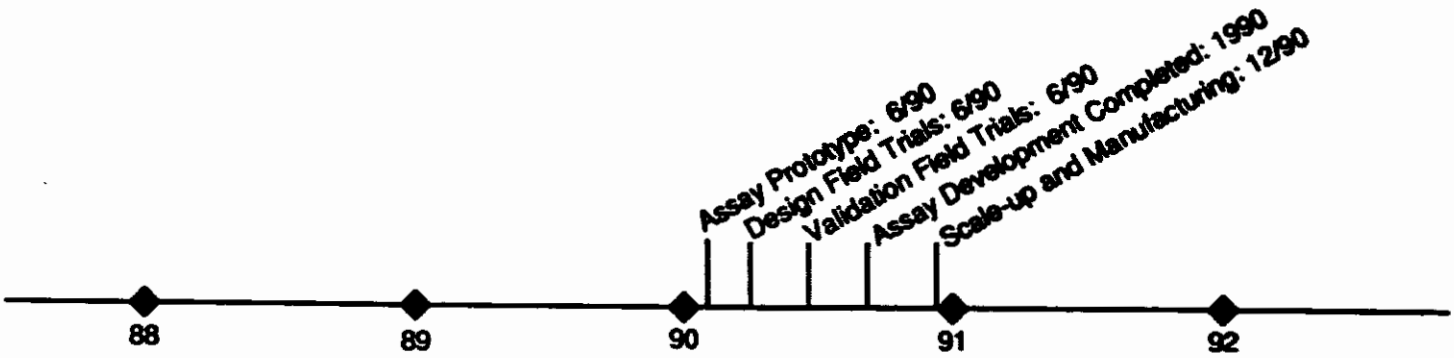
Progress to Date

A hybrid cell line producing monoclonal antibodies to *P. falciparum* had been previously developed by the P.I. This line, F2W22C1, was found to be a low producer upon thawing and even after recloning, and the P.I. was not able to successfully reestablish this line. A competitive ELISA approach, using polyclonal rabbit serum for both capture and detection to detect parasites in blood, is under development. Preliminary experiments indicate that the test method is feasible but presently requires improvement to be practical. When applied to clinical samples, the test sensitivity was 87% (cutoff: >10% inhibition) for 103 cases of falciparum malaria, and cross-reacted with 31% of 29 cases of vivax malaria, and 0% of 39 healthy controls. Level of parasitemia did not correlate well with EIA values.

Although the competitive assay produced a fairly high sensitivity, it still has the shortcomings of being a two-day assay, has less than maximal sensitivity, cross reacts with vivax malaria, and gives fluctuating results with low-grade parasitemia. These shortcomings will be improved in Year II of this project. The P.I. will also take the opportunity to reclone or reproduce an equivalent of the 22C1 monoclonal antibody with which he will redevelop the antigen capture immunoassay he originally planned to develop as part of Year I goals. In addition, the P.I. has agreed to supplement DiaTech efforts in specimen collection and will serve as a field testing laboratory for technologies developed in other DiaTech projects.

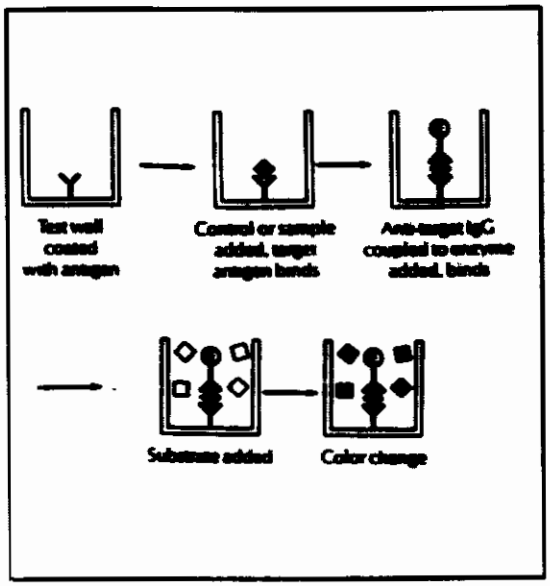
Funding Amount	Year 1: $ 14,960 Year 2: $ 37,011 Total: $ 51,971
Period	January 1, 1988 - September 26, 1990

Timeline for Development



February 19, 1990
HS01302V

Title	Development of Malaria Antigen ELISA
Number	890800232
P.I.	Alister Voller, Ph.D., D.Sc.
Institution	Institute of Zoology, Zoological Society of London
Status	Active



Objectives

The P.I.'s laboratory has undertaken further development, optimization, and validation of the monoclonal antibody ELISA assay for PfHRP-2 serum malarial antigen using the reagents supplied by Dr. Diane W. Taylor, another DiaTech subgrantee. It is anticipated that a diagnostic kit specific for *P. falciparum* will result from this effort.

Significance

More than 1.5 billion people live in malarious areas of the world. Worldwide, an estimated 250-300 million cases of malaria occur each year. In tropical Africa alone, an estimated 160-200 million people are infected annually and more than 1 million people die, predominantly infants and small children. Microscopic diagnosis, the current standard, requires considerable technical skill and is tedious and time-consuming, especially when large numbers of samples must be examined. An improved method of diagnosis such as a high-volume microtest ELISA method will hopefully improve these present diagnostic limitations.

Progress to Date

The transfer of reagents with diagnostic potential from a P.I. more interested in basic research, to another P.I. more interested or more qualified for diagnostic kit development is new for DiaTech. This may allow for a more rapid development cycle, especially if a technology does not have an identified commercial potential, yet can be applied to public health problems of the developing world. If successful, DiaTech may decide to solicit more

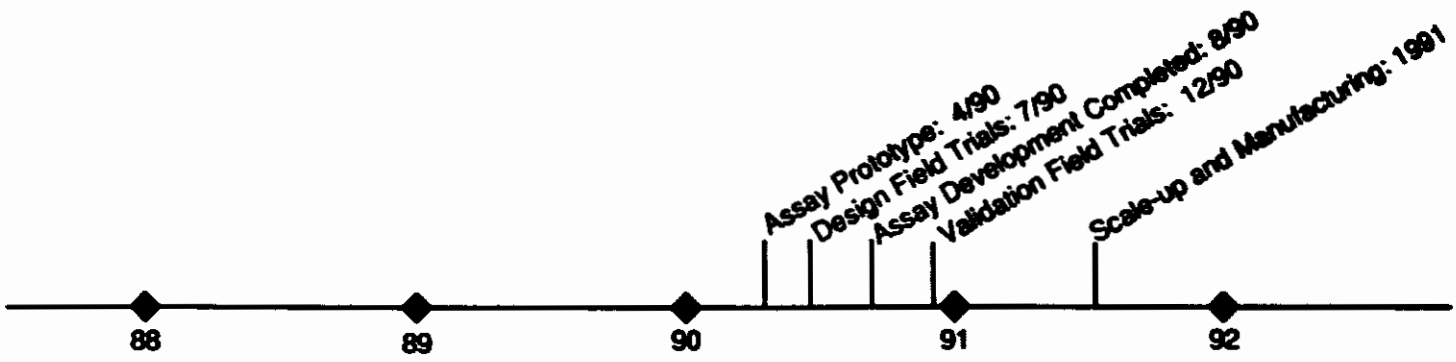
"piggyback" projects as technologies move from the laboratory bench and through the development pipeline.

The reagents, test protocols, preliminary data, and reference materials were transferred to the P.I.'s laboratory in the latter part of 1989. Testing on reference specimens initially revealed a high false-positive and significant false-negative rate. Fortunately, Dr. Taylor provided other MAbs, which were used to prepare conjugates for evaluation with one another in a checkerboard manner. The results revealed that several combinations of MAbs used for capture and signal were much better than the original system. In particular, 1E1 used as capture and 2G12 for signal gave very high positive and low negative values. Further development and optimization of this MAb pair and testing on a more extensive panel of specimens is now under way.

Funding Amount Total: $ 29,500

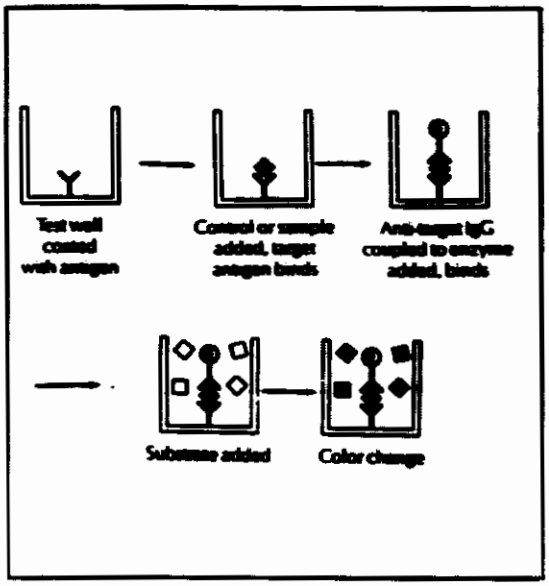
Period September 1, 1989 - August 1, 1990

Timeline for Development



February 19, 1990
MS01276V

Title	Development of an ELISA to Monitor Quinine Levels in Malarious Patients
Number	860300049
P.I.	Alister Voller, Ph.D., D.Sc.
Institution	Institute of Zoology, Zoological Society of London
Status	Completed



Objectives

To develop an inhibition immunoassay for quinine in serum and plasma.

Significance

In parts of the world where chloroquine resistance is widespread, quinine is often used to treat falciparum malaria. Quinine has a narrow therapeutic window; thus, the ability to monitor plasma drug levels is important to patient management. This assay may also be useful as an indirect measure of the emergence of drug-resistant strains by enabling distinction between treatment failures due to poor compliance or poor absorption and failures due to true breakthrough infection.

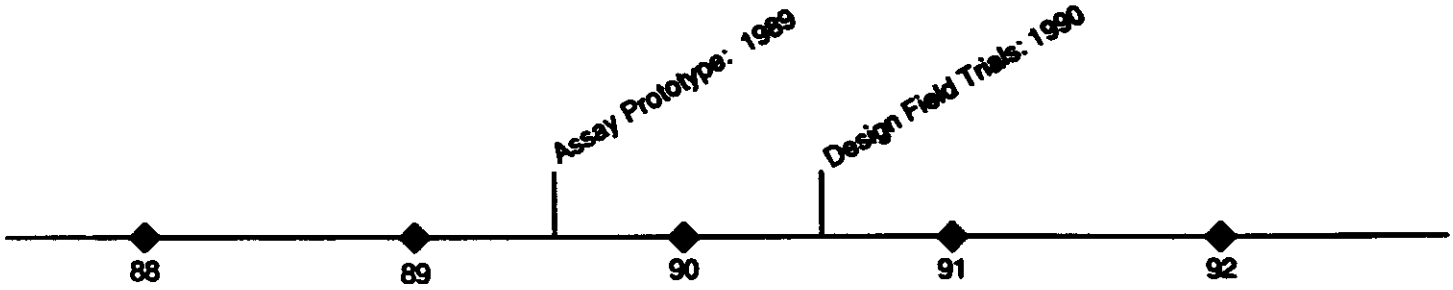
Progress to Date

Antiserum to quinine was raised in rabbits, purified, and labeled with HRP. Polyvinyl chloride microtiter plates were coated with a conjugate of quinine/TBG and tested in an inhibition ELISA with artificially reconstituted plasma samples. As presently configured, the assay requires approximately 3.5 hours to perform. A publication indicates that the assay is sensitive and reproducible. A needed step in assay verification was supplied in December 1989, when Dr. Karen Snowden of the Liverpool School of Tropical Medicine requested quinine ELISA kits for a therapeutic study to be conducted in Malawi in early 1990. The P.I. will work with Dr. Snowden to prepare and test the kits. The quinine assay is also currently being used to test a new formulation of sugar coated quinine for pediatrics, and is currently being evaluated on 200 patients in London.

Funding Amount Total: $ 19,948

Period March 1, 1987 - February 29, 1988

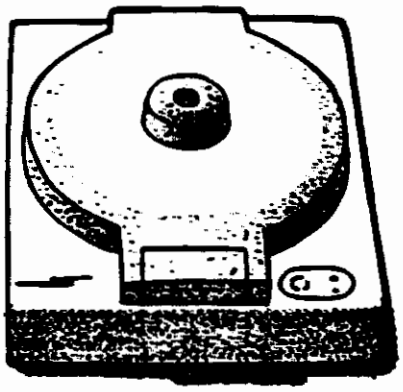
Timeline for Development



February 19, 1990
HS01275V

Other

Title	Provision of Assistance to DiaTech Management and Sample Collection
Number	861100161
P.I.	Supawat Chutivongse, M.D.
Institution	The Queen Saovabha Memorial Institute of The Thai Red Cross Society, Bangkok
Status	Active



Objectives

The purpose of this primary subagreement is to set up the infrastructure for a team of technical and field-experienced advisors in a developing world site. The QSMI is an ideal field test center for diagnostic technologies.

Significance

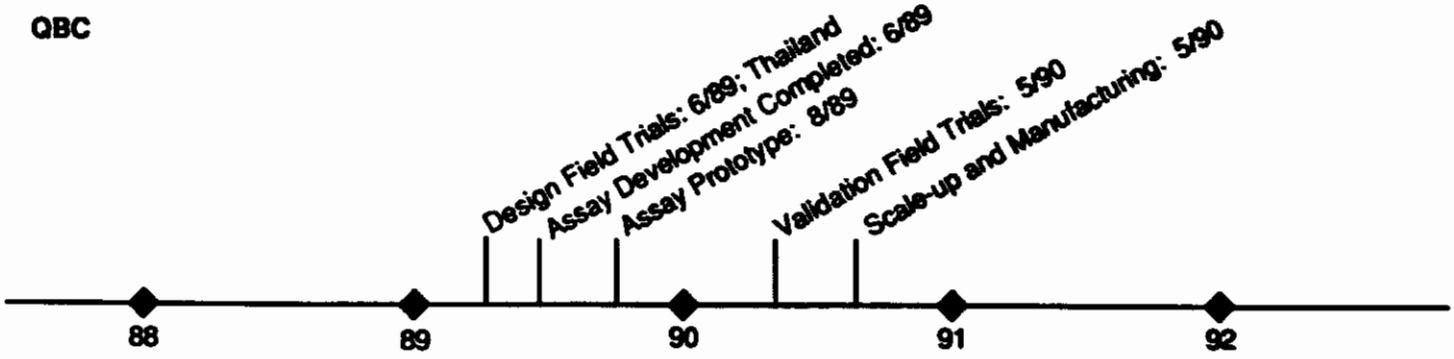
The QSMI team has conducted many activities in the early stages of DiaTech. Their involvement will be even more significant later in DiaTech when technologies are ready to be field tested and finally transferred to the developing world. During that phase of DiaTech, QSMI can serve as a model program for technology transfer as well as a regional center for this activity.

Progress to Date

During the first two years of the subagreement, the QSMI team has served as advisors to the DiaTech Technical Director on many occasions through participation in workshops, review of proposals, identification of needs, resources, and priorities from the developing world perspective. As a biological resource to DiaTech, they are continuing studies on site and in the field, including: (1) the evaluation of the Quantitative Buffy Coat instrument with a new UV microscope objective developed for resource-poor environments by Becton Dickinson, (2) continued testing of a pre-production model Artel hemoglobinometer, (3) evaluation of a biochemistry kit and compact, battery-operated centrifuge for field use, and (4) continued collection of biological samples for the Biological Resources Bank.

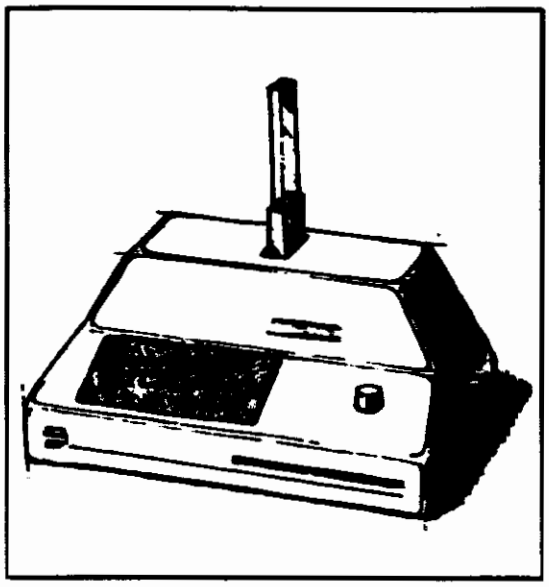
Funding Amount	Year 1: $ 76,690 Year 2: $ 81,040 Year 3: $ 69,640 Total: $227,370
Period	April 1, 1987 - May 31, 1990

Timeline for Development



February 19, 1990
HS01306V

Title	Development of a Direct-Reading Hemoglobinometer
Number	861000156
P.I.	Richard H. Curtis, Ph.D.
Institution	Artel, Inc.
Status	Active



Objectives

To develop a direct-reading, hand-held or portable hemoglobinometer for field use. Undiluted whole blood will be hemolyzed and measured photometrically in a short path length cuvette. The reading will be displayed electronically in grams per deciliter.

Significance

Hemoglobin is one of the most frequently performed laboratory tests in the developing world. Determination of hemoglobin concentration is especially relevant in falciparum malaria-endemic areas, in which severe anemia, especially in children, is a frequent consequence. A WHO meeting on appropriate laboratory technologies attended by the Technical Director concluded that a low-cost, accurate hemoglobinometer was a valuable and needed diagnostic tool for use in primary health care in the developing world.

Progress to Date

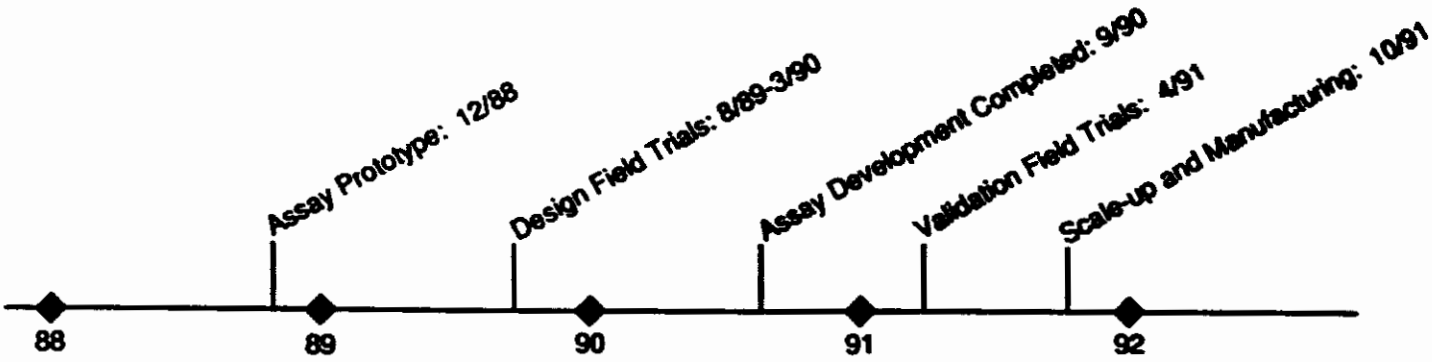
A prototype instrument has been developed and was first tested for clinical efficacy at the Maine Medical Center. Initial results indicated that the accuracy of the device fell within 5% of the standard method of determination, an acceptable margin of error. DiaTech took delivery of two prototype devices in December 1988. These were evaluated in both the DiaTech laboratory and at Johns Hopkins. Both evaluations confirmed that the device provides acceptable accuracy and is easy to use. A field trial was then conducted by the QSMI/Thai Red Cross Society at a refugee camp near the Thai-Cambodian border. The device performed very well until the battery recharger malfunctioned, a short in the

electrical system developed, and the cuvettes became unusable. The device was then returned to Artel for inspection.

Phase II funding was recently approved for development of a preproduction model. Approximately 20 devices are to be manufactured with updated software, circuitry, and a sturdy case construction more appropriate for sustained field use. Distribution of pre-production stage devices to a variety of field sites, including A.I.D. missions and WHO regional offices, is planned. This will allow more complete assessment of the application of the hemoglobinometer, and will allow DiaTech to reach a decision regarding its full production. Concerns remain regarding providing a standard for calibration and producing an injection mold which will produce a supply of accurate, inexpensive cuvettes. Because tolerances for cuvettes must be precise, they are currently hand-made, which is laborious and obviously not cost-effective. The budget for Year II includes $11,477 to finance the production of a precision injection mold, for the cuvette.

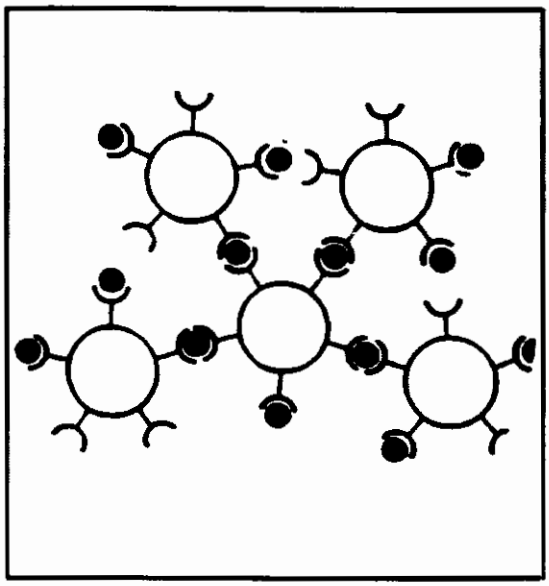
Funding Amount	Year 1:	$ 36,719
	Year 2:	$117,716
	Total:	$154,435
Period	May 1, 1987 - September 26, 1990	

Timeline for Development



February 19, 1990
HS01274V

Title	Provision of Technical Assistance to Management and Laboratory Resources for the DiaTech Program
Number	860900001
P.I.	Noel R. Rose, M.D., Ph.D.
Institution	The Johns Hopkins University School of Hygiene and Public Health
Status	Active



Objectives

The goal of the primary subagreement arranged with The Johns Hopkins University (JHU) is for the JHU team to provide technical advisory assistance and laboratory resources to the DiaTech project. The team of consultants includes experts in all the identified priority disease areas who also have knowledge of diagnostic capabilities and resources available in the developed and developing world.

Significance

The primary subagreement expands the technical capabilities and resources of the DiaTech project.

Progress to Date

In the first three years, the JHU team participated in the DiaTech program in mostly an advisory role, reviewing proposals, monitoring progress of ongoing subagreements, participating in site visits of DiaTech subgrantees, participating in DiaTech workshops, publicizing the DiaTech program, assessing resources and needs in diagnostics technologies, and establishing collaborative relationships with field experts in the developing world. The role played by JHU experts in this arena is becoming increasingly more active as the DiaTech technologies are moved out of the laboratory and into field testing situations. Another area of activity for JHU was the establishment of the Biological Resources Bank and collection, maintenance, and distribution of reagents and specimens. JHU

laboratory resources are available to DiaTech for the stages of the program when technologies need to be tested and evaluated.

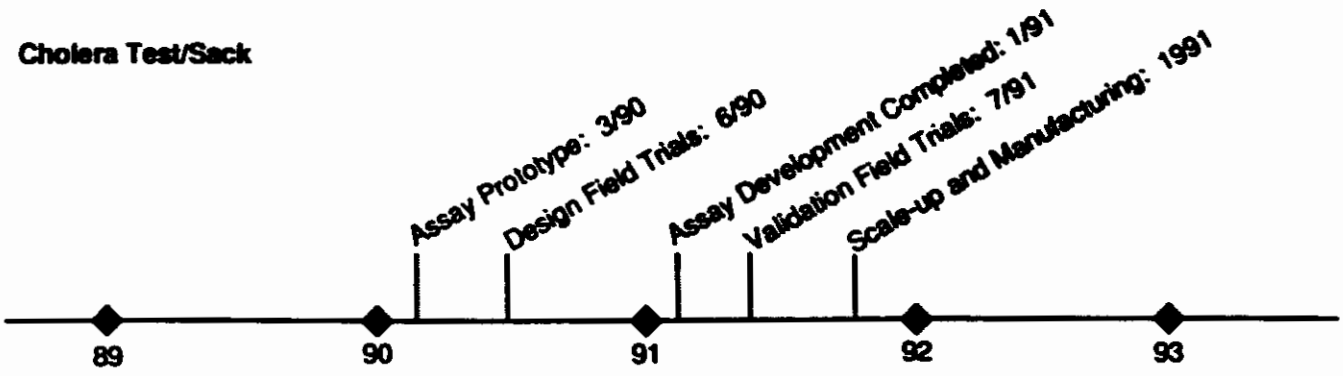
Fourth and fifth year activities in addition to those described above include or included: (1) evaluation of a test for *E. hystolytica* in Egypt, (2) laboratory evaluation of the Artel hemoglobinometer, (3) development of a co-agglutination method for culture-accelerated detection of *Vibrio cholerae* and planning for validation-stage field testing of the method, (4) review of existing commercial tests for ARI and selection of the most robust tests for field testing, (5) assembly of a proficiency panel of mycobacterial and malarial specimens for testing by DiaTech subgrantees, and (6) provision of a standardized, high-quality source of *Staphylococcus aureus* Cowan strain A for development of co-agglutination tests for projects within the DiaTech program.

The DiaTech program has also approved subagreements submitted by collaborators of JHU consultants. For example, a collaborator of Brad Sack, Richard Oberhelman, is assessing the utility of an oligonucleotide probe for detection of *Shigella* in a field test in Peru; Nils Carlin, a collaborator of David Sack, has recently been approved to develop a rapid immunoassay for detection of *Shigella* antigen, which, if successful, he intends to field test at a Native American reservation in Arizona.

Funding Amount	Year 1-3: $232,086 Year 4-5: $149,498 Total: $381,576
Period	October 1, 1986 - September 26, 1990

Timeline for Development

Cholera Test/Sack



February 19, 1990
HS01305V

116

Acute Respiratory Infections/Tuberculosis

Title	Monoclonal Antibodies for the Diagnosis of Parainfluenza Virus Infections
Number	860300047
P.I.	Larry J. Anderson, M.D.
Institution	Centers for Disease Control/Atlanta
Status	Completed

Objectives

To develop and characterize monoclonal antibodies (MAbs) against parainfluenza viruses 1, 2, 3, and 4. To determine their suitability in indirect immunofluorescence assays (IFA), enzyme-linked immunosorbent assays (ELISA), immunoblots, and time resolved fluoroimmunoassays (TRFIA).

Significance

Prompt and specific diagnosis of viral infections is increasingly more important as advances in viral therapy are made. Conventional methods for isolation of many viruses are time-consuming and require skills and facilities that are not routinely available in developing countries.

Progress to Date

To date, Dr. Anderson has completed fusions to yield antibodies to parainfluenza virus types 1, 2, and 3 (P1, P2, and P3). As a result of these fusions, 12 MAbs have been developed against P2, 27 against P3, and 13 against P1. Several of the P2 MAbs appear to be type-specific and broadly reactive by immunofluorescence. These MAbs have been evaluated in a time-resolved fluoroimmunoassay and in a biotin/avidin-amplified ELISA. In these initial tests, they reacted broadly and specifically for the respective paraflu serotypes. The initial set of P1 and P3 MAbs were not type-specific by IFA, and could be combined with the P2 MAbs to produce an IFA test which would recognize P1-P3 without identifying the virus type. These antibody reagents will be useful epidemiologic tools, but the assays being evaluated are too complex for developing world use. The P.I.'s request for additional funding to develop simpler EIA and IFA assays emphasizing RSV and paraflu has been approved (see project number 880200210). EIA tests for influenza have been found to lack in sensitivity.

Funding Amount	Total: $ 30,962 (funded by PASA)
Period	September 1, 1987 - August 31, 1988

Title	Development of Monoclonal Antibodies to Pneumococcus
Number	860700085
P.I.	Michael A. Apicella, M.D.
Institution	The Research Foundation of the State University of New York
Status	Completed

Objectives

To develop monoclonal antibodies to pneumococcal serotypes 1, 2, 4, 5, 6, 14, and 19.

Significance

Acute respiratory disease due to pneumococcus is a major cause of childhood morbidity and mortality in the developing world. Recent studies indicate that *Streptococcus pneumoniae* capsular serotypes 1, 2, 5, 6, and 7 account for 66% of the serotypes causing pneumococcal disease in central Africa. In Goroka, Papua New Guinea, *H. influenzae*, *S. pneumoniae*, or both were isolated from blood or lung aspirates of over half the children with pneumonia. Interest has been heightened by the potential for prevention of pneumococcal and hemophilus disease with vaccination. To appropriately apply a vaccine, methods must be improved to rapidly define the nature and serological type of the pathogen causing respiratory disease and to more rapidly and accurately diagnose hemophilus and pneumococcal respiratory infection in populations in remote areas. This project will develop monoclonal antibody reagents to the capsular antigens of the common pneumococcal serotypes which would be utilized in such an immunoassay.

Progress to Date

Pneumococcal polysaccharide was obtained as a gift from Philip Vella of Merck Research and used to immunize mice. Using standard fusion techniques, the P.I. reports that he has been successful in producing MAb clones specific to types 5, 6, and 18. These represent three of six serotypes the P.I. had originally contracted to make. The P.I.'s request for second year funding has been rejected at the recommendation of the TAG. Since this subagreement was completed, the P.I. sent a quantity of MAb to types 5, 6, and 18 in ascites to PATH/DiaTech. These MAbs have been evaluated in Dr. Richard Facklam's laboratory at the CDC in January 1990. Only type 5 was positive in the agglutination test and the quell test. Type 6 and type 18 were negative on both tests. The P.I. also reports a patented invention (No. 4,859,421), entitled "Disposable Antigen Concentrator and

Detector," which was developed and tested with the aid of DiaTech funds. The device is claimed as useful for antigen concentration from specimen extracts. It is similar to other commercial immunoconcentrator devices, but, in addition, all essential reagents to perform an assay are contained within the device.

Funding Amount Total: $ 57,760

Period May 1, 1988 - April 30, 1989

Title	ELISA for Rapid Diagnosis of Influenza
Number	860700075
P.I.	J. Donald Coonrod, M.D.
Institution	University of Kentucky/University of Rochester
Status	Completed

Objectives

To evaluate and modify, as needed, a recently developed ELISA for diagnosis of influenza by detection of influenza core antigens in respiratory secretions and in culture; and to compare ELISA and culture for diagnostic efficacy using nasopharyngeal secretions.

Significance

Prompt and specific diagnosis of viral infections is increasingly important as advances in viral therapy are made. Conventional methods for isolation of many viruses are time-consuming and require skills and facilities that are not routinely available in developing countries. Monoclonal antibodies to influenza antigens have been used to study influenzal epidemiology and antigenic drift, but their application to immunodiagnosis is just beginning.

Progress to Date

Using a solid phase (1/4 inch plastic bead) ELISA, influenzal core antigens can be detected with acceptable sensitivity when cultured virus is used as specimen. The P.I. has used his own antibodies or those produced in the laboratory of Alan Kendal (CDC). However, sensitivity with clinical specimens or virus-spiked normal nasal secretions has been much poorer. Preliminary studies suggest that this is due to inactivation of antigen (core, matrix, and nucleoprotein) in nasal wash specimens. Heating specimens to between 60°C and 100°C for 5-30 minutes reduces the loss of activity, i.e., improves sensitivity. Experiments with other viruses suggest that this inactivation may be a widespread phenomenon. Brief acidification, followed by neutralization to disrupt immune complexes, appears to improve detectability somewhat but has not improved sensitivity to where an ELISA test would be useful. The P.I. has not succeeded in developing a sensitive ELISA using antibodies to other virus epitopes, i.e., hemagglutinin. He now feels that there is typically insufficient antigen in nasal secretions for an ELISA method to succeed. DFA, although only about 75% sensitive compared to culture and a more subjective method, may be a better culture-independent method.

Funding Amount	Total: $ 24,934
Period	January 1, 1988 - December 31, 1988

Title	Feasibility Study: Advanced Agglutination Assay
Number	860700095
P.I.	Dean S. Milbrath, Ph.D.
Institution	Minnesota Mining and Manufacturing Co. (3M)
Status	Completed

Objectives

To demonstrate feasibility of a novel color-endpoint agglutination assay for detection of the capsular polysaccharide of *Streptococcus pneumoniae*. Sensitivity and specificity will be compared to a commercially available latex agglutination assay using artificially reconstituted specimens.

Significance

Agglutination assays offer many advantages for developing world use, including simplicity, rapidity, and a comparatively long shelf life. However, they are typically less sensitive than enzyme-labeled immunoassays and are subjective in interpretation. The assay proposed by 3M uses a novel, colored, particulate support, suspended in a separation medium; antibody- or antigen-mediated agglutination of the sensitized support results in color separation. The 3M assay is thus easier to read than a conventional agglutination assay. In addition, the reaction is reversible so the entire process can be repeated without the need for additional sample.

Progress to Date

An agglutination assay using 3M's proprietary particulate support was demonstrated, the sensitivity of which is reported to between 1 and 3 ng/mL of pneumococcal CPS in urine. While such a lower limit of detection is comparable to that reported for conventional latex-based assays, the 3M assay takes longer to produce a result. The reported sensitivity limits are unclear upon closer inspection of the progress report. To date, the P.I. has not succeeded in demonstrating the color-separation in this model system. The TAG has reviewed the final report and is pessimistic about the technology and the ability of the P.I. to move it forward. The P.I. desires continued funding of this project. A cost-sharing agreement for overhead has been discussed, but his study is arguably too risky be extended as a formal proposal.

Funding Amount	Total: $ 11,560
Period	June 1, 1987 - January 31, 1988

February 19, 1990
HS01287V

Diarrheal Diseases/Typhoid Fever

Title	Development of Immunoassays for Detection of *Salmonella typhi* and *Salmonella paratyphi* A and B
Number	860700134
P.I.	Steven B. Binion, Ph.D.
Institution	Land O'Lakes, Inc.
Status	Completed

Objectives

To obtain and test individual colostrum samples for activity. To produce colostral whey protein concentrates for further testing and use in immunoassay kit formulation. To run ELISA or coagglutination assays to demonstrate colostral antibodies specific for *S. typhi* Vi antigen and *S. paratyphi*.

Significance

Polyclonal antibodies are well-suited for use in a variety of immunoassay applications, their usefulness being limited primarily by quantity and variability in responses of outbred animals. The use of immunized dairy cows for antibody production is well documented in literature and is attractive due to the large quantities of colostrum that can be obtained containing immunoglobulin concentrations of about 5% by weight.

Progress to Date

Four cows were immunized with Vi antigen-positive *C. freundii* bacteria but failed to produce high titers of antibody. Two of the four cows responded with increasing serum titers of 2-4X over background, but no antibody could be detected in colostrum or milk. The immunizing regimen used has been successful for other antigens. A possible reason for this failure includes high dose tolerance of this particular carbohydrate antigen in bovines. The P.I. wishes to continue his studies using purified Vi antigen to be obtained from Myron Levine; however, the risk of failure seems high, and the cost-effectiveness over conventional methods of immunization seems low.

Funding Amount	Total: $ 21,224
Period	January 1, 1988 - June 30, 1988

February 19, 1990
MS01268V

Title	Development of an Indium Slide Assay for the Rapid Detection of Antigen or Antibody
Number	860700142
P.I.	C. Lynne Burek, Ph.D.
Institution	The Johns Hopkins University
Status	Completed

Objectives

To develop an assay based on the indium slide principle for detection of antibody to *S. typhi* O antigens.

Significance

The indium slide assay is potentially rapid, simple, and visually interpretable. As such, it should be applicable for use in field sites where trained personnel and equipment are often unavailable.

Progress to Date

Indium Slide Immunoassay (ISI) protocol developed:

- Lipopolysaccharide antigen *Salmonella typhosa* 0901 is coated onto slides followed by BSA (1 mg/ml), a masking agent. Stability of antigen is excellent.
- Sera are incubated, diluted 1:10 for one hour, then rinsed.
- Reaction is amplified by adding 50 mg/ml of anti-human IgG for 30 minutes.
- Optical density changes of 0.05 can be detected.

Pre/post-typhoid vaccine sera were used in a feasibility study. Results were analyzed qualitatively (spot intensity) and quantitatively (compared to ELISA). Weak sera were detected. Good correlation was noted between the intensity of the spots on the slide and results of ELISA. The P.I. would like to test her assay in the field. A testing procedure for whole blood has also been developed. Remaining concerns include the uncertain supply of indium-coated plastic to construct dipsticks, their ultimate cost of production since they

are a specialty item, the relatively long assay time, stability of components, and lack of a meaningful assay system developed to date. A renewal of the project has been requested but not initiated.

Funding Amount	Total: $ 25,109
Period	November 1, 1987 - April 30, 1988

Title	Disposable Diagnostic Device for *E. coli* Enterotoxins
Number	870600191
P.I.	Harvey Brandwein, Ph.D.
Institution	Genetic Diagnostics Corporation
Status	Terminated

Objectives

To prepare immunoreactive solid phase materials by covalent attachment of STa-BSA and/or purified LT to one or more membrane materials (nylon, activated paper, immunodyne, etc.). To prepare anti-STa or anti-LT monoclonal antibody-alkaline phosphatase conjugates via heterobifunctional coupling reagents. To titrate the assay components above to generate maximally sensitive competitive ELISA protocols for STa and LT in aqueous solutions. To combine STa and LT solid phases and antibody enzyme conjugates into "11 port device" and single test rotary device being manufactured by the Pall Corporation (Glen Cove, NY). To validate the assays on 200 to 300 clinical samples (culture supernatants). To design device packaging and highly simplified user instructions.

Significance

Although ETEC are probably the major bacterial cause of infectious diarrhea in the world, diagnosis is of interest primarily from an epidemiologic viewpoint. The assay to be developed by GDC is of broad generic interest because it promises to be both simple and sensitive. LT and STa were chosen as models because the investigators have experience with, and reagents, for them.

Progress to Date

The subagreement has been terminated. Monoclonal antibody reagents prepared in the first phase of the study, mouse ascites and enzyme conjugates, and a quantity of purified toxin have been transferred to PATH for future studies. The P.I. had called DiaTech in August 1988 to report that GDC was experiencing cash flow problems, had terminated their research staff, and was no longer in the position to complete the research for which he had contracted. Dr. Herbert Platt, the CEO of GDC, confirmed that cash flow problems existed and that no R&D operations remained. A quarterly progress report was requested and received a few weeks later. This report was found to be garbled and written by someone only vaguely familiar with the project. It indicated that work had been performed but was not of high quality. A site visit to Dr. Platt confirmed that GDC was not in a

position to immediately resume R&D efforts. However, Dr. Platt agreed to give DiaTech access to purified antigen and monoclonal antibodies for continued test development. DiaTech, with A.I.D. approval, contractually terminated the project as of February 1, 1989. At the time of termination, half of the original $92,365 grant had been disbursed.

Funding Amount	Total: $ 46,182
Period	February 1, 1988 - January 31, 1989

Title	Feasibility Study: DNA Probe for *Giardia lamblia*
Number	870800202
P.I.	Thomas D. Edlind, Ph.D.
Institution	Medical College of Pennsylvania
Status	Completed

Objectives

The first objective will be to identify several short nucleotide sequences present in *Giardia* rRNA but absent from the host, normal flora, and unrelated pathogens. Oligonucleotide probes targeted to these sequences have several advantages over longer probes: (1) the desired level of specificity can be easily controlled; (2) probes can be chemically synthesized in large quantities at low cost, with the potential for incorporating a nonisotopic label during synthesis; (3) the size differential between the rRNA target and the probe permits separation by a variety of techniques; and (4) nonspecific binding is minimal. The second objective will be to develop a protocol for *Giardia* rRNA extraction from stool specimens which is rapid and simple, yet does not sacrifice sensitivity or specificity. Initial studies have employed a standard multi-step phenol extraction/ethanol precipitation. New procedures have been introduced which permit one-step solubilization and immobilization of sample (Bresser et al., 1983) or solubilization and hybridization in the same solution (Kohne, 1986). However, application of these procedures to stool specimens has not yet been described.

Significance

G. lamblia is prevalent worldwide, wherever fecal contamination of drinking water occurs, and has been associated with chronic, prolonged diarrhea and malabsorption in children. Current practice relies on microscopy for detecting cysts in stool specimens and is known to be relatively insensitive without multiple stool exams. A diagnostic assay will be useful primarily for epidemiological purposes. Apart from the relative value of improving diagnosis of *G. lamblia* infection, the technology embodied in this proposal is of interest in its own right. Although hybridization assays are typically thought of as involving the detection of DNA targets by DNA probes, either the target or the probe can be replaced by RNA. The present proposal utilizes rRNA, which is present in multiple copies per cell, as the target, thereby leading to enhanced sensitivity.

Progress to Date

About 1,200 nucleotides from the 3' end, and an additional 800 nucleotides from the 5' end of the 23S subunit of rRNA have been sequenced and the sequence compared to that of six other groups of organisms. Based on this analysis, three candidate targets were identified and probes for these sequences synthesized. More probes will be identified with complete sequence analysis. Prior to completion of sequence analysis and probe design, the P.I. has been performing preliminary hybridizations with a 1.6 kbp restriction fragment which is specific for *Giardia* RNA. However, when this probe was tested with spiked stool samples, high backgrounds due to the high level of normal stool RNA were found. A panel of stool specimens from infected and normal patients was received from a collaborator and were blotted against the 1.6 kbp probe. A range of positives was obtained, as well as a few negative results. The code for the samples has not been broken. RNA probes for stools remain a considerable challenge due to the large amount of RNA and RNAse normally found. The P.I. did not request follow-on funding. In addition, the TAG considers *Giardia* diagnostics to be of lower priority.

Funding Amount Total: $ 15,000

Period February 1, 1988 - July 31, 1988

Title	Preparation of Monoclonal Antibodies for Detection of EPEC
Number	860700137
P.I.	Myron M. Levine, M.D.
Institution	University of Maryland/Center for Vaccine Development
Status	Completed

Objectives

To purify the 94 kDa outer membrane protein encoded by the EAF plasmid found in most EPEC strains; to prepare monoclonal antibodies against the purified protein; and to develop assays incorporating these reagents which will be useful for detecting EPEC in clinical specimens.

Significance

Enteropathogenic *E. coli* (EPEC) have been shown conclusively to play an etiologic role in human diarrhea. Numerous studies have shown them to be among the leading causes of pediatric, bacterial diarrhea throughout the developing world, where they have a propensity to cause persistent disease. EPEC are generally identified by O-serogrouping, which is a resource-intensive procedure. A more reliable, but still resource-intensive, method of identifying EPEC is by the phenomenon of localized adherence. This property is mediated by a 60 MDa plasmid, the EPEC adherence factor or EAF plasmid, which encodes a 94 kDa outer membrane protein (OMP). Because this protein is expressed at the bacterial cell surface, is antigenic, and is either identical or shares determinants across a variety of EPEC strains, identification of EPEC by immunodetection of this protein is a realizable goal which should greatly simplify diagnosis of EPEC infections. Such an assay can be expected to be useful for epidemiologic purposes and also in patient care, since antibiotic therapy has been shown to be effective when administered early in the course of disease.

Progress to Date

The 94 kDa OMP was purified by excision of stained bands from SDS-PAGE gels. Because other, immunologically distinct bands co-migrate with the 94 kDa OMP, other methods of purification have been used, including the use of longer gels, isoelectric focusing, and 2D-gel electrophoresis. A convalescent serum from a patient with diarrheal disease is the only reagent available for assessing progress in purification techniques. None of these methods has proven entirely satisfactory to date, but sufficient material for

immunization of mice has been obtained despite limitations. Sera from these mice are specifically reactive with the protein of interest and fusions were in progress. Rabbit immunizations have also been undertaken using whole bacteria, but none of the resultant antisera has recognized the 94 kDa OMP. The final report for this project has been received. Second year funding was applied for; however, due to slow progress and a strong research orientation (as opposed to development), the project was given a low priority for renewal.

Funding Amount Total: $ 57,104

Period May 1, 1987 - April 30, 1988

Title	Colony-ELISA for Detection of Bacteria Producing Shiga or Shiga-like Toxins
Number	860500063
P.I.	Alison D. O'Brien, Ph.D.
Institution	Henry M. Jackson Foundation for the Advancement of Military Medicine, Uniformed Services University of the Health Sciences
Status	Completed

Objectives

To develop a colony-blot ELISA for SLT-II using monoclonal antibodies, and to test the feasibility of direct detection of toxins in stool.

Significance

E. coli producing elevated levels of Shiga-like toxin (SLT) I or II have been implicated as the cause of pediatric diarrhea, hemorrhagic colitis in adults and children, and hemolytic uremic syndrome in children. Currently, there is no assay which will permit detection in stool cultures of SLT-producing *E. coli* (EHEC). Such an assay will be useful as an epidemiologic tool for evaluating the importance of EHEC as agents of disease in the developing world.

Progress to Date

During Year I, Shiga-like toxin II (SLT-II) was obtained from toxin-converting phage plaque preparations and purified by ion exchange chromatography and chromatofocusing. The purified material was used to immunize mice at sublethal doses. After toxoiding, the toxoided material proved to be a better immunogen. A total of 10 hybrid cell lines, reactive with SLT-II by ELISA, were derived from two fusions, one each with glutaraldehyde-treated toxin and with formaldehyde-treated toxin. Antibody from five of the lines was neutralizing and all five recognized epitopes associated with the A subunit, as assessed by radioimmune precipitation (RIP). By colony-ELISA, two of the monoclonals (11E10 and 2E1) recognize SLT-II-producing *E. coli*. During Year II, additional fusions have been successful in generating monoclonal antibodies capable of recognizing the SLT-IIv, a variant toxin found in approximately 30% of SLT-II-producing strains, and currently missed by the antibodies in hand. A "cocktail" of monoclonals will then be formulated which will recognize all SLT-II-producing *E. coli*. In addition, use of a monoclonal antibody from

Nancy Strockbine of the CDC which reacts with and neutralizes both SLT-II and SLT-IIv will be tested in the colony ELISA developed. The prospects for direct detection of toxin in stool by use of simplified assays have yet to be assessed. Unfortunately, data from the field suggest that shiga-toxins do not seem to be a developing-world disease, so follow-on activities for diagnostic kit development seem uncertain at this time.

Funding Amount	Year 1: $ 55,621 Year 2: $ 52,205 Total: $107,826
Period	January 15, 1987 - March 31, 1989

Title	Detection of Enteropathogenic *E. coli* (EPEC) with the EPEC-Specific Carbohydrate Receptor
Number	861000158
P.I.	Gary K. Schoolnik, M.D.
Institution	Stanford University School of Medicine
Status	Completed

Objectives

To develop a simplified assay for EPEC in stool using the observation made in the P.I.'s laboratory that a beta-linked oligosaccharide from the urine of patients with GMI ganglio-sidosis binds all tested EPEC strains. Synthetic oligosaccharides will be derivatized and covalently bound to activated latex beads and to cellulose paper strips annealed to plastic dipsticks. The specificity of the receptor-bound matrices for EPEC and EAEC strains will be determined with diverse ETEC, EHEC, and EIEC strains and with non-pathogenic *Escherichia coli* commensals isolated from the stools of healthy volunteers. Stool inoculation studies will be performed in which serially diluted inocula of selected EPEC and EAEC strains will be introduced into liquified stools from normal volunteers. Each of the test formats that perform well in the stool inoculum studies will be assessed with stools submitted by patients presenting with diarrhea to the oral rehydration treatment facilities in the District II Health Center in Chiapas, Mexico.

Significance

Enteropathogenic *E. coli* (EPEC) have been shown conclusively to play an etiologic role in human diarrhea. Numerous studies have shown them to be among the leading causes of pediatric, bacterial diarrhea throughout the developing world where they have a propensity to cause persistent disease. EPEC are generally identified by O-serogrouping, which is a resource-intensive procedure. A more reliable, but still resource-intensive, method of identifying EPEC is by the phenomenon of localized adherence. Assays for EPEC can be expected to be useful primarily for epidemiologic purposes, but also in patient care, since antibiotic therapy has been shown to be effective when administered early in the course of disease.

Progress to Date

The P.I. has developed and tested the DiaTech-sponsored EPEC assay in Mexico in an area where the degree of infant mortality due to enteric disease was very high (approximately 140 deaths per 1,000 live births). Unanticipated problems appeared during the field trial phase of the study. First, other EPEC serotypes were identified in addition to the O111 which reacted with the test reagent. Unlike the O111 serotype, no inhibition of binding to HeLa cells was observed with the 0111 oligosaccharide, indicating that these serotypes possessed a different receptor-adhesion system. This obviously rendered the test reagent useless. Second, the number of EPEC strains isolated from children at the Mexico field site was unexpectedly low; instead a different "diffuse adhering" *E. coli* was the most common pathogen. Therefore, the P.I. indicated that the DiaTech-funded assay appeared to be only second best when compared to a DNA probe assay. No follow-on funding for further test optimization or testing was requested.

Funding Amount Total: $ 53,310

Period August 1, 1987 - July 31, 1988

Title	Development of a Field-Suitable Immunoassay for the Detection of *Salmonella* Vi Antigen in Urine
Number	860700104
P.I.	Suraj B. Singh, Ph.D.
Institution	Dynamac Corporation
Status	Terminated

Objectives

To develop reagents and technology for the purification of Vi antigen of *Salmonella typhi* and specific antibody; and to standardize ELISA for Vi antigen detection and determine optimum conditions for the test.

Significance

Typhoid fever caused by *Salmonella typhi* remains a major clinical and public health problem in developing countries. It may occur throughout the year in certain regions, without a well-defined clinical syndrome, and is difficult to differentiate from other diseases. Typhoid fever has a high morbidity, and complications are frequently encountered in untreated patients. The asymptomatic carrier state is common and remains a key link in the epidemiology of this malady. Vi antigen of *S. typhi* is correlated with virulence and immunogenicity of the organism and has been recognized as an important diagnostic marker in clinical specimens. It has been shown that Vi antigen is consistently present in urine of typhoid patients and carriers.

Progress to Date

Vi antigen was purified from acetone-killed *C. freundii* by enzymatic digestion, phase separation, alcohol concentration, and cetavalon precipitation according to published methods. Yield from the initial preparations was very low (0.5mg/gm cells), but use of a more mucoid strain has resulted in higher yields. Purified antigen gave a single immunoprecipitin band in Ouchterlony double diffusion assays. This antigen was found to be pyrogenic when used to immunize rabbits, presumably because of LPS contaminants. Additional rabbits were thus immunized with whole cell preparations of *C. freundii*. Antisera containing considerable titers of anti-Vi were obtained which could be used in EIA. These antisera are contaminated with anti-LPS, which should not be a concern since this LPS differs serologically from *Salmonella*. Rheumatoid factor-like activity observed in rabbit

antisera was removed by heat and high speed centrifugation. These rabbit antisera await evaluation. Since there has been a turnover of personnel at Dynamac and a low level of interest in continuation of the project within the company, they have been notified that DiaTech does not intend to pursue development of this project.

Funding Amount	Total: $ 26,970
Period	November 1, 1987 - September 30, 1988

Malaria

Title	Development and Evaluation of DNA Probe Technology for the Diagnosis of Malaria in Volunteers
Number	860300045
P.I.	James B. Kaper, Ph.D.
Institution	University of Maryland/Center for Vaccine Development
Status	Completed

Objectives

To evaluate three different radiolabeled DNA probes for *P. falciparum* and the optimum conditions for their use using reconstituted and spiked samples. All five probes will then be evaluated using blood samples obtained from experimentally infected volunteers. All stages of infection, from pre-patient through treatment, will be examined and compared to ELISA and microscopy (thick film).

Significance

Falciparum malaria is a major cause of morbidity and mortality in the developing world. DNA probes represent an important new technology in the diagnosis of infectious diseases and are particularly attractive whenever a large number of samples must be screened. Previous reports have shown that DNA probes can detect parasitemias as low as 0.0001% in a 10-microliter blood sample. This project will systematically compare cloned and synthetic probes at various stages of infection and disease with each other and with ELISA and microscopy.

Progress to Date

Three cloned probes to a twenty-one base pair repeated sequence, found in all strains of *P. falciparum* examined to date, were evaluated in a dot hybridization assay on nitrocellulose. The probes were radiolabeled by nick translation or random priming. Sample preparation ranged from simple lysis, either in a proteinase K/SDS/EDTA buffer or in water, followed by extraction in $CsCl_2$/TFAA, to a more complex phenol extraction. All three probes were capable of detecting a 1% parasitemia, but none were capable of detecting parasitemia in the challenge recipients, in whom parasitemia was as low as 0.002%. Specificity was acceptable with all but one of the three probes. The time required to complete a typical assay was approximately 48 hours, although actual hands-on time per sample was

only about 1 hour. Additional funding for a second year of work has not been sought by the investigator.

Funding Amount	Total: $ 67,856
Period	February 1, 1987 - July 31, 1988

Title	Development and Evaluation of an ELISA for the Detection of *P. falciparum* Infection in *in vitro* Erythrocyte Cultures and Clinical Malaria Infection in Volunteers
Number	860700133
P.I.	Genevieve A. Losonsky, M.D.
Institution	University of Maryland
Status	Completed

Objectives

To develop an ELISA for the detection of ring-stage antigens of *P. falciparum* using monoclonal and polyclonal antibodies provided by Dr. Robert Reese (Agouron Institute, San Diego, CA). Preliminary data suggest that antibodies to the RESA antigen may be the best choice. Initial experiments will utilize material from culture; subsequent experiments will utilize real patient samples from experimentally infected volunteers.

Significance

More than 1.5 billion people live in malarious areas of the world. Worldwide, an estimated 250-300 million cases of malaria occur each year. In tropical Africa alone, an estimated 160-200 million people are infected annually and more than 1 million people die, predominantly infants and small children. Microscopic diagnosis requires technical skill and is tedious and time-consuming, especially when large numbers of samples must be examined. An improved method of diagnosis will also facilitate vaccine trials.

Progress to Date

Six monoclonal antibodies obtained from Dr. Reese and two polyclonal antisera directed at various antigens, some of which are associated with the membrane of the infected red cell and some of which with the merozoite surface, have been evaluated as the capture antibody in an indirect ELISA for the detection of ring-stage parasites. As antigen, SDS-lysed red cells or freeze-thawed red cells have been used. Capture of parasite antigens was visualized by the addition of *Aotus* hyperimmune serum, followed by addition of alkaline phosphatase-conjugated anti-*Aotus* Ig. Of the eight antibodies evaluated, the best recognized the RESA (p155) antigen and was capable of detecting parasitemia as low as 0.00015% in a three-hour assay. The anti-RESA antibody also detects solubilized antigens that may be released from the red cell, suggesting that serum or whole blood may be the

best specimen for analysis. However, this assay failed to produce results when clinical samples were used. A second monoclonal directed to the merozoite surface antigen (gp185/p83) gave variable results depending upon the passage tested. This latter antibody, and another to the p300 Ag, appear to pick up antigens or antigen fragments in the urine of experimentally infected humans by ELISA, but Western blot analysis was inconclusive.

The P.I. has submitted a second year work plan and budget; however, due to inconsistent Year I results and the uncertain supply of monoclonal antibodies from Dr. Reese, this project has been given a low priority for further funding.

Funding Amount	Total: $ 54,258
Period	April 1, 1987 - March 31, 1988

Other

Title	Development of Dilution Sensors for ORS Solutions
Number	861000155
P.I.	Gilbert Pacey, Ph.D.
Institution	Miami University (Ohio)
Status	Terminated

Objectives

To develop a simple test to indicate when the concentration of sodium (and, by inference, glucose and potassium) in rehydrated ORS exceeds safe limits.

Significance

Diarrheal illnesses are a major health problem in the developing world. Treatment is usually supportive in nature, i.e., fluids are given orally or intravenously to prevent or reverse dehydration. Sachets of oral rehydration salts are now available in the developing world and have been shown to be an effective means of fluid replacement when properly used. A simple test is needed which can be used by village health care workers to assess whether or not sodium levels are within safe limits following reconstitution of the sachet. While this test is not intended to quality control manufacture of ORS, it will in effect do so because the sensor measures concentration.

Progress to Date

Certain macrocyclic compounds are able to selectively complex alkali metal ions. If measurable functionalities are introduced into these molecules, they can be used to signal the presence and amount of a specific ion, for example, by changing color. The P.I. has pioneered the synthesis of such compounds, known as chromogenic crown ethers, and has achieved detection in the ppb range for Na, K, and Li ions. The major technical hurdle in this project has been to achieve a loading of the indicator which achieves a visually discernable color change within the desired concentration range. Since the concentration we wish to detect is relatively high, very low loadings of the indicator are necessary, making the resultant color change less intense than desired.

DiaTech has received filter paper impregnated with sensor from the P.I. for evaluation. First indications are that the color change is visible, but subtle over the critical 20-30 mM range, and must include triethanolamine-HCl buffer for color intensification. Further studies,

including development of a dipstick assay, will have to be performed by DiaTech laboratory personnel. With further development, solutions to the technical development problems appear to be within reach, but still more critical at this stage is better product definition. This project was terminated in late 1989 by mutual agreement.

Funding Amount	Year 1: $ 52,390 Year 2: $ 18,360 Total: $ 70,750
Period	March 1, 1987 - February 28, 1989

Title	Rapid Diagnostic Tests for the Detection of Visceral Leishmaniasis
Number	870300169
P.I.	Richard D. Pearson, M.D.
Institution	University of Virginia School of Medicine
Status	Completed

Objectives

To develop and evaluate latex agglutination tests to identify circulating leishmanial antigens that are present in sera of patients with clinically apparent visceral disease. The sensitivities and specificities of the latex agglutination tests will then be determined for donors who have asymptomatic, oligosymptomatic, or progressive leishmaniasis to identify the test best suited for field use.

Significance

Visceral leishmaniasis caused by the protozoal pathogen *Leishmania donovani* remains an important public health problem in multiple rural areas of South America, Africa, and India. In general, the disease is similar throughout the world, but the age groups affected vary from region to region. In South America, children are most frequently involved. Diagnosis is confirmed by identifying amastigotes in tissue or isolating promastigotes in culture. Death occurs in the great majority of victims if they are not treated. Even with appropriate therapy, the diagnosis is often made late in the course of infection, and the death rate approaches 10%. A diagnostic method is needed to identify individuals who are infected and likely to develop full blown disease. Since the majority of victims live in rural areas where sophisticated laboratory methodologies cannot be applied, it is imperative that diagnostic tests be easy to perform, require little or no equipment, and be sensitive as well as specific.

Progress to Date

Immune complexes from patients with confirmed visceral leishmaniasis were precipitated from serum and used to immunize mice. From the first fusion, several MAbs were cloned which appeared to have appropriate specificity to leishmanial antigens. MAb 7C10, which appeared to show the strongest preferential binding to immune complexes in sera, but unfortunately when optimized in an immunoassay format, was found to be directed against a human immunoglobulin determinant rather than a parasite antigen. A subsequent fusion

produced two MAbs with apparent specificity for leishmanial antigen. However, at this time A.I.D. decided against renewal of all parasitology projects other than for malaria, and the project was discontinued. The P.I. has since worked on the immunoassay when possible, although no other funding was identified for continuation of his effort, and now thinks he has a specific, practical assay.

Funding Amount Total: $ 37,250

Period May 1, 1988 - May 31, 1989

Title	Development of *O. volvulus* Diagnostics for Third World Use
Number	860300051
P.I.	Alfred M. Prince, M.D.
Institution	New York Blood Center
Status	Completed

Objectives

To generate monoclonal antibodies against species- and stage-specific antigens of the parasite *O. volvulus* using microfilariae and L_3 and L_4 stage larvae. Once appropriate antibodies are generated, antigen detection assays employing them will be developed.

Significance

Onchocerciasis is found throughout Central and Western Africa, as well as in Central America and the northern latitudes of South America. The disease is estimated to be more prevalent today than one hundred years ago, despite intensive control efforts. Recent therapeutic success using ivermectin has made early diagnosis a highly desirable goal. Infection is currently diagnosed by skin biopsy, an invasive and resource-intensive procedure. Serological assays have proven to be of little value due to cross-reactions with other parasite species.

Progress to Date

This project was slow to start due to turnover and recruiting of key personnel. To date, five monoclonal antibodies which react with *Onchocerca* genus antigens and specific to stage L3 were produced by intrasplenic inoculation of mice. However, none of the MAbs reacted to detergent-extracted L3 surface proteins by Western blot or immunoprecipitation, and therefore, the nature of the epitopes remain undefined. Carbohydrate epitopes, however, have been ruled out since immune reactions are destroyed by proteases. Use of various MAbs, polyclonal antisera, and probes have resulted in production of a number of clones. OV-3, a protein from the outer epicuticular layer, was isolated with MAb 229. Eleven clones, eight of which produced fusion proteins, were identified with chimp immune serum and a 70 kD heat shock protein. The clones are in various stages of sequencing

with the aim that species-specific regions can be identified for use in development of serodiagnostic tests. This project was discontinued because of direction from A.I.D. to not renew any parasitology projects other than for malaria.

Funding Amount	Total: $121,071
Period	April 1, 1987 - December 31, 1988

Attachment 1

NeoRx: a prescription for its troubles

by James E. Lalonde
Times business reporter

NeoRx Corp. executives arrived at the company's annual meeting today armed with what they hope will be a powerful prescription for the ailing biotechnology firm.

It was bitter tasting medicine: a mixture of layoffs, research cutbacks and a plan to reduce debt.

But the firm hopes it will sweeten the company's stock price.

It has been a rough year for the six-year-old company. Its stock recently hit the $2 range, less than one-third its price a year ago. The stock was trading at $2.25 this morning.

The Food and Drug Administration has delayed expected approval of NeoRx's first product, a cancer-detection drug. Its losses have continued, once again totaling more than $13 million, or 95 cents per share, for the year ending last Sept. 30, nearly unchanged from the previous year.

Revenues, largely from research contracts, totaled $6.8 million, up 12 percent.

"I think the key issue in people's minds is why has the price of the company's stock fallen so precipitously?" said Dr. Robert Abbott, president and chief executive officer, in an interview before today's meeting.

"We think that people are concerned about the magnitude of our expenditures relative to what the potential is of product sales in the next five years."

NeoRx is developing two types of cancer-related products, which use man-made or monoclonal antibodies linked with radioactive substances to either detect (image) or find and kill cancer cells in the body.

Until last month, however, a third or more of NeoRx's $21 million in gross expenditures were directed at therapeutical products, yet revenues from that type of products could not be expected to hit balance sheets for at least five years.

Abbott now concedes that, in addition to being tangled in a slower and more-complex-than-expected FDA approval process, NeoRx made a strategic error in letting its product-research program go in too many grand, long-term directions – at the expense of short-term products.

The time and expense of bringing a biotechnology product to market "has been underestimated by everybody," Abbott said.

"... We allowed ourselves to expand into areas which were of interest to each of the key contributors of the company," he said. "Since it was all cancer-related, we rationalized that this was still maintaining focus.

"What was happening is that we were defocusing from short-term pay-back investments to long-term pay-back investments. ... So we had to step back and terminate those. And, in fact, that has led to the departure of some real positive contributors to the company.

"So what we decided to do was to give up the long-term projects, cut back on our (cash) burn rate, put all of our resources behind the imaging products," said Abbott, "and that gives us a longevity factor lasting into 1992," when the company hopes to become profitable.

Abbott said NeoRx will continue to work on longer-term cancer therapeutic drugs, for treating various cancers using rhenium radionuclides attached to monoclonal antibodies, but that that research will be funded largely by partnerships with other drug companies, not shareholder equity.

The company expects FDA approval this summer of its first cancer-imaging product, OncoTrac Melanoma, designed to detect a form of skin cancer. Abbott estimates that even at its peak, that product will probably bring in only about $5 million a year, and then only after three years following approval.

The other product under FDA licensing scrutiny, for detection of small cell lung cancer, is expected to be approved for sale in the summer of 1991. It holds more financial promise.

Meanwhile, NeoRx announced [illegible] for a new facility at Canyon Park near Bothell.

NeoRx has filed a registration statement with the Securities and Exchange Commission seeking to offer some shareholders a new series of preferred stock. The new stock will be offered at the rate of 40 shares of preferred stock for each $1,000 in principal amount of debentures outstanding.

The exchange is an accounting move which would shift up to $28 million from NeoRx's debt ledger to the shareholder-equity column.

Instead of paying interest on the bonds, NeoRx would pay dividends on the preferred stock, which would be convertible to common stock at $5.50 per share.

Abbott said that the move is being taken to quell concerns that NeoRx might fail to meet financial criteria required to maintain its listing on the National Association of Securities Dealers automated quotation systems.

NeoRx reported a loss of $4.57 million, or 38 cents a share, for its first quarter ended Dec. 31. The loss, including a $1.4 million one-time restructuring charge, compared with a net loss of $3.9 million, or 27 cents a share, a year earlier.

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GEORGE STEINMETZ—ONYX

A pact that provides 'the resources to accomplish the dream I set out to do': *Founder Swanson*

Deep Pockets for Biotech

Genentech's deal points the way for a troubled field

A foreign competitor is closing in on one of America's brightest technologies—a worldwide power respected for its ruthless efficiency, business smarts and wealth. Japan? Nope. Switzerland. Basel-based Roche Holding, the parent of Hoffmann-La Roche, announced this month that it will plunk down $2.1 billion for 60 percent of Genentech, America's biggest biotechnology drug company. The deal gives Genentech money to beef up drug-development efforts and frees it from the whims of Wall Street. Roche gets to take a big step into the biotech field. Both companies stressed that Genentech would retain its autonomy, though Roche can buy the rest of the company within the next five years. The deal "gives us the resources to accomplish the dream that I set out to do," says Genentech founder Robert Swanson.

The Genentech sale is a part of a massive reshaping of the field of biotechnology—the business of producing everything from growth hormones to bacteria that protect plants from frost by inserting genes into living organisms. The past year saw 550 strategic alliances in medical and biotechnology companies. The dollar volume in mergers should also rise this year—perhaps by 25 percent, estimates researcher Julie Eskay of the New York-based Wilkerson Group (chart). This isn't Genentech's first linkup: even before it went public in 1979, it sold a 25 percent stake to Ohio-based chemical company Lubrizol for $10 million. California-based venture capitalist Robert Kunze predicts that further consolidation is inevitable. "Biotech and new developments in life sciences have spawned nearly 300 companies," he says. "It would be surprising to me if 30 of those exist independently by the end of the decade."

Genentech didn't act out of desperation. It was *healthy*, with two strong products and $400 million in revenues last year. But the company realized that it would take even deeper pockets to compete in the '90s. Biotech's costs and risks are astronomical. Robert Fildes, founder of Cetus Corp., estimates that bringing a drug to market can take 10 years and $150 million. "You need access to capital—for the long time frame and the enormous amounts of money involved." Even if companies get their drugs past sluggish federal regulatory agencies and hostile environmental groups, they still have to contend with a cutthroat market: the pharmaceutical industry has recently been transformed by a rash of mergers creating such powerhouses as SmithKline Beecham and Bristol-Myers Squibb.

While the business is getting more expensive, funding is harder to come by. The stock market's love affair with biotech has cooled. "There were companies in the early '80s that consisted of not much more than a mouse, a microbiologist and a venture capitalist—and you didn't need the last two to go public," jokes Jim Barrett, president of Genetic Therapy, Inc. in Gaithersburg, Md. "But that kind of stuff came to a screeching halt in '85 and '86." In 1986, initial public offerings brought biotech companies $1.2 billion; last year it was around $300 million. Biotech stocks have fallen into a slump.

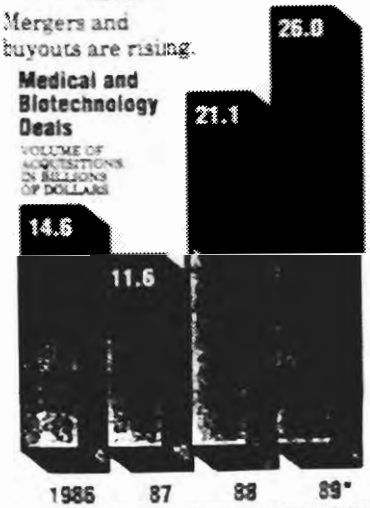
Stamp of approval: Giants like Roche need these deals as much as the biotech firms do. Venture capitalist Kunze notes that while the big players "have become very good at the 'D' part of 'R&D'," most of them "have not been very good at the 'R' side." Buying undervalued biotech companies gives the major pharmaceuticals a chance to enter a market most investors shunned in the early days as too speculative.

Because the Genentech deal represents a stamp of approval from big business, many biotech executives applaud it. "It speaks better than anything else of the commercial value of biotech today and its future," says Fildes of Cetus. "I think it's a win-win-win type of situation for the independent companies as well as the industry as a whole." Some biotech experts say that there will always be room for companies that think small, so long as they judiciously license the technology they develop. "It's a question of how much you want to grow," says James Cavanaugh, president of Health Care Investment Corp., a venture capital fund. But from publishing to semiconductors, more and more American companies are concluding that making it in the '90s means being big enough to play a global game. Says Barrett, "If there are higher returns in the States, [foreign businesses will] invest in the States—whether it's biotech or Rockefeller Center." The only risk is that as the trend accelerates, the biggest winners won't be American—and that the nation could lose its dominance in another technology it pioneered.

JOHN SCHWARTZ with DANIEL GLICK in Washington

Is Bigger Better?

Mergers and buyouts are rising.



*ESTIMATE. SOURCES: WINDHOVER INFORMATION INC.; THE WILKERSON GROUP

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Attachment 2

Attachment 2

TECHNICAL ADVISORY GROUP MEMBERS

Name	Dates of Service
Thomas M. Buchanan, M.D. Chief, Immunology Research Laboratory Pacific Medical Center Seattle, Washington	1986 - present
David F. Clyde, M.D., Ph.D. Senior Associate School of Hygiene and Public Health The Johns Hopkins University Baltimore, Maryland	1986
Richard R. Facklam, Ph.D. Chief, Laboratory Section Respiratory Diseases Branch Centers for Disease Control Atlanta, Georgia	1988-present
Stanley Falkow, Ph.D. Chairman, Department of Medical Microbiology Stanford University Stanford, California	1986
William Greenough, M.D. Professor of Medicine Division of Geriatric Medicine The Johns Hopkins University Baltimore, Maryland	1986-1987
Pascal Imperato, M.D. Professor and Chairman, Department of Preventive Medicine and Community Health State University of New York Brooklyn, New York	1986-1989
Gerald T. Keusch, M.D. Professor of Medicine Chief, Division of Geographic Medicine and Infectious Diseases Tufts University School of Medicine Boston, Massachusetts	1986-present
Myron M. Levine, M.D., D.T.P.H. Professor and Director Center for Vaccine Development University of Maryland Baltimore, Maryland	1986-present

Adetokunbo O. Lucas, M.D. Program Chair, Strengthening Human Resources in Developing Countries Carnegie Corporation of New York New York, New York	1987-present
Fabio Luelmo, Ph.D. Global Programme on AIDS World Health Organization Geneva, Switzerland	1986-1988
Michael N. Oxman, M.D. Professor of Medicine and Pathology University of California, San Diego San Diego, California	1986-present
James C. Richards, Ph.D. Director, Business Planning and Development Gene-Trak Systems Framingham, Massachusetts	1989-present
Alister Voller, Ph.D., D.Sc. Head, Applied Immunology Unit The Zoological Society of London London, United Kingdom	1986-present

Ex Officio Members

Supawat Chutivongse, M.D. Director, Science Division Queen Saovabha Memorial Institute The Thai Red Cross Society Bangkok, Thailand	1986-present
Gordon W. Perkin, M.D. President Program for Appropriate Technology in Health Seattle, Washington	1986-present
Noel R. Rose, M.D., Ph.D. Professor and Chairman, Department of Immunology and Infectious Diseases School of Hygiene and Public Health The Johns Hopkins University Baltimore, Maryland	1986-present

Ad Hoc Member

Don de Savigny, Ph.D. Senior Program Officer Health Sciences Division International Development Research Centre Ottawa, Ontario, Canada	1988-present

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Gene-Trak Systems
Framingham, Massachusetts

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Professor and Chairman, Department of Immunology and Infectious Diseases
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Senior Program Officer
Health Sciences Division
International Development Research Centre
Ottawa, Ontario, Canada

Attachment 3

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PROPOSALS RECEIVED BY DIATECH PROJECT

Number	P.I.	Institution	Title	Status
860100001-6			PRELIMINARY SUBMISSIONS	DELETED
860100007	SCHILLER EL	UNIFORMED SERVICES UNIV	SEE 860700108	DELETED
860100008	TRANTALO DJ	DYNATECH R/D COMPANY	DEVELOPMENT OF A DIAGNOSTIC SYSTEM FOR IDENTIFICATION OF MALARIAL ANTIGENS	REJECTED
860100009	TOMPKINS LS	STANFORD UNIVERSITY	SEE 860700096	DELETED
860100010	VANDER JAGT DL	UNIVERSITY OF NEW MEXICO	DEVELOPMENT OF ENZYME-LINKED DIAGNOSTIC TESTS FOR MALARIA	REJECTED
860100011		K.T. BR LABORATORIES	DEVELOPMENT OF A SIMPLE IMMUNODIAGNOSTIC TEST FOR MALARIA SUITABLE FOR PERFORMANCE IN THE PHYSICIAN'S OFFICE	REJECTED
860100012	EDEN-MCCUTCHAN	MELOY LABORATORIES INC	A RECOMBINANT DNA APPROACH TO DETECTION AND SPECIES IDENTIFICATION	REJECTED
860100013	RUBIN H	UNIV OF PENNSYLVANIA	CONTROL OF DNA SYNTHESIS	DELETED
860100014	LIZARDI P	NATIONAL UNIV OF MEXICO	HIGH SENSITIVITY PHYCOFLUOR DETECTION OF PARASITE NUCLEIC ACIDS	REJECTED
860100015	HERRINGTON DA	UNIVERSITY OF MARYLAND	SEE 860300045	DELETED
860100016	LOSONSKY GA	UNIVERSITY OF MARYLAND	SEE 860700133	DELETED
860100017	LIZARDI P	NATIONAL UNIV OF MEXICO	SEE 860100014	DELETED
860100018	STRICKLAND GT	UNIVERSITY OF MARYLAND	ASSESSMENT OF ELISA IN DETECTION OF MALARIAL ANTIGENS	DELETED
860100019	KEUSCH GT	TUFTS UNIVERSITY	MONOCLONAL ANTIBODY ELISA FOR DETECTION OF SHIGA TOXIN	DELETED
860100020	ROBERTS DR	UNIFORMED SERVICES UNIV	SEE 860700107	DELETED
860200021	FLINT J	SCRIPPS CLINIC	DEVELOPMENT OF DIAGNOSTIC ASSAYS FOR HUMAN MALARIA	DELETED
860200022	BURKOT TR	JOHNS HOPKINS UNIVERSITY	SEE 860700144	DELETED
860200023	MAYUNGA EG	UNIFORMED SERVICES UNIV	RAPID DIAGNOSIS OF SCHISTOSOMIASIS	REJECTED
860200024	REED SG	ISSAQUAH HEALTH RESEARCH	DEVELOPMENT OF A SPECIFIC DIAGNOSTIC FOR VISCERAL LEISHMANIASIS	REJECTED
860200025	STRICKLAND GT	UNIVERSITY OF MARYLAND	EVALUATION OF AN INEXPENSIVE MCARTHUR MICROSCOPE FOR RAPID DETECTION OF MALARIA INFECTIONS IN THE FIELD	RESUBMISSION REQUESTED

860200026	BEAL CB	INT HEALTH SERVICES	DUPLICATION	DELETED
860200027	BEAL CB	INT HEALTH SERVICES	SEE 860700111	DELETED
860200028	BEAL CB	INT HEALTH SERVICES	DUPLICATION	DELETED
860200029	BEAL CB	INT HEALTH SERVICES	DUPLICATION	DELETED
860200030	MCMANUS DP	IMPERIAL COLLEGE OF SCIENCE	SEE 86700090	DELETED
860200031	BLACK RE	JOHNS HOPKINS UNIVERSITY	CYSTICERCOSIS: AN OLD DISEASE WITH NEW POSSIBILITIES FOR DIAGNOSIS	DELETED
860200032	STUART K	ISSAQUAH HEALTH RESEARCH	DIAGNOSTIC PROBES FOR GIARDIA	DELETED
860200033	STUART K	ISSAQUAH HEALTH RESEARCH	DIAGNOSTIC PROBES FOR MALARIA	DELETED
860200034	STUART K	ISSAQUAH HEALTH RESEARCH	METHODS FOR UTILIZING DNA PROBES	DELETED
860200035	CANTELLA RA	UNIV CAYETANO HEREDIA	INDIRECT IMMUNOFLUORESCENT TEST FOR MALARIA	DELETED
860300036	UNGAR BLP	NIH	CONVERSION OF CONVENTIONAL ELISA SYSTEMS TO ELISA SYSTEMS LINKED TO A PARTICLE AGGLUTINATION SYSTEM	DELETED
860300037	UNGAR BLP	NIH	FIELD TEST OF AN ELISA FOR DETECTION OF ENTAMOEBA HISTOLYTICA ANTIGENS IN FECAL SPECIMENS	DELETED
860300038	UNGAR BLP	NIH	FIELD APPLICATION OF AN ELISA FOR DETECTION OF SPECIFIC ANTI-CRYPTOSPORIDIUM ANTIBODIES	DELETED
860300039	UNGAR BLP	NIH	DEVELOPMENT OF AN ELISA FOR DETECTION OF CRYPTOSPORIDIUM ANTIGENS IN FECAL SPECIMENS	DELETED
860300040	UNGAR BLP	NIH	FIELD TEST OF AN ELISA FOR DETECTION OF GIARDIA LAMBLIA ANTIGENS IN FECAL SPECIMENS	DELETED
860300041	LONDNER MV	THE HEBREW UNIVERSITY	SEE 860700118	DELETED
860300042	BUREK CL	JOHNS HOPKINS UNIVERSITY	SEE 860700142	DELETED
860300043	ARTHUR RR	JOHNS HOPKINS UNIVERSITY	DEVELOPMENT OF MALARIA ANTIGEN DETECTION SYSTEM UTILIZING AN ELISA AND SPECIFIC PABS OR MABS	REJECTED
860300044	KEUSCH GT	TUFTS UNIVERSITY	DETECTION OF PLASMODIUM FALCIPARUM USING A SYNTHETIC DNA PROBE	DELETED
860300045	KAPER JB	UNIVERSITY OF MARYLAND	DEVELOPMENT AND EVALUATION OF DNA PROBE TECHNOLOGY FOR THE DIAGNOSIS OF MALARIA IN VOLUNTEERS	FUNDED
860300046	KAGAN IG	SERO-IMMUNO DIAGNOSTICS	SEE 860700089	DELETED

860300047	ANDERSON LJ	CENTERS FOR DISEASE CONTROL	MONOCLONAL ANTIBODIES FOR THE DIAGNOSIS OF PARAINFLUENZA VIRUS INFECTIONS	FUNDED
860300048	VOLLER A	ZOOLOGICAL SOC OF LONDON	MALARIA ANTIGEN DETECTION IMMUNOASSAY	DELETED
860300049	VOLLER A	ZOOLOGICAL SOC OF LONDON	DEVELOPMENT OF AN ELISA TO MONITOR QUININE LEVELS IN MALARIOUS PATIENTS	FUNDED
860300050	BUESING MA	UNIFORMED SERVICES UNIV	DEVELOPMENT OF OLIGONUCLEOTIDE PROBES FOR THE RAPID DIAGNOSES OF MALARIA PARASITES	REJECTED
860300051	PRINCE AM	THE NEW YORK BLOOD CENTER	DEVELOPMENT OF O. VOLVULUS DIAGNOSTICS FOR USE IN THE DEVELOPING WORLD	FUNDED
860300052	PAPPAS MG	COVALENT TECHNOLOGY CORP	VISUAL IMMUNOASSAY (VIA) FOR RAPID DETECTION OF HEPATITIS B AND CHOLERA ENTERO-TOXIN: A FEASIBILITY STUDY	FUNDED/TERMINATED
860400053	SACK RB	JOHNS HOPKINS UNIVERSITY	DEVELOPMENT OF ELISA ASSAYS FOR DETECTION OF THE HEAT LABILE (LT) AND HEAT STABLE (ST) ENTEROTOXINS OF ESCHERICHIA COLI	REJECTED
860400054	DRASAR BS	LONDON SCHOOL OF HYGIENE	STUDIES ON IMPROVED ASSAY METHODS FOR THE DETECTION OF THE HEAT LABILE AND HEAT STABLE TOXINS OF ESCHERICHIA COLI FOR USE IN EPIDEMIOLOGICAL STUDIES IN DEVELOPING COUNTRIES	REJECTED
860400055	GERBER MS	AMREF	THE ROLE OF THE LABORATORY IN THE RATIONAL USE OF DRUGS	DELETED
860500056	MELBER B	BATTELLE	SCREENING AND ASSESSMENT MODULE: OPERATIONS RESEARCH COMPONENT FOR DIATECH	REJECTED
860500057	STRYER L	STANFORD MEDICAL SCHOOL	HIGH SENSITIVITY PHYCOFLUOR OF DNA AND SURFACE ANTIGENS	REJECTED
860500058	GERBER MS	AMREF	A LONGITUDINAL STUDY TO INVESTIGATE CHLOROQUINE SUSCEPTIBILITY IN VITRO OF P. FALCIPARUM IN KENYA	DELETED
860500059	GERBER MS	AMREF	A PROSPECTIVE TRIAL TO COMPARE THE PROTECTIVE EFFECTS OF PALUDRINE MOSQUITO NETS AND PLACEBO AGAINST MALARIA	DELETED
860500060	GERBER MS	AMREF	PROPOSAL FOR THE STUDY OF THE EPIDEMIOLOGY OF AIDS IN KENYA: 1986	DELETED
860500061	MARTIN WJ	UNIV OF SOUTHERN CALIFORNIA	NON-RADIOISOTOPIC ULTRASENSITIVE DETECTION OF AMPLIFIED P. VIVAX GENES	FUNDED
860500062	JONES CL	COVALENT TECHNOLOGY CORP	VISUAL DNA ASSAY (VDA) FOR RAPID DIAGNOSIS OF MALARIA IN DEVELOPING NATIONS	REJECTED
860500063	O'BRIEN AD	UNIFORMED SERVICES UNIV	COLONY-ELISA FOR DETECTION OF BACTERIA PRODUCING SHIGA OR SHIGA-LIKE TOXINS	FUNDED
860500064	DOENHOFF MJ	LONDON SCHOOL OF HYGIENE	IDENTIFICATION OF DIAGNOSTIC AND PROPHYLACTIC ANTIGENS FOR SCHISTOSOMA HAEMATOBIUM	DELETED

860500065	LAWRENCE PJ	LITMUS CONCEPTS INC.	A RELIABLE THRESHOLD-LIMIT TEST TO DETERMINE EXCESSIVE NA/K LEVELS IN ORS	REJECTED
860600066	LAWRENCE PJ	LITMUS CONCEPTS INC.	A SELF-CONTAINED R*A*C*E*S TEST TO DETECT BLOOD IN URINE	REJECTED
860700067	ANDERSSON N	CENTRO DE INVESTIGACION	LARGE SCALE USE OF EPHP PLASTIC MICROSCOPES IN TUBERCULOSIS DIAGNOSIS	REJECTED
860700068	SRISART P	CHULALONGKORN UNIVERSITY	THE EARLY DIAGNOSIS OF LEPTOSPIROSIS BY THE DETECTION OF ANTIGEN	REJECTED
860700069	PHANUPHAK P	CHULALONGKORN UNIVERSITY	THE EARLY DIAGNOSIS OF ENTERIC FEVER BY THE DETECTION OF SALMONELLA TYPHOSA ANTIGEN	REJECTED
860700070	PHANUPHAK P	CHULALONGKORN UNIVERSITY	PROSPECTIVE EVALUATION OF THE LEUKOCYTE MIGRATION INHIBITION FACTOR (LIF) TEST IN THE DIAGNOSIS OF ACTIVE TUBERCULOSIS	REJECTED
860700071	RATANAVARARAKSA	CHULALONGKORN UNIVERSITY	AN APPROPRIATE RAPID TECHNIQUE TO DIAGNOSE INFECTION DUE TO STREPTOCOCCUS PNEUMONIAE	REJECTED
860700072	NILSSON LA	UNIVERSITY OF GOTEBORG	DEVELOPMENT OF A SIMPLIFIED SOLID PHASE TECHNIQUE FOR DETERMINATION OF THE NUMBER OF LIVE EGGS EXCRETED DURING THE COURSE OF SCHISTOSOMA MANSONI INFECTION	REJECTED
860700073	GREENWOOD BM	MEDICAL RESEARCH COUNCIL	DIAGNOSIS OF RESPIRATORY INFECTIONS BY DETECTION OF BACTERIAL ANTIGENS IN URINE	REJECTED
860700074	GREENWOOD BM	MEDICAL RESEARCH COUNCIL	A SEARCH FOR MALARIA ANTIGENS IN THE URINE OF CHILDREN WITH ACUTE MALARIA	REJECTED
860700075	COONROD JD	UNIVERSITY OF KENTUCKY	ELISA FOR RAPID DIAGNOSIS OF INFLUENZA	FUNDED
860700076	MA WS	ANIMAL BIOTECH CORPORATION	DIPSTICK IMMUNOASSAY TO DETECT STREPTOCOCCUS PNEUMONIAE IN SPUTUM	REJECTED
860700077	MA WS	ANIMAL BIOTECH CORPORATION	DIPSTICK IMMUNOASSAY TO DETECT SALMONELLA (S. TYPHI AND S. PARATYPHI) IN BLOOD	REJECTED
860700078	TEKLU B	HARBORVIEW MEDICAL CENTER	EVALUATION OF THE MCARTHUR MICROSCOPE IN DIAGNOSING TUBERCULOSIS	REJECTED
860700079	TEKLU B	HARBORVIEW MEDICAL CENTER	EVALUATION OF THICK AND THIN MALARIA SMEARS USING FIELD'S STAIN	REJECTED
860700080	TEKLU B	HARBORVIEW MEDICAL CENTER	EVALUATION OF THE MCARTHUR MICROSCOPE IN DIAGNOSING MALARIA	REJECTED
860700081	HOFFMAN AS	UNIVERSITY OF WASHINGTON	THE PREPARATION OF SPECIAL NON-FOULING SURFACES TO INCREASE THE SENSITIVITY OF IMMUNOASSAYS AND DNA PROBES	REJECTED
860700082	HOFFMAN AS	UNIVERSITY OF WASHINGTON	A NOVEL SEPARATION TECHNIQUE FOR IMMUNOASSAYS OR DNA PROBES	REJECTED

860700083	PETCHCLAI B	MAHIDOL UNIVERSITY	LABORATORY DIAGNOSIS OF TUBERCULOUS MENINGITIS BY ENZYME-LINKED IMMUNOSORBENT ASSAY	REJECTED
860700084	PETCHCLAI B	MAHIDOL UNIVERSITY	ENZYME-LINKED IMMUNOSORBENT ASSAY AMPLIFIED BY BIOTIN AVIDIN AND REVERSED PASSIVE HEMAGGLUTINATION TEST FOR IMMUNODIAGNOSIS OF PNEUMONIA DUE TO HAEMOPHILUS INFLUENZAE TYPE B AND S. PNEUMONIAE	REJECTED
860700085	APICELLA MA	SUNY AT BUFFALO	DEVELOPMENT OF MONOCLONAL ANTIBODIES TO PNEUMOCOCCAL CAPSULAR POLYSACCHARIDES 1 6 5 2 AND 7	FUNDED
860700086	SINGH AK	RUSH MEDICAL COLLEGE	RAPID ELISA FOR TYPHOID VI ANTIGEN USING A NOVEL ENZYME WITH A SHARP COLOR READOUT	REJECTED
860700087	KOLK AHJ	ROYAL TROPICAL INSTITUTE	DEVELOPMENT OF A FIELD ASSAY FOR THE DETECTION OF MYCOBACTERIUM TUBERCULOSIS IN SPUTUM	FUNDED
860700088	MAIZELS RM	UNIVERSITY OF LONDON	IMMUNODIAGNOSIS OF HUMAN FILARIASIS	REJECTED
860700089	KAGAN IG	SERO-IMMUNO DIAGNOSTICS	SEROLOGIC DETECTION OF GIARDIA LAMBLIA ANTIGEN IN STOOL SPECIMENS (FEASIBILITY STUDY)	FUNDED
860700090	MCMANUS DP	IMPERIAL COLLEGE OF SCIENCE	DEVELOPMENT AND FIELD TESTING OF DIAGNOSTIC REAGENTS FOR HUMAN HYDATIDOSIS	REJECTED
860700091	STEINHOFF MC	UNIVERSITY OF WASHINGTON	RAPID DIAGNOSIS OF STREPTOCOCCAL DISEASE: A FIELD EVALUATION IN INDIA	DELETED
860700092	HARTE RA	MICROBIOLOGICAL ASSOCIATES	A FIELD INSTRUMENT AND REAGENT SYSTEM FOR RAPID QUANTIFICATION OF ANTIBODIES	REJECTED
860700093	KHARE GP	IMMU-CORE INC.	PRODUCTION OF A QUALITATIVE ENZYME LINKED IMMUNOSORBENT SYSTEM (ELISA) FOR DETECTION OF GROUP A STREPTOCOCCUS AND PERFORMANCE OF CLINICAL TESTING IN DEVELOPED AND DEVELOPING COUNTRIES	REJECTED
860700094	WIEDERMANN BL	CHILDREN'S HOSPITAL	A FIELD-SUITABLE ASSAY FOR DETECTION OF HAEMOPHILUS INFLUENZAE TYPE B IN BODY FLUIDS	REJECTED
860700095*	MILBRATH D	3M	PREPARATION AND CHARACTERIZATION OF POLYCLONAL BOVINE ANTIBODIES SPECIFIC FOR S. PNEUMONIAE AND H. INFLUENZA TYPE B	FUNDED
860700096	TOMPKINS LS	STANFORD UNIVERSITY	DEVELOPMENT OF NUCLEIC ACID PROBES TO DETECT CAMPYLOBACTER IN FECAL SAMPLES	REJECTED
860700097	VELTRI RW	AMERICAN BIOTECHNOLOGY CO	DEVELOPMENT OF A FIELD SUITABLE ASSAY FOR THE DETECTION OF HEMOPHILUS INFLUENZA AND STREPTOCOCCUS PNEUMONIAE ANTIGENS	REJECTED
860700098	MAXIM PE	AMERICAN BIOTECHNOLOGY CO	PREPARATION OF MONOCLONAL ANTIBODIES TO THE SALMONELLA VI ANTIGEN	REJECTED

860700099	COONROD JD	VETERANS ADMINISTRATION	EFFICACY OF LATEX AGGLUTINATION AND CO-AGGLUTINATION FOR DETECTION OF TYPE-SPECIFIC PNEUMOCOCCAL POLYSACCHARIDES IN SPUTUM	REJECTED
860700100	KLEBBA PE	KLM TECHNOLOGIES	SOLID-STATE BIOPROBE FOR DETECTION OF DISEASE-CAUSING MICROORGANISMS	REJECTED
860700101	SHAH HP	IGEN INC.	RAPID FIELD DETECTION OF ROTAVIRUS	REJECTED
860700102	CLEVELAND PH	UNIVERSITY OF CALIFORNIA	A TWENTY-FIVE MINUTE FIELD DIAGNOSTIC TEST FOR MICROORGANISMS	REJECTED
860700103	ZAHORCHAK R	THE UNIVERSITY OF ALABAMA	DEVELOPMENT OF RAPID DIAGNOSTIC TESTS FOR FIELD APPLICATION FOR THE DETECTION OF SELECTED PROTOZOA	REJECTED
860700104	SINGH SB	DYNAMAC CORPORATION	DEVELOPMENT OF A FIELD-SUITABLE IMMUNOASSAY FOR THE DETECTION OF SALMONELLA VI ANTIGEN IN URINE	FUNDED/TERMINATED
860700105	BRANDWEIN HJ	GENETIC DIAGNOSTICS CORP	FIELD-READY IMMUNOASSAY SYSTEM FOR DIARRHEAL PATHOGENS	REJECTED
860700106	EVANS JA	UNIV SOUTHERN MISSISSIPPI	FEASIBILITY STUDY OF A SIMPLE SENSITIVE DEVICE FOR IDENTIFYING MICROORGANISMS	REJECTED
860700107	ROBERTS DR	UNIFORMED SERVICES UNIV	A DIPSTICK ASSAY FOR DETECTION OF MALARIA SPOROZOITES IN MOSQUITOES	REJECTED
860700108	SCHILLER EL	UNIFORMED SERVICES UNIV	EARLY DETECTION OF ONCHOCERCIASIS BY IMMUNODIAGNOSIS USING PURIFIED ANTIGENS	REJECTED
860700109	BEAL CB	INT HEALTH SERVICES	MALARIA DIAGNOSIS IN A FLEXIBLE CAPILLARY CHANNEL SYSTEM	REJECTED
860700110	EAPEN J	INT HEALTH SERVICES	A RAPID DIAGNOSIS OF TYPHOID FEVER BY ANTIGEN DETECTION	REJECTED
860700111	BEAL CB	INT HEALTH SERVICES	DETECTION OF PARASITIC AGENTS OF DIARRHEA BY THE PLASTIC ENVELOPE METHOD (PEM)	REJECTED
860700112	EAPEN J	INT HEALTH SERVICES	A SCREENING TEST FOR CHOLERA	REJECTED
860700113	BEAL CB	INT HEALTH SERVICES	A SPUTUM SAMPLING METHOD FOR TUBERCULOSIS DIAGNOSIS	FUNDED
860700114	WEBBER RJ	RES AND DIAG ANTIBODIES	DEVELOPMENT OF FIELD SUITABLE ELISA KITS FOR S. TYPHI AND S. PARATYPHI	REJECTED
860700115	VAN EYS GJ	ROYAL TROPICAL INSTITUTE	DETECTION AND CLASSIFICATION OF LEISHMANIA PARASITES BY DNA HYBRIDIZATION	REJECTED
860700116	REICHELDERFER	ADVANCED LABORATORY TECH	APPLICATION OF A SIMPLE ECONOMICAL SLIDE AGGLUTINATION TEST FOR THE DETECTION OF ANTIBODY TO HTLV-III IN LATIN AMERICA	REJECTED
860700117	YEAGER H	GEORGETOWN UNIVERSITY	DETECTION OF MYCOBACTERIAL ANTIGENS IN SPUTUM	REJECTED
860700118	LONDNER MV	THE HEBREW UNIVERSITY	DIAGNOSIS OF PLASMODIUM FALCIPARUM ANTIGENS AND ANTIBODIES BY DOT ELISA	REJECTED

860700119	TACKET CO	UNIVERSITY OF MARYLAND	EVALUATION OF ANTI-FIMBRIAL MONOCLONAL ANTIBODY AND A DNA PROBE FOR RAPID DETECTION OF ENTEROHEMORRHAGIC ESCHERICHIA COLI	REJECTED
860700120	ALONSO JL	LABORATORIOS ANDROMACO	DEVELOPMENT AND CHARACTERIZATION OF MONOCLONAL REAGENTS TO S. TYPHI AND S. PARATHYPI A AND B	REJECTED
860700121	TAYLOR DW	GEORGETOWN UNIVERSITY	A MONOCLONAL ANTIBODY ANTIGEN-CAPTURE ASSAY FOR DIAGNOSIS OF MALARIA	FUNDED
860700122	COOPER BW	HARTFORD HOSPITAL	AN ELISA TO DETECT SALMONELLA TYPHI IN BODY FLUIDS USING MONOCLONAL ANTIBODIES TO CELL WALL ANTIGENS	REJECTED
860700123	LANG DR	SYNTRO CORPORATION	DEVELOPMENT OF A SENSITIVE MALARIA DIAGNOSTIC	REJECTED
860700124	CRAWFORD JT	MCCLELLAN MEM VA HOSPITAL	GENETIC PROBES FOR THE DETECTION OF MYCOBACTERIUM TUBERCULOSIS	FUNDED
860700125	ANDRADE JD	UNIVERSITY OF UTAH	DIPSTICK SELF-LUMINESCENT IMMUNOASSAY USING A NOVEL OPTICAL SEPARATION TECHNIQUE: FEASIBILITY STUDIES	REJECTED
860700126	DE LIBERO G	UNIVERSITY OF MILANO	ISOLATION OF M. TUBERCULOSIS SPECIFIC DNA PROBES TO SCREEN BIOLOGICAL SAMPLES	REJECTED
860700127	FUCCILLO DA	MICROBIOLOGICAL ASSOC INC	DEVELOPMENT OF ANTIBODY AND ANTIGEN DETECTION ASSAYS FOR INFECTIOUS DISEASES	REJECTED
860700128	SANBORN WR	PORTABLE RAPID DIAG TECH	COMPARISON OF SERO-DIAGNOSTIC TESTS FOR RAPID DIAGNOSIS OF ENTERIC FEVERS	REJECTED
860700129	GRANDICS P	STEROGENE INC.	PREPARATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES CAPABLE OF DIFFERENTIATING HAEMOPHILUS INFLUENZAE TYPE B (HITB)	REJECTED
860700130	SORIANO TF	PEM TECH INC.	RAPID FIELD DETECTION OF INVASIVE OUTER MEMBRANE PROTEINS OF SHIGELLA SPECIES AND ENTEROINVASIVE E. COLI	REJECTED
860700131	SORIANO T	PEM TECH INC.	DETECTION OF LT AND ST E. COLI ENTEROTOXINS USING A RAPID SELF-CONTAINED ELISA	REJECTED
860700132	SORIANO TF	PEM TECH INC.	RAPID FIELD DETECTION OF CFA-I, CFA-II AND CFA-III FROM E. COLI AND MRNA FROM SALMONELLA	REJECTED
860700133	LOSONSKY GA	UNIVERSITY OF MARYLAND	DEVELOPMENT AND EVALUATION OF AN ENZYME LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF P. FALCIPARUM INFECTION IN IN VITRO ERYTHROCYTE CULTURES AND CLINICAL MALARIA INFECTION IN VOLUNTEERS	FUNDED
860700134	BINION SB	LAND O'LAKES INC.	DEVELOPMENT OF IMMUNOASSAYS FOR DETECTION OF S. TYPHI AND S. PARATYPHI A AND B	FUNDED

860700135	TRANTALO DJ	DYNATECH R/D COMPANY	DESIGN AND TESTING OF RAPID DIAGNOSTIC KITS FOR FIELD USE IN DEVELOPING COUNTRIES	REJECTED
860700136	TRANTOLO DJ	DYNATECH R/D COMPANY	A STUDY OF THE IMPLICATIONS FOR DIAGNOSTIC TECHNOLOGIES IN DEVELOPING COUNTRIES	REJECTED
860700137	LEVINE	UNIVERSITY OF MARYLAND	PREPARATION OF A MONOCLONAL ANTIBODY FOR IMPROVED AND SIMPLIFIED DETECTION OF ENTEROPATHOGENIC ESCHERICHIA COLI	FUNDED
860700138	HARITH AE	ROYAL TROPICAL INSTITUTE	APPLICATION OF A DIRECT AGGLUTINATION TEST FOR VISCERAL AND MUCOCUTANEOUS LEISHMANIASIS IN BRAZIL	REJECTED
860700139	THONGKRAJAI P	KHON KAEN UNIVERSITY	DEVELOPMENT OF AN ELISA TEST FOR DETECTION OF MYCOBACTERIAL ANTIGENS IN EXTRAPULMONARY TUBERCULOSIS	REJECTED
860700140	SRIPA B	KHON KAEN UNIVERSITY	RAPID AURAMINE O STAINING TECHNIQUE FOR MYCOBACTERIA	REJECTED
860700141	SRIPA B	KHON KAEN UNIVERSITY	A NEW, SIMPLE AND RAPID STAINING TECHNIQUE FOR MYCOBACTERIA USING CLOTH DYE	REJECTED
860700142	BUREK CL	JOHNS HOPKINS UNIVERSITY	DEVELOPMENT OF THE INDIUM SLIDE IMMUNOASSAY FOR DIAGNOSIS OF TYPHOID FEVER	FUNDED
860700143	RAVDIN JI	UNIVERSITY OF VIRGINIA	DEVELOPMENT OF LECTIN SPECIFIC DNA HYBRIDIZATION PROBES FOR DIAGNOSIS OF INTESTINAL AMEBIASIS	FUNDED
860700144	BURKOT TR	JOHNS HOPKINS UNIVERSITY	EVALUATION OF MALARIA INTERVENTION STRATEGIES IN DEFINED EPIDEMIOLOGICAL AREA	REJECTED
860700145	PAWLAK JW	BIOGENEX LABORATORIES	FIELD-SUITABLE MONOCLONAL TESTS FOR THE DETECTION OF S. TYPHI/PARATYPHI IN BLOOD	REJECTED
860700146	SORIANO TF	CHILD HEALTH AND SAFETY	DETECTION OF ENTEROTOXIGENIC AND ENTEROINVASIVE E. COLI BY DNA COLONY HYBRIDIZATION	REJECTED
860700147	CLEVELAND PH	UNIVERSITY OF CALIFORNIA	DEVELOPMENT OF A TWENTY-FIVE MINUTE FIELD DIAGNOSTIC TEST FOR MALARIA, MEASLES, RUBELLA, CHLAMYDIA TRACHOMATIS AND HTLV-III	REJECTED
860700148	GRANDICS P	STEROGENE INC	DEVELOPMENT OF FIELD-SUITABLE IMMUNOASSAY FOR THE DETECTION OF CAPSULAR POLYSACCHARIDE OF HAEMOPHILUS INFLUENZAE TYPE B (HITB) IN URINE	RESUBMISSION POSTPONED
860700149	MITCHELL ML	T SMITH RESEARCH INSTITUTE	ASSAY OF SOMATOMEDIN-C IN BLOOD SPOTTED ON FILTER PAPER	REJECTED
860700150	KLIKS MM	UNIVERSITY OF HAWAII	APPLICATION OF ELISA'S FOR RAPID PREPATENT DIAGNOSIS OF DRACUNCULIASIS	REJECTED
860700151	BARNA-VETRO I	HUMAN INSTITUTE	DOT-IMMUNOASSAY FOR MYCOBACTERIUM TUBERCULOSIS ANTIBODIES	REJECTED
860900152	KHALIL MM	AINS SHAMS UNIVERSITY	PROPOSAL FOR SUBCONTRACT ON ACUTE DIARRHEAL DISEASES	REJECTED
860900153	KHALIL MM	AINS SHAMS UNIVERSITY	PROPOSAL FOR FILARIASIS	REJECTED

860900154	BOUZOUBAA K	INSTITUT HASSAN II	DEVELOPMENT OF RAPID AND ADAPTED BIOCHEMICAL AND SEROLOGICAL TESTS FOR THE DIAGNOSIS OF SOME BACTERIAL DIARRHEAL DISEASES IN MOROCCO	REJECTED
861000155	PACEY GE	MIAMI UNIVERSITY	DEVELOPMENT OF DILUTION SENSORS FOR ORS SOLUTIONS	FUNDED/TERMINATED
861000156	CURTIS RH	ARTEL INC.	DIRECT-READING HEMOGLOBINOMETER FOR DEVELOPING-WORLD USE	FUNDED
861000157	GILMAN RH	UNIV CAYETANO HEREDIA	CYSTICERCOSIS: AN OLD DISEASE WITH NEW POSSIBILITIES FOR DIAGNOSIS AND TREATMENT DETECTION OF ANTIGEN IN HUMANS AND ANIMALS	REJECTED
861100158	SCHOOLNIK GK	STANFORD UNIVERSITY	DETECTION OF ENTEROPATHOGENIC E. COLI (EPEC) WITH THE EPEC-SPECIFIC CARBOHYDRATE RECEPTOR	FUNDED
861100159	MIOTTI PG	JOHNS HOPKINS UNIVERSITY	A SELF CONTAINED ENZYMATIC MEMBRANE IMMUNOASSAY FOR THE DIAGNOSIS OF RESPIRATORY INFECTIONS	REJECTED
861100160	MCLAUGHLIN G	UNIVERSITY OF ILLINOIS	DETECTION OF MALARIA PARASITES USING ENZYME-LINKED SYNTHETIC DNA PROBES	FUNDED
861100161	CHUTIVONGSE S	THAI RED CROSS SOCIETY	PROVISION OF FIELD TESTING AND ADVISORY SERVICES TO THE DIATECH PROJECT	FUNDED
861200162	SUTHAT F	MAHIDOL UNIVERSITY	SCREENING FOR HEMOGLOBINOPATHIES AT PRIMARY HEALTH CARE LEVEL	REJECTED
861200163	BUCHANAN TM	UNIVERSITY OF WASHINGTON	DEVELOPMENT AND TESTING OF DIAGNOSTIC REAGENTS FOR M. TUBERCULOSIS	FUNDED
861200164*	GUERRANT RL	UNIVERSITY OF VIRGINIA	STUDIES OF POTENTIALLY SIMPLER METHODS FOR DETECTION OF FECAL LEUKOCYTES AS A GUIDE TO INFLAMMATORY DIARRHEA	FUNDED
870100165	SENARATH D	UNIVERSITY OF PERADENIYA	A W. BANCROFTI DNA LIBRARY, SPECIES SPECIFIC CLONED ANTIGENS AND IMMUNODIAGNOSIS OF FILARIASIS	REJECTED
870100166	KELLERMAN GH	PROTATEK INTERNATIONAL INC	DEVELOPMENT OF A FIELD DIAGNOSTIC TEST: SHIGELLOSIS	REJECTED
870200167	CHEN BQ	PRC	HEMORRHAGIC FEVER WITH RENAL SYNDROME VIRUS MONOCLONAL ANTIBODY	REJECTED
870200168	JEGEDE O	UNIVERSITY OF IBADAN	SEROLOGICAL STUDIES OF THE EPIDEMIOLOGY OF AMOEBIASIS IN SOUTH WEST NIGERIA	REJECTED
870300169	PEARSON RD	UNIVERSITY OF VIRGINIA	RAPID DIAGNOSTIC TESTS FOR THE DETECTION OF VISCERAL LEISHMANIASIS	FUNDED
870300170	KHALID K	INSTITUT AGRONOMIQUE ET VETERINAIRE HASSAN II	VALIDATION OF THE ELISA FOR THE IMMUNODIAGNOSIS OF HUMAN FASCIOLIASIS IN MOROCCO	REJECTED
870500171	VOID			

870500172*	KANTOR IN	CENTRO PANAMERICANO DE ZOONOSIS	EVALUATION OF AN ENZYME LINKED IMMUNOSORBENT ASSAY FOR THE SEROLOGICAL DIAGNOSIS OF BACILOSCOPY NEGATIVE ADULTS AND CHILDREN'S TUBERCULOSIS	REJECTED DUE TO AID RESTRICTIONS
870500173	ANTHONY RL	UNIVERSITY OF MARYLAND	RAPID DIAGNOSIS OF CUTANEOUS AND VISCERAL LEISHMANIASIS	REJECTED
870500174*	DEUTSCH ME	MARSHALL DIAGNOSTICS, INC.	APPLICATIONS OF A UNIQUE LAMINATED MATERIAL TO RAPID CLINICAL TESTS	REJECTED
870500175	PHAIRE L	TUSKEGEE UNIVERSITY	USE OF MONOCLONAL ANTIBODIES FOR DEVELOPMENT OF AN ENZYME-LINKED IMMUNODIAGNOSTIC TEST FOR FALCIPARUM MALARIA IN WEST AFRICAN CHILDREN	REJECTED
870500176	STRICKLAND GT	UNIVERSITY OF MARYLAND	DEVELOPMENT AND EVALUATION OF AN ELISA TO DETECT E. HISTOLYTICA ANTIGENS	REJECTED
870500177	DANIEL TM	CASE WESTERN RESERVE UNIV	PRACTICAL TUBERCULOSIS ELISA	FUNDED
870500178*	MAIZELS RM	IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY	DEVELOPMENT OF MONOCLONAL ANTIBODIES AGAINST WUCHERERIA BANCROFTI STAGE 3 LARVAE	REJECTED
870500179*	CHUKWUMA FO	CHUKS MEDICAL DIAGNOSTICS LABORATORIES	SCREENING A LARGE SECTION OF THE POPULATION FOR GENOTYPE IN ORDER TO ELMINATE THE INCIDENCE OF SICKLE CELL DISEASE	REJECTED
870500180*	SAVANAT T	MAHIDOL UNIVERSITY	TWO-SITE ELISA FOR DETECTION OF PLASMODIUM FALCIPARUM ANTIGEN	FUNDED
870500181*	WATSON-WILLIAMS EJ	UGANDA RED CROSS	DEVELOPMENT AND FIELD TESTING IN UGANDA OF A DOT EIA TEST FOR HIV I	REJECTED
870600182	EL-BADRY AM	SENSOR DIAGNOSTICS INC.	VISUAL ENZYME IMMUNOASSAY FOR RESPIRATORY SYNCYTIAL VIRUS	REJECTED
870600183	HEWLETT EL	UNIVERSITY OF VIRGINIA	A DIAGNOSTIC PROBE FOR STUDY OF B. PERTUSSIS INFECTION AND ITS EPIDEMIOLOGY	DEFERRED
870600184	YOLKEN RH	JOHNS HOPKINS UNIVERSITY	PRACTICAL AND SENSITIVE HYBRIDIZATION ASSAYS FOR THE DIAGNOSIS OF RESPIRATORY INFECTIONS	REJECTED
870600185	LAL AA	BIOMEDICAL RESEARCH INST	DEVELOPMENT OF MALARIAL DIAGNOSTIC AND SEROEPIDEMIOLOGICAL PROBES BASED ON RIBOSOMAL RNA	REJECTED
870600186	ZIPP AP	SENSOR DIAGNOSTICS INC.	VISUAL ENZYME IMMUNOASSAY FOR STREPTOCOCCUS PNEUMONIAE	REJECTED
870600187	NASH P	BIO-METRIC SYSTEMS INC.	RAPID MULTIENZYME KIT FOR DETECTING DIARRHEAL DISEASES	REJECTED
870600188	NASH P	BIO-METRIC SYSTEMS INC.	DEVELOPMENT OF A FIELDABLE ENZYME IMMUNOASSAY FOR MALARIA SPOROZOITE DETECTION	REJECTED
870600189*	APICELLA MA	RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW	DEVELOPMENT OF A RAPID DIAGNOSTIC SYSTEM FOR BACTERIAL PNEUMONIA	RESUBMISSION REQUESTED

		YORK		
870600190*	HARITH AE	ROYAL TROPICAL INSTITUTE	A DIRECT AGGLUTINATION (DA) ASSAY FOR THE DIAGNOSIS OF VISCERAL AND MUCOCUTANEOUS LEISHMANIASIS	REJECTED
870600191	BRANDWEIN HJ	GENETIC DIAGNOSTICS CORP	DISPOSABLE DIAGNOSTIC DEVICE FOR E. COLI ENTEROTOXINS	FUNDED/TERMINATED
870600192	ALI MI	ICDDR,B	A STUDY OF KNOWLEDGE AND PRACTICE RELATED TO DIARRHOEA AND ORAL REHYDRATION THERAPY IN MATLAB	REJECTED
870600193*	BOCTOR FN	SPECIALTY LABORATORIES INC.	RAPID DIAGNOSIS OF MYCOBACTERIAL MENINGITIS	RESUBMISSION REQUESTED
870600194	LUCIUS R	INSTITUT FUR TROPENHYGIENE	DEVELOPMENT OF A FIELD APPLICABLE SEROLOGICAL TEST FOR DETECTION OF HUMAN INFECTIONS WITH BRUGIA MALAYI AND WUCHERERIA BANCROFTI	REJECTED
870600195	EDINGTON MA	UNIVERSITY COLLEGE/UK	METHODS FOR MONITORING BACTERIOLOGICAL WATER QUALITY IN 3RD WORLD VILLAGES	REJECTED
870600196	SUTTIPANT S	MAHIDOL UNIVERSITY	DEVELOPMENT OF VI MONOCLONAL ANTIBODIES FOR THE DIAGNOSIS OF TYPHOID FEVER	FUNDED
870600197	BENAVENTE L	UNIVERSIDAD PERUANA CAYETANO HEREDIA	ESTABLISHMENT OF A BANK OF BIOLOGICAL SPECIMENS FOR THE DIAGNOSIS OF TYPHOID FEVER AT CAYETANO HEREDIA UNIVERSITY, LIMA, PERU	UNDER REVIEW BY DBRS
870800198*	GASANGWA DK	EXOCH UNIVERSAL ENTERPRISES	DEVELOPMENT AND PRODUCTION OF DIAGNOSTIC TECHNOLOGIES FOR INFECTIOUS DISEASES: HUMAN TUBERCULOSIS IN UGANDA	REJECTED
870800199	KAPSENBERG JG	RIJKSINSTITUUT VOOR VOLKSGEZONDHEID	DEVELOPMENT OF ELISA METHODS FOR INTRATYPIC DIFFERENTIATION OF POLIOVIRUS STRAINS AND ANTIBODIES	REJECTED
870800200	VINAYAGMOORTHY	UNIVERSITY OF JAFFNA	MANUFACTURING DISPOSABLE BACTERIAL TEST KITS FOR (A) URINE (B) WATER	REJECTED
870800201*	BERRY S	JOHNS HOPKINS UNIVERSITY	FEASIBILITY OF THE USE OF THE ELISA FOR RSV ANTIGEN DETECTION	REJECTED
870800202*	EDLIND TD	THE MEDICAL COLLEGE OF PENNSYLVANIA	DNA PROBE FOR GIARDIA LAMBLIA	FUNDED
871200203	VAN ALPHEN AJW	UNIVERSITY OF AMSTERDAM	DEVELOPMENT OF A TEST FOR THE DIAGNOSIS OF ACUTE RESPIRATORY TRACT INFECTIONS BY HAEMOPHILUS INFLUENZAE IN CHILDREN	REJECTED
871200204	ECHEVERRIA P	AFRIMS	DEVELOPMENT AND FIELD TESTING OF BIOTINYLATED CLONED DNA PROBES TO IDENTIFY VEROCYTOTOXIN 1 AND 2 ESCHERICHIA COLI	REJECTED
871200205*	STIBBS HH	GIARDIA SPECIALTY DIAGNOSTICS, INC.	LATEX AGGLUTINATION AND BEAD-ELISA FOR DIAGNOSIS OF GIARDIASIS AND AMOEBIASIS	TO BE CONSIDERED AT FUTURE DATE
871200206	VOID			
871200207*	MORSHED MG	LIFE SCIENCES INSTITUTE	DEVELOPMENT OF A LOW COST KIT FOR BACTERIOLOGICAL WATER QUALITY TEST IN RURAL AREAS OF DEVELOPING COUNTRIES	REJECTED

880100208*	FARMER-OGOGO	SAN MATEO, CA	ASSAYS FOR THE RAPID DETECTION OF GIARDIA LAMBLIA	RESUBMISSION REQUESTED
880200209	GRINSTEIN S	CHILDREN'S HOSPITAL RICARDO GUTIERREZ	DEVELOPMENT OF SIMPLE, ANTIGEN-SPECIFIC TESTS AS TOOLS FOR CHAGAS' DISEASE	REJECTED
880200210	ANDERSON LJ	CENTER FOR INFECTIOUS DISEASES/CDC	DEVELOPMENT OF ANTIGEN AND ANTIBODY IMMUNOASSAYS FOR THE PARAINFLUENZA VIRUSES AND RESPIRATORY SYNCYTIAL VIRUS	FUNDED
880200211*	MANNING DD	UNIVERSITY OF WISCONSIN	A MINK AND FERRET MODEL FOR INTESTINAL DISEASE CAUSED BY CAMPYLOBACTER JEJUNI	REJECTED
880300212*	SOMMERFELT H JOHN J	UNIVERSITY OF BERGEN	DNA HYBRIDIZATION IN STOOL BLOTS, WATER SAMPLES AND WEANING FOODS FOR THE IDENTIFICATION OF ENTEROTOXIGENIC ESCHERICHIA COLI	REJECTED
880400213	GOPO JM	UNIVERSITY OF ZIMBABWE	ROUTINE DIAGNOSIS TO DETERMINE THE PREVALENCE AND STATUS OF SALMONELLAE SPP., IN RURAL AND URBAN DRINKING WATER SOURCES; DAIRY AND DAIRY PRODUCTS; MEAT AND MEAT PRODUCTS; POULTRY AND POULTRY PRODUCTS; AND CLINICAL DIARRHOEA USING A SALMONELLA-SPECIFIC DNA PROBE	REJECTED
880400214	PARKINSON AJ	CDC/ANCHORAGE	DEVELOPMENT OF AN ENZYME IMMUNOASSAY FOR THE RAPID DETECTION OF PNEUMOCOCCAL C POLYSACCHARIDE	FUNDED
880500215	LEINONEN M	NATIONAL PUBLIC HEALTH INSTITUTE/FINLAND	EVALUATION OF RAPID SEROLOGICAL DIAGNOSTIC METHODS FOR BACTERIAL ACUTE LOWER RESPIRATORY INFECTIONS (ALRI) OF CHILDREN IN DEVELOPING COUNTRIES	REJECTED
880600216	MORRIS JG	UNIVERSITY OF MARYLAND	FIELD STUDIES OF A SYNTHETIC DNA PROBE FOR PATHOGENIC YERSINIA SPECIES	REJECTED
880600217	LAMBILLOTTE D	LMD LABORATORIES, INC.	TESTING STOOLS FOR MULTIPLE ANTIGENS	REJECTED
880600218	REESE RT	THE AGOURON INSTITUTE	DETECTION OF PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX ANTIGENS USING MONOCLONAL ANTIBODIES	REJECTED
880600219	REESE RT	THE AGOURON INSTITUTE	NEW APPROACHES TO PRODUCING MONOSPECIFIC REAGENTS AGAINST MALARIAL PARASITE ANTIGENS	REJECTED
880700220	MURALIDHARAN R	ASSOCIATION FOR RURAL SOCIAL & HEALTH AFFAIRS	COMMUNITY HEALTH PROGRAMME FOR INDUSTRIAL POLLUTED AREA	REJECTED
880800221	SAUL AJ	QUEENSLAND INSTITUTE OF MEDICAL RESEARCH	DEVELOPMENT OF MALARIAL SERODIAGNOSTIC TESTS SUITABLE FOR LARGE-SCALE FIELD USE	REJECTED
881100222	WELCH DF	UNIVERSITY OF OKLAHOMA	DIAGNOSIS OF MYCOBACTERIAL INFECTIONS BASED ON GAS-CHROMOTOGRAPHIC ANALYSIS OF CEREBROSPINAL FLUID, SERUM, OR SPUTUM	REJECTED
881200223	DE ESTRADA MB	NATIONAL HEALTH INSTITUTE	ENTAMOEBA HISTOLYTICA AND AMEBIASIS IN NORTH, CENTER AND SOUTH	REJECTED

			FROM PERU	
881200224*	PARKINSON AJ	ARCTIC INVESTIGATIONS LABORATORY	FIELD APPLICABILITY OF POLYCLONAL ENZYME IMMUNOASSAY FOR DETECTION OF RESPIRATORY VIRAL ANTIGEN	REJECTED
890100225	LIM PL	UNIVERSITY OF HONG KONG	THE APPLICATION OF TUBE LATEX IMMUNOASSAYS TO THE DIAGNOSIS OF TYPHOID FEVER	DEFERRED
890100226	OBERHELMAN R	JOHNS HOPKINS UNIVERSITY	A CLINICAL FIELD TRIAL OF A NUCLEIC PROBE FOR THE DETECTION OF SHIGELLA INFECTIONS	FUNDED
890300227	KABBA SB	COMMUNITY DEVELOPMENT COUNCIL	RURAL SANITATION RESEARCH: PHASE III	REJECTED
890600228	ROBINSON HW	SAN JOSE STATE UNIVERSITY	FLUOROCHROME STAINING FOR THE RAPID DIAGNOSIS OF FECAL PROTOZOA AND HELMINTHS	REJECTED
890600229	CARLIN N	NATIONAL BACTERIOLOGICAL LABORATORY	DEVELOPMENT OF A RAPID AND SENSITIVE DETECTION ASSAY OF SHIGELLA FLEXNERI ANTIGEN IN STOOL SAMPLES	FUNDED
890600230*	CABELLO F	NEW YORK MEDICAL COLLEGE	DEVELOPMENT OF DIAGNOSTIC ASSAYS FOR TYPHOID FEVER	FUNDED
890800231	WRIGHT J	GULL FOUNDATION FOR MEDICAL RESEARCH	A MEMBRANE-BASED ASSAY FOR PNEUMOCOCCAL POLYSACCHARIDE	REJECTED
890800232	VOLLER A	ZOOLOGICAL SOCIETY OF LONDON	DEVELOPMENT OF MALARIA ANTIGEN ELISA	FUNDED
890800233	HOFFMAN A	UNIVERSITY OF WASHINGTON	A NOVEL APPROACH TO A DIAGNOSTIC DIPSTICK	REJECTED
891200234	BACHARACH U	HEBREW UNIVERSITY	DEVELOPMENT OF A NEW DIAGNOSTIC METHOD FOR MALARIA USING ANTIBODIES AND DNA PROBES TO PLASMODIAL ORNITHINE DECARBOXYLASE	DEFERRED
891200235	GLASS R	EMORY UNIVERSITY	THE EPIDEMIOLOGIC IMPORTANCE OF NOVEL VIRAL AGENTS DIARRHEA	PENDING REVIEW
891200236*	FRANKEL G	WEIZMANN INSTITUTE OF SCIENCE	DETECTION OF SALMONELLA TYPHI IN FECES AND BLOOD USING DNA AMPLIFICATION	DEFERRED
900100237*	HULL AE	NEW ENGLAND MEDICAL CENTER HOSPITAL	FIELD STUDY OF A COLONY BLOT ASSAY TO DETECT SHIGELLA AND SLT PRODUCING E. COLI	PENDING REVIEW
900100238	SNOWDEN KJ	LIVERPOOL SCHOOL OF TROPICAL MEDICINE	DIFFERENTIAL DIPSTICK FECAL ANTIGEN DETECTION TEST FOR TWO PROTOZOANS	PENDING REVIEW
900100239	CARRILLO C	UNIVERSIDAD PERUANA CAYETANO HEREDIA	USE OF DNA PROBE FOR THE DETECTION OF CHRONIC CARRIERS OF SALMONELLA TYPHI	DEFERRED

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*Feasibility Studies

Attachment 4

DiaTech Subagreements by Disease Area
Date: 02/16/90

Number	Institution	PI	Title
ARI/TB			
860300047	Centers for Disease Control	Anderson, Larry J.	Production and characterization of MAbs against parainfluenza
860700075	University of Kentucky Research Foundation	Coonrod, J. Donald	ELISA for the rapid diagnosis of influenza
861200163	University of Washington (Year II)	Buchanan, Thomas M.	Development and testing of reagents for M. tuberculosis
860700087	Koninklijk Instituut voor de Tropen (Yr II)	Kolk, Arend H.J.	Development of field assay for detection of M. tuberculosis
860700124	Biomedical Research Foundation	Crawford, Jack T.	Genetic probes for the detection of M. tuberculosis
860700095	Minnesota Mining and Manufacturing Company	Milbrath, Dean S.	Advanced agglutination assay for S. pneumoniae
860700075	University of Rochester	Betts, Robert	Development of an ELISA for rapid diagnosis of influenza
860700085	Research Foundation of SUNY	Apicella, Michael A.	Development of monoclonal antibodies to pneumococcus
870500177	Case Western Reserve University (Year II)	Daniel, Thomas M.	Practical tuberculosis ELISA
860700113	International Health Services	Beal, Charles B.	A sputum sampling method for tuberculosis diagnosis
880400214	Centers for Disease Control/Anchorage-Yr II	Parkinson, Alan J.	Enzyme immunoassay for pneumococcal C polysaccharide
880200210	Centers for Disease Control (Year II)	Anderson, Larry J.	Antigen and antibody immunoassays for respiratory viruses
Diarrhea/Typhoid			
860500063	Henry M. Jackson Foundation (Year II)	O'Brien, Alison D.	Colony-ELISA for detection in stool of E. coli
861000158	Stanford University Medical Center	Schoolnik, Gary K.	Detection of EPEC with the EPEC-specific carbohydrate receptor
860700143	University of Virginia (Year III)	Ravdin, Jonathan I.	Development of a lectin-specific ELISA for amebiasis
860700104	Dynamac Corporation	Singh, Suraj B.	Field-suitable ELISA for detecting salmonella vi antigen
860700142	The Johns Hopkins University/DIID	Burek, C. Lynne	Indium slide assay for diagnosis of typhoid fever
860700134	Land O'Lakes	Binion, Steven B.	Development of assays for detection of S. typhi
860700137	University of Maryland at Baltimore/CVD II	Levine, Myron M.	Preparation of MAb for detection of enteropathogenic E. coli
870600196	Mahidol University/Faculty of Medicine	Suttipant Sarasombath	Development of Vi monoclonal antibodies for typhoid fever
870600191	Genetic Diagnostics Corporation	Brandwein, Harvey	Disposable diagnostic device for E. coli enterotoxins
861200164	University of Virginia Dept of Geog Medicine	Guerrant, Richard L.	Studies of simpler methods of detection of fecal leukocytes
870800202	Medical College of Pennsylvania	Edlind, Thomas D.	DNA probe for Giardia lamblia
860300052	Covalent Technologies Corporation	Pappas, Michael G.	Visual immunoassay for hepatitis B & cholera entero-toxin
860700089	Sero-Immuno Diagnostics, Inc.	Kagan, Irving	Serologic detection of Giardia lamblia antigen in stool
890100226	The Johns Hopkins University/DIH	Oberhelman, Richard	Clin. field trial of a nucleic acid probe for shigella
890600230	New York Medical College	Cabello, Felipe	Development of diagnostic assays for typhoid fever
890600229	National Bacteriological Laboratory	Carlin, Nils	Diagnostic assay for the detection of shigella in stool
Malaria			
860300045	University of Maryland at Baltimore/CP	Kaper, James B.	Evaluation of five probes for the diagnosis of malaria
860300049	Institute of Zoology, Zoological Society	Voller, Alister	ELISA for the measurement of plasma quinine levels
860500061	Professional Staff Association (Year II)	Martin, W. John	Nonradioactive detection/P. vivax & P. falciparum in blood
860700121	Georgetown University (Year III)	Taylor, Diane Wallace	Development of monoclonal antibody-based assay for malaria
860700133	University of Maryland at Baltimore/CVD I	Losonsky, Genevieve A.	Development of ELISA for detection of P. falciparum
861100160	University of Illinois (Year II)	McLaughlin, Gerald	Detection of malaria parasites using synthetic DNA probes
870500180	Mahidol Univ/Faculty of Trop Med (Year II)	Savanat Tharavanij	Two-site ELISA for the detection of P. falciparum antigen
890800232	The Zoological Society of London	Voller, Alister	Development of malaria antigen ELISA
Other			
860900001	The Johns Hopkins University/Primary I	Rose, Noel R.	Provision of assistance to management/laboratory resources
861000155	Miami University (Year II)	Pacey, Gilbert E.	Development of dilution sensors for ORS solutions
861100161	Queen Saovabha Memorial Institute/Primary II	Supawat Chutivongse	Provision of assistance to management and sample collection
860300051	The New York Blood Center	Prince, Alfred M.	Development of O. volvulus diagnostics for third world use
860400156	Artel, Inc.	Curtis, Richard	Development of a direct-reading hemoglobinometer
870300169	University of Virginia School of Medicine	Pearson, Richard D.	Rapid diagnostic tests for detection of visceral Leishmaniasis
860900002	University of Maryland- Subprimary	Strickland, G. Thomas	Technical assistance to DiaTech

Attachment 5

DIATECH
PROPOSAL REVIEW GUIDELINES

CRITERIA FOR RECOMMENDATION

- The project must further the goals of DiaTech.
- The project must have technical merit.
- Project personnel must be competent to perform the work planned.
- The applicant institution must have adequate facilities, equipment, and financial management resources.
- The project must have a high likelihood of achieving its stated goals within the expressed time frame and budget.
- The budget must be appropriate to the tasks described.

GENERAL GUIDELINES

Individual reviewers are expected to review the criteria for recommendation and to read all proposals under consideration. The number of proposals assigned to a given reviewer will vary, depending on the number of proposals received. If the assigned reviewer is unable to review the proposals submitted, the reviewer will advise project management within three days of receipt so that an alternate reviewer may be found. Reviewers will not circulate proposals to other colleagues for comment without the prior approval of project management.

Reviewers are asked to provide a narrative explanation of the strengths and weaknesses of each proposal in relation to the criteria for recommendation. Deficiencies, ambiguities, and needed clarifications in individual proposals should be addressed. This summary document need not be lengthy, but it should explain the basis for the individual recommendation. This written narrative should be returned to the Technical Director by overnight courier.

Reviewers should clearly justify why a particular proposal is unacceptable and not capable of being made acceptable through negotiation. For acceptable proposals, reviewers should outline what additional information, if any, is needed to make a final recommendation. This should generally be limited to two or three points in a given proposal before consideration should be given to judging the proposal as unacceptable.

In addition to providing a narrative review, reviewers are asked to score proposals according to the criteria provided. This will be especially important in instances where the entire review process is conducted by mail, since differences in the tone of the narrative review may be more imagined than real.

CONDUCT OF REVIEW

Assigned reviewers, usually no more than two in number, will provide their narrative reviews and score sheets to the Technical Director by the assigned date. The Technical Director will collate the reviews, average the scores, and make comments, where pertinent. If the reviews of a given proposal are markedly discordant, the Technical Director may,

at his discretion, seek an additional review; refer the proposal to the TAG for a final recommendation; or attempt to negotiate a consensus between the original reviewers. The TAG will review all acceptable formal proposals for a final recommendation, prior to any award being made, for appropriateness of the reviewers' assessments and for relevance to DiaTech's goals. Proposals which were judged unacceptable by the reviewers will not be forwarded to the TAG. DiaTech staff will coordinate review of all proposals received and will furnish a written summary of the reviewers' comments to the applicant, but reviewers' names will not be released. There is no provision for rebuttal of a review of appeal of the TAG's decision. All grants require the express approval of A.I.D. before funding is awarded.

/wb
MT00098V

Attachment 6

DIATECH-SPONSORED PUBLICATIONS

ARI/TB

LARRY J. ANDERSON, Centers for Disease Control

Hierholzer JC, Bingham PG, Coombs RA, Johansson KH, Anderson LJ, Halonen PE. Comparison of monoclonal antibody time-resolved fluoroimmunoassay with monoclonal antibody capture-biotinylated detector enzyme immunoassay for respiratory syncytial virus and parainfluenza virus antigen detection. *Journal of Clinical Microbiology* 27:1243-1249 (1989).

J. DONALD COONROD, University of Kentucky

Coonrod JD, Karathanasis P, Betts RF, Donofrio JC. Enzyme-linked immunosorbent assay of core antigens for clinical diagnosis of influenza. *Journal of Medical Virology* 25:399-409 (1988).

JACK T. CRAWFORD, Veterans Administration Medical Center

Direct detection of *M. tuberculosis* in clinical material using a DNA probe assay. (In preparation 1989)

THOMAS M. DANIEL, Case Western Reserve University

Ferguson LE, Daniel TM. Comparison of *Mycobacterium tuberculosis* antigens 5 and 6 for ELISA serodiagnosis of tuberculosis. (*Clinical Research*, Abstract submitted 1989).

Steele BA, Daniel TM. Evaluation by decision analysis of the potential role of serodiagnosis of tuberculosis in a clinic in Bolivia. Abstract in *American Review of Respiratory Diseases* 139:A184 (1989).

Daniel TM, Ferguson LE, Sanson AJ. Evaluation of *Mycobacterium tuberculosis* antigen 6 for use in enzyme-linked immunosorbent assay for the serodiagnosis of tuberculosis. Abstract in *American Review of Respiratory Diseases* 139:A394 (1989).

Wallis RS, Alde SLM, Havlir DV, Amir-Tahmasseb M, Daniel TM, Ellner JJ. Identification of potentially protective antigens of *Mycobacterium tuberculosis* using human monoclonal antibodies derived from pleural fluid lymphocytes. Abstract in *Clinical Research* 37:567A (1989).

Havlir D, Wallis R, Boom WH, Chervenak K, Daniel T, Ellner JJ. *In vitro* blastogenic response to native and recombinant antigens of *Mycobacterium tuberculosis*. (*Clinical Research*, Abstract submitted 1989)

Salata RA, Sanson AJ, Malhotra IJ, Phillips NB, Harbue M, Daniel TM. Purification and epitope characterization of the 30,000 dalton native antigen of *Mycobacterium tuberculosis*. (In preparation 1990)

Sada ED, Ferguson LE, Daniel TM. An enzyme-linked immunoabsorbent assay (ELISA) for the serodiagnosis of tuberculosis using a 30,000 dalton native antigen of *Mycobacterium tuberculosis*. (In preparation 1990)

AREND H. KOLK, Koninklijk Instituut voor de Tropen

Verstijnen CPHJ, Kuijper S, Schöningh R, Bruins JJM, van Ketel R, Groothuis DG, Kolk AHJ. Identification of *Mycobacterium tuberculosis* with monoclonal antibodies. In: *The XII International Congress for Tropical Medicine and Malaria*; 18-23 September 1988, Amsterdam: Excerpta Medica (International Congress Series 810) (1988).

Verstijnen CPHJ, Schöningh R, Kuijper S, Bruins J, van Ketel RJ, Groothuis DG, Kolk AHJ. Rapid identification of *Mycobacterium tuberculosis* with a panel of monoclonal antibodies in western blot and in immunofluorescence. (*Research in Microbiology*, Accepted for publication 1989)

Schöningh R, Verstijnen CPHJ, Kuijper S, Kolk, AHJ. Enzyme immunoassay for the identification of heat killed mycobacteria belonging to the *Mycobacterium tuberculosis* and the *Mycobacterium avium* complex derived from early cultures. (*Journal of Clinical Microbiology*, Submitted for publication 1989)

Verstijnen CPHJ, Schuitema ARJ, van Embden JDA, Kolk AHJ, Thole JER, Hermans PWM. Specific detection of *M. tuberculosis* by polymerase chain reaction. Poster presentation, Symposium of The Molecular Biology of Mycobacteria, September 1989, University of Surrey, U.K.

Schöningh R, Verstijnen CPHJ, Kuijper S, Kolk AHJ. Identification and detection of *M. tuberculosis* in early cultures or sputum by monoclonal antibodies. Abstract poster, International Symposium of the Dutch Society of Immunology, 14-16 December 1989, The Hague.

Hermans P, Schuitema ARJ, van Scolingen D, Verstijnen CPHJ, Bik EM, Thole JER, Kolk AHJ, van Embden JDA. Specific detection of *M. tuberculosis* complex strains by polymerase chain reaction. (*Journal of Clinical Microbiology*, Submitted for publication 1989)

Verstijnen K, Kuijper S, Schöningh R, Bruins J, van Ketel R, Groothuis D, Kolk AHJ. Identification of *M. tuberculosis* with monoclonal antibodies. (Submitted for publication 1989)

ALAN J. PARKINSON, Centers for Disease Control

Parkinson AJ, Davidson M, Rabiego M. Enzyme immunoassay for the detection of pneumococcal C polysaccharide in sputum of patients with presumptive pneumococcal pneumonia. Abstract, 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, October 24-26 1988, Los Angeles, CA.

Parkinson AJ, Clift SE, Rabiego M, Davidson M. Detection of pneumococcal C polysaccharide by enzyme immunoassay in Alaska Native children with chronic otitis media. Abstract, 29th Interscience Conference on Antimicrobiol Agents and Chemotherapy, September 17-20, 1989, Houston, TX.

Parkinson AJ, Davidson M, Campbell J, Facklam R, Rabiego M. Enzyme immunoassay for the detection of pneumococcal C polysaccharide in sputum of patients with presumptive pneumococcal pneumonia. (*Journal of Clinical Microbiology*, In preparation 1989)

DIARRHEA/TYPHOID

THOMAS D. EDLIND, Medical College of Pennsylvania

Edlind TD, Sharetzsky C, Cha ME. Ribosomal RNA of the primitive eukaryote *Giardia lamblia*: Large subunit domain I and potential processing signals. (Submitted for publication 1989)

ALISON D. O'BRIEN, Uniformed Services University of the Health Sciences

Perera LP, Marques LRM, O'Brien AD. Isolation and characterization of monoclonal antibodies to shiga-like toxin II of enterohemorrhagic *Escherichia coli* and use of the monoclonal antibodies in a colony enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology* 26:2127-31 (1988).

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Saxena SK, O'Brien AD, Ackerman EJ. Shiga toxin, shiga-like toxin II variant, and ricin are all single-site RNA *N*-glycosidases of 28 S RNA when microinjected into *xenopus* ocytes. *Journal of Biological Chemistry* 264:596-601 (1989).

JONATHAN I. RAVDIN, University of Virginia

Ravdin JI, Jackson TF, Petri WA, Murphy CF, Ungar BL, Gathiram V, Skilogiannis J, Simjee AE. Association of serum anti-adherence lectin antibodies with invasive amebiases and asymptomatic infection with pathogenic *Entamoeba histolytica*. (*Journal of Infectious Diseases*, Submitted for publication 1989)

Ravdin JI, Jackson TF, Petri WA, Murphy CF, Ungar BL, Gathiram V, Skilogiannis J, Simjee AE. Serum anti-adherence lectin antibodies as a marker of invasive amebiasis and asymptomatic pathogenic *Entamoeba histolytica* infection. (*New England Journal of Medicine*, Submitted for publication 1989)

MALARIA

W. JOHN MARTIN, University of Southern California

Shibata D, Martin WJ, Arnheim N. Analysis of DNA sequences in forty year old paraffin embedded thin tissue section: A bridge between molecular biology and classical histology. *Cancer Research* 48:4564-66 (1988). (Hard Copy Requested)

Shibata D, Forrester K, Martin WJ, Arnheim N, Perucho M. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. *Cell* 53:549-54 (1988). (Hard Copy Requested)

Shibata D, Fu Y, Gupta J, Shah K, Arnheim N, Martin WJ. The detection of human papillomavirus in normal and dysplastic tissue by the polymerase chain reaction. *Lab Investigation* 59:555 (1988). (Hard Copy Requested)

Shibata D, Appleman M, Causey D, Leedom J, Martin WJ, Arnheim N. Detection of cytomegalovirus DNA in peripheral blood of HIV-infected patients. *Journal of Infectious Diseases* 158:1185-92 (1988). (Hard Copy Requested)

Shibata DK, Arnheim N, Martin WJ. Detection of human papilloma virus in paraffin-embedded tissue using the polymerase chain reaction. *Journal of Experimental Medicine* 167:225-30 (1988).

Kiyabu M, Shibata D, Arnheim N, Martin WJ, Fitzgibbons P. Detection of human papillomavirus in formalin-fixed invasive squamous carcinomas using the polymerase chain reaction. *American Journal of Surgical Pathology* 12: 221-24 (1989). (Hard Copy Requested)

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GERALD L. MCLAUGHLIN, University of Illinois

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McLaughlin, GL. Use of synthetic DNA oligomers to analyze the repetitive DNA families of *Plasmodium falciparum*. Abstract, 38th Meeting of the American Society of Tropical Medicine and Hygiene. Honolulu, HI (1989).

McLaughlin GL, Decrind C, Dayal-Drager R, Subramanian S, King H, Greenwood BM. Evaluation of a prototype assay for falciparum malaria detection in The Gambia. (In preparation 1989)

Marich JE, Ruth JL, McLaughlin GL. Microscopic detection of *Plasmodium falciparum* using enzyme-linked synthetic DNA probes. (In preparation 1989)

McLaughlin GL, Decrind C, Dayal-Drager R, Hassan-King M, Subramanian S, Greenwood BM. Evaluation of a rapid, nonisotopic DNA hybridization method for falciparum malaria diagnosis in The Gambia. (In preparation 1989)

SAVANAT THARAVANIJ, Mahidol University

Srisin K, Savanat T, Ratanaporn K, Chutrudee V, Danai B. Two-site immunoradiometric assay for detection of *Plasmodium falciparum* antigen in blood using monoclonal and polyclonal antibodies. *Journal of Clinical Microbiology* 25:1467-71 (1987).

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Savanat T. Development of an ELISA for detection of *Plasmodium falciparum* in whole blood. (*Southeast Asian Journal of Tropical Medicine and Public Health*, Submitted for publication 1989)

DIANE W. TAYLOR, Georgetown University

Taylor DW, Parra M, Evans CB, Quakyi IA, Howard RJ. Use of a monoclonal antibody "antigen capture" assay for the diagnosis of malaria. Abstract, 3rd International Conference on Malaria and Babesiosis, Annecy, France (1987).

Parra ME, Taylor DW. Presence of histidine rich protein 2 (Pf HRP-2) in the sera of people infected with *Plasmodium falciparum*. Poster, American Society of Tropical Medicine and Hygiene, Honolulu, HI (1989).

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Rodriguez-del Valle M, Taylor DW. Identification of malarial antigens in the urine of individuals with *Plasmodium falciparum* infections. Poster, American Society of Tropical Medicine and Hygiene, Honolulu, HI (1989).

Rodriguez-del Valle M, Taylor DW. Malarial antigens in urine of individuals with *Plasmodium falciparum* infection. Abstract, 602nd Meeting of the Helminthological Society of Washington, Washington, D.C. (1989).

Parra ME, Howard RJ, Quakyi IA, Evans CB, Small ME, Wilde H, Taylor DW. Use of monoclonal antibodies for the detection of the histidine-rich protein-II (PfHRP-2) in sera of individuals with acute *Plasmodium falciparum* infection. (*American Journal of Tropical Medicine and Hygiene*, In preparation 1990)

Taylor DW, Evans CB, Quakyi IA, Changkasiri S, Kennedy L, Lee S, Marsh K, Howard RJ. Identification of a conserved antigen that circulates in the sera of mice and man during acute malarial infection. (*American Journal of Tropical Medicine and Hygiene*, In preparation 1990)

Rodriguez del Valle M, Quakyi IA, Evans CB, Taylor DW. Identification of *Plasmodium falciparum* antigens in the urine of individuals infected with malaria. (*Parasite Immunity*, In preparation 1990)

Limpaiboon T, Taylor DW, Kao V, Jones G, Geysen HM, Saul A. Characterization of a *Plasmodium falciparum* epitope recognized by a monoclonal antibody with broad isolate and species specificity. (*Infection and Immunity*, In preparation 1990)

Parra ME, Howard RJ, Taylor DW. Detection of histidine-rich protein-II (PfHRP-2) in the sera of humans with acute *Plasmodium falciparum* infection. (*Infection and Immunity*, In preparation 1990)

ALISTER VOLLER, The Zoological Society of London

Eslava A, Morgan M, Voller A, Bidwell DE. ELISA--a possible alternative to establish a therapeutic drug monitoring system in severe and complicated falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 82:683-85 (1988).

OTHER

RICHARD H. CURTIS, Artel, Inc.

Rundell C, Wiley T. (Curtis R). Evaluation of a Portable Hemoglobinometer. Abstract for presentation at Clinical Chemistry 1989, October 14, 1989, Morristown, NJ.

RICHARD D. PEARSON, University of Virginia

Evans TG. (Pearson RD). Development of monoclonal antibodies to assay for circulating antigen in visceral leishmaniasis. *Memorias Do Instituto Oswaldo Cruz* 83(S):471-74 (1988).

Evans TG, Pearson RD. Identification of leishmanial antigens in the sera of patients with American visceral leishmaniasis. *Infection and Immunity* 56:3139-44 (1988).

Evans TG, Krug EC, Wilson ME, Vasconcelos AW, Alencar JE, Pearson RD. Evaluation of antibody responses in American visceral leishmaniasis by ELISA and immunoblot. *Memorias Do Instituto Oswaldo Cruz* 84:157-66 (1989).

Evans TG, Vasconcelos IAB, Lima JW, Teixeira JM, McAullife IT, Lopes UG, Pearson RD, Vasconcelos AW. Canine visceral leishmaniasis in northeast Brazil: assessment of serodiagnostic methods. *American Journal of Tropical Medicine and Hygiene* 42:118-23 (1990).

SUPAWAT CHUTIVONGSE, The Thai Red Cross Society

Pornsilapatip J, Intragumtornchai T, Hanvanich M, Namsiripongpum V, et al. An evaluation of a new cell counting and malaria identification system (QBC) in a tropical hospital setting. (Submitted for publication 1989)

Wilde H, Chutivongse S. Advice for travelers to southeast Asia. (In press 1989)

Pornsilapatip J, Namsiripongpun V, Wilde H, Hanvanich M, Chutivongse S. Detection of plasmodia in acridine orange stained capillary tubes (the QBC system). (In preparation 1990)

Wilde H, Innis B, Pornsilapatip J, Alexander B, Glias C. (Chutivongse S). Tetracycline responsive fevers at Thai Kampuchean border displaced persons camps. (In preparation 1990)

PATH/DIATECH

Free, MJ, Hargreaves WR, Stansfield SK. Collaborative project in diagnostics for the improvement of health care in developing countries. Abstract, 13th Annual Conference of the National Council for International Health, June 1986, Washington, D.C.

Auditore-Hargreaves K. Diagnostic technology for community health. Abstract, 17th Congress of the International Association of Medical Laboratory Technologists, August 1986, Stockholm, Sweden, p. 58-62.

Auditore-Hargreaves K, Farrand WP. Integration of diagnostics into primary health care programs. Abstract, International Congress of the World Federation of Public Health Associations, March 22-27, 1987, Mexico City, Mexico.

Auditore-Hargreaves K, Miesowicz FM, Schwartz D, Voller A. Appropriate technologies for the rapid diagnosis of infectious diseases. Abstract, TekMed Conference--Health for All in the Future--What Appropriate Technologies?, May 1987, Lyons, France.

Spielberg F, Kabeya CM, Ryder RW, Quinn TC, Bender TR, Auditore-Hargreaves K. Assessment of rapid, qualitative HIV screening assays in Kinshasa, Zaire. Abstract, 15th Annual Conference of the National Council for International Health, May 1988, Washington, D.C.

Kabeya CM, Spielberg F, Kifuani NK, Ryder R, Heyward W, Quinn T. Comparison of rapid HIV antibody screening assays, Mama Yemo Hospital, Kinshasa, Zaire. Abstract, IV International Conference on AIDS, Book 2, p. 262 (1988).

Spielberg F, Auditore-Hargreaves K, Heyward WL, Ryder RW, Quinn TC, Tam MR, et al. Rapid, instrument-free assays can be used in tandem for screening and confirmation of HIV infection. Abstract, V International Conference on AIDS (1989).

Spielberg F, Kabeya CM, Ryder RW, Kifuani NK, Harris J, Bender TR, Heyward WL, Quinn TC. Field testing and comparative evaluation of rapid, visually read screening assays for antibody to human immunodeficiency virus. *The Lancet I*(8638):580-84 (1989).

Spielberg F, Kabeya CM, Quinn TC, Ryder RW, Kifuani NK, Harris J, Dender TR, Heyward WL, Tam MR, Auditore-Hargreaves K. Performance and cost-effectiveness of a dual rapid assay system for screening and confirmation of HIV-1 seropositivity. *Journal of Clinical Microbiology*, 28:303-06 (1990).

February 20, 1990
MS01230V

Attachment 7

FIELD TESTING AND COMPARATIVE EVALUATION OF RAPID, VISUALLY READ SCREENING ASSAYS FOR ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS

FREYA SPIELBERG[1] CLAIRE MULANGA KABEYA[2]
ROBERT W. RYDER[2,3] N. K. KIFUANI[4]
JEFFREY HARRIS[5] THOMAS R. BENDER[5]
WILLIAM L. HEYWARD[3] THOMAS C. QUINN[6]

Program for Appropriate Technology in Health, Seattle, USA;[1] Projet SIDA, Kinshasa, Zaire;[2] US Centers for Disease Control, Atlanta;[3] Mama Yemo Hospital, Kinshasa;[4] US Agency for International Development, Washington, DC;[5] National Institute of Allergy and Infectious Diseases (Laboratory of Immunoregulation), Bethesda[6]

Summary Five rapid, visually read assays for detection of antibody against human immunodeficiency virus (HIV) were evaluated on fresh serum samples from 4000 prospective blood donors at Mama Yemo Hospital, Kinshasa, Zaire. The sensitivity of the assays, based on 214 specimens positive by western blot, ranged from 84·6% to 99·1%. The specificity, based on 3664 samples negative by enzyme-linked immunosorbent assay (ELISA) or western blot, ranged from 92·7% to 98·8%. Three readers scored each test result independently; disagreement about test interpretation occurred in 1·2–8·3% of the specimens. There was no correlation between assay performance and assay principle (agglutination or dot immunobinding) or antigen source (viral lysate or recombinant). Assays such as these can be readily implemented in a developing country transfusion centre, where blood screening by ELISA is not practicable.

Introduction

TRANSFUSION-ASSOCIATED transmission of human immunodeficiency virus (HIV) remains a severe problem in many developing countries even though it is the most readily preventable mode of HIV transmission. In many central African countries, blood banking is not technically or economically feasible so the time between donation and transfusion is short. Yet in these countries the demand for blood is often great and the seroprevalence of HIV infection in the general population is high.[1,2] To implement routine screening of blood for HIV in developing countries effectively, simple and inexpensive assays are needed, which give reliable results in less time than the conventional method of enzyme-linked immunosorbent assay (ELISA).

Several HIV screening assays have been described which meet one or more of these criteria.[3-8] When some of these assays were evaluated on African blood specimens, performance ranged from poor[8] to excellent.[3-6] However, differences in size of the population sampled and in method among the various studies (eg, fresh or frozen specimens, blinding) make these results difficult to compare. We therefore undertook a study to evaluate the performances of five rapid, qualitative screening assays on fresh serum samples collected from 4000 prospective blood donors in Kinshasa, Zaire; Zairois laboratory staff carried out and interpreted the assays.

Methods

Samples

Serum was collected from 4000 prospective blood donors as part of a blood donation screening programme at Mama Yemo (3090) and Ngaliema (910) Hospitals in Kinshasa, Zaire. Each sample,

TABLE I—COMPARISON OF ASSAY RESULTS WITH WESTERN BLOT EVALUATION OF INITIAL TEST ONLY

—	Cambridge	Du Pont	Fujirebio	Salck	UCD	ELISA
True-negative	3503	3620	3530	3499	3397	3593
True-positive	184	211	212	181	197	211
False-negative	30	3	2	33	17	3
False-positive	161	44	134	165	267	71
Sensitivity (%)	85·98	98·60	99·07	84·58	92·06	98·60
Specificity (%)	95·61	98·80	96·34	95·50	92·71	98·06
Reader variability (%)*	4·0	1·2	2·0	3·4	8·3	..

$$\text{*Reader variability} = \left(\frac{\text{samples with disagreement on initial test}}{\text{total number of samples tested}}\right) \times 100$$

TABLE II—COMPARISON OF ASSAY RESULTS WITH WESTERN BLOT: EVALUATION OF DUPLICATE VISUAL ASSAY TESTS

—	Cambridge	Du Pont	Fujirebio	Salck	UCD	ELISA
True-negative	3629	3650	3598	3642	3588	3648
True-positive	171	208	208	165	178	205
False-negative	43	6	6	49	36	9
False-positive	35	14	66	22	76	16
Sensitivity (%)	79·91	97·20	97·20	77·10	83·18	95·79
Specificity (%)	99·04	99·62	98·20	99·40	97·93	99·56

(HIV-specific bands were not reproducibly as strong as the weak positive control). The vast majority of the samples excluded because of incomplete data were concordantly negative in the assays for which data were available. Therefore, specificity values would have improved, albeit only very slightly, for all the assays had these samples been included. No one assay would have been been affected more than another. Of the remaining 3878 samples, 214 were positive by western blot (5·5% prevalence). None of the 400 initially negative samples on which western blots were done was positive.

Table I compares the initial test results of the five rapid assays and the Wellcome ELISA relative to the western blot result. The number of positive samples (true-positives) correctly identified ranged from 181 to 212; 211 were positive by ELISA. The number of initially reactive specimens which were not positive by western blot (false-positives) ranged from 44 to 267, with 71 initially reactive by ELISA. There was little disagreement among readers about the interpretation of a given test result, but variability was least with the Du Pont assay (1·2%) and greatest with the UCD assay (8·3%); both of these are colour endpoint assays.

If repeatably reactive results are considered (table II), the number of true-positives ranged from 165 to 208, with 205 by ELISA. The number of repeatably reactive false-positive samples ranged from 14 to 76, with 16 repeatably reactive by ELISA.

TABLE III—RESULTS OF RETESTING OF MASTER VIAL SPECIMENS THAT WERE ORIGINALLY DISCORDANT

—	Cambridge	Du Pont	Fujirebio	Salck	UCD
False-negatives on original testing of fresh specimens					
Total	43	6	6	49	36
Number retested	41	6	6	45	33
Number still negative on retesting	13	1	1	16	10
Sensitivity (%)*	93·9	99·5	99·5	92·5	95·3
False-positives on original testing of fresh specimens					
Total	35	14	66	22	76
Number retested	28	13	61	18	70
Number still positive on retesting	8	2	47	8	23
Specificity (%)*	99·8	99·9	98·7	99·8	99·4

*Only discordant samples were retested from the master vials. The recalculated sensitivity and specificity assume that the master vials not retested would have been concordant with the initial-repeat results obtained on the aliquots.

All available false-positive and false-negative specimens were retested on the reference aliquots that had been stored frozen. These specimens were relabelled with random codes so that they could not be identified when included in our daily testing regimen. For all assays the numbers of false-positive and false-negative samples were lower on retesting (table III). The percentage disagreement between initial or repeat test results and reference results for positive discordants was twice as large for the Cambridge, Salck, and UCD assays as for the Du Pont and Fujirebio assays, whereas for negative discordants it was two (Cambridge, UCD) to five (Fujirebio) times as large for some of the assays as for the Du Pont assay.

Assay Characteristics and Ease of Use

The rapid assays evaluated in this study shared various characteristics which make them suitable for use in developing countries. All five gave a visual endpoint within 2 h. Electrically powered instruments were not required to carry out or interpret any assay, although an illuminated magnifying lens and a rotator are recommended by the manufacturer for use with the Cambridge assay and were used for all but the first 350 specimens in this study. There

TABLE IV—CHARACTERISTICS OF FIVE RAPID ASSAYS FOR ANTIBODIES TO HIV

—	Cambridge	Du Pont	Fujirebio	Salck	UCD
Performance time (min)	5	5	120	30	30
Assay description	Latex agglutination	Colloidal gold dot blot	Gelatin agglutination	Haemagglutination	AP dot blot
Assay stability—packaged†*					
4°C	12 mo	>6 mo	12 mo	24 mo	6 mo
25°C	12 mo	2 mo	NA	8 mo	1·5 mo
37°C	1 mo	1 mo	NA	1 mo	NA
Smallest quantity to reconstitute	No reconstitution	10 tests	24 tests	No reconstitution	10 tests
Assay stability—working strength†					
4°C	12 mo	2 mo	7 days	12 mo	3 days
25°C	12 mo	5 days	NA	8 mo	1 day
37°C	1 mo	3 days	NA	1 mo	NA
Cost per test (US $)‡	1·75	2·75	1·45	0·65	0·53

*The smallest kit size is generally 100 tests per kit.
†Washout components.
‡Price for Mama Yemo Hospital, Kinshasha, Zaire, for 10 000 tests.
NA = not available; AP = alkaline phosphatase.

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The availability of technologically appropriate screening assays presents local health care systems with many options from which to choose on the basis of the financial, logistic, and technical requirements of the site(s) where screening will be conducted. International agencies such as the World Health Organization Global Programme on AIDS can play an important role, by using data like those collected in this study to guide developing countries in their decisions.

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Performance and Cost-Effectiveness of a Dual Rapid Assay System for Screening and Confirmation of Human Immunodeficiency Virus Type 1 Seropositivity

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Recent studies have shown that rapid, instrument-free assays for the detection of antibody to human immunodeficiency virus (HIV) can be as sensitive and specific as enzyme-linked immunosorbent assay (ELISA) for screening of donated blood in developing countries. Currently, however, specimens which test positive on a screening assay must still be confirmed by Western blot (immunoblot), a method which is not feasible in most developing-country laboratories. We examined whether a testing hierarchy which utilizes neither conventional ELISA nor Western blot can be reliably used for screening and confirmation of HIV infection in a high-risk population. In a retrospective analysis of 3,878 specimens which were screened for antibody to HIV in Kinshasa, Zaire, we observed that a testing hierarchy consisting of duplicate HIVCHEK screening assays followed by duplicate Serodia-HIV confirmatory assays resulted in correct confirmation of all ELISA- and Western blot-positive specimens. We conclude that such a testing hierarchy can produce highly accurate results for identification of positive specimens in routine HIV testing and provides a practical alternative to conventional methods of HIV screening and confirmation.

Transfusion of unscreened blood continues to be an important route of transmission of human immunodeficiency virus (HIV) in many developing countries (3, 7). Recently, a number of instrument-free assays to detect the presence of antibody to HIV have been described, allowing minimally trained laboratory staff to rapidly and accurately screen donated blood prior to transfusion (1, 5–7, 9; P. K. Chun, E. C. Albert, R. L. Cybulski, and J. P. Galvin, Abstr. Int. Symp. African AIDS, 1987). As with enzyme-linked immunosorbent assay (ELISA) methods, however, specimens which test positive on such assays must still be confirmed by the time-consuming, costly, and highly technique-dependent method of Western blotting (immunoblotting).

We have recently shown that rapid, instrument-free assays can replace the ELISA as the primary method of blood screening for HIV in a developing country (6). For five such assays in our study, sensitivity ranged from 77.1 to 97.2% and specificity ranged from 97.9 to 99.6% compared with Western blot results. Two of the assays compared favorably with a conventional ELISA method which in our study yielded a sensitivity of 95.8% and a specificity of 99.6%. In the present paper, we analyze the data of that study from the perspective of using one rapid, instrument-free HIV screening assay to confirm the results of another and demonstrate that not only the conventional ELISA but also the Western blot can be eliminated in developing-country transfusion centers.

MATERIALS AND METHODS

Serum and collection of specimens. Serum was collected from 4,000 blood donors between November 1987 and May 1988 as part of a blood donation screening program at Mama Yemo (n = 3,090) and Ngaliema (n = 910) hospitals in Kinshasa, Zaire. Specimens were assigned randomly generated code numbers when they were obtained and were tested within 36 h of donation. When retesting or Western blotting was performed, specimens were held for as long as 1 week at 4°C without added preservatives. Six screening assays from different manufacturers, including a competitive microdilution ELISA method, were performed on each specimen. All initially positive specimens as well as every tenth negative specimen were subjected to Western blot for confirmation. The two rapid, instrument-free assays which compared most favorably with ELISA are considered in this analysis.

Laboratory methods. Serodia-HIV (Fujirebio Inc., Tokyo, Japan) is a gelatin particle agglutination assay which uses a purified lysate of HIV type 1 (HIV-1)-infected cells as antigen (9). A 25-μl portion of a test specimen was diluted 1:4 in microdilution well 1 and serially diluted twofold through microdilution wells 2 and 3. Serum in well 2 was mixed with unsensitized (negative-control) particles and serum in well 3 was mixed with HIV-1-sensitized particles by using disposable droppers. The settling pattern was observed after a 2-h incubation at ambient temperature.

HIVCHEK (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) is a second-generation immunofiltration assay which uses a recombinant HIV-1 envelope protein as antigen (Chun et al., abstract). One drop of undiluted serum

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ing assay and Serodia-HIV was used as the confirmatory assay (Table 1); six of these, all from the indeterminate category, were true positives as judged by Western blot. When Serodia-HIV was used for screening and HIVCHEK was used for confirmation, there were 72 repeatably reactive specimens which could not be confirmed, 5 of which were true positives by Western blot. Unless demonstrated otherwise, these unconfirmed specimens would have to be subjected to additional confirmatory testing, such as Western blot.

We then examined how many of those positive specimens which could not be confirmed as positive were repeatably negative on the rapid confirmatory assay yet Western blot positive. For the combination of HIVCHEK screening and Serodia-HIV confirmation, there were no such specimens, while for the inverse combination, only one was found (Table 1). Thus, had HIVCHEK been used in duplicate as the screening assay and Serodia-HIV been used in duplicate as the confirmatory assay on this sample population, no true positives would have been missed because of incorrect confirmatory test results. If the inverse combination had been used, however, only one true positive specimen would have been missed. Thus, for this category (11 for HIVCHEK screening plus Serodia-HIV confirmation and 62 for Serodia-HIV screening plus HIVCHEK confirmation), an argument may be made that no further testing of these samples is warranted.

There were a few initially positive specimens which were negative on the first confirmatory test and positive on the repeat test or vice versa. Nine specimens fell into this indeterminate category when duplicate HIVCHEK assays were used to screen and duplicate Serodia-HIV assays were used for confirmation (Table 1). With the inverse combination of Serodia-HIV screening and HIVCHEK confirmation, there were 10 indeterminate specimens from a total of 3,878 specimens. Six of the 9 specimens in the former category and 5 of the 10 specimens in the latter category were Western blot positive. This result indicates that all indeterminate specimens should be blotted.

Specificity of the dual rapid assay system. We then asked how many specimens were repeatably positive on the screening assay and repeatably positive on the confirmatory assay and yet were Western blot negative. No false-positives were found, regardless of which assay was used for screening and which was used for confirmation (Table 1).

DISCUSSION

Adoption of routine screening of donated blood for HIV antibodies has been hampered in many developing countries by the lack of blood-banking facilities, equipment, technically trained staff, and financial resources. Yet the demand for blood in these countries is often great and seroprevalence of HIV infection in the general population can be significant, making transfusion an important route of transmission (2).

The availability of rapid, instrument-free assays for the detection of antibody to HIV may facilitate blood screening, provided that the assays are priced affordably and can be used reliably by minimally trained laboratory staff. We and others have evaluated a variety of these assays under a range of laboratory settings and found that under many conditions, performance as good as or better than that of conventional ELISA can be obtained (1, 4, 6, 8; L. Ndongala, J. Rowland, H. Francis, M. P. Duma, M. Kasali, and T. C. Quinn, Abstr. IIIrd Int. Conf. AIDS, 1:166, 1987).

While a single or repeatably positive result on a rapid, instrument-free assay may be sufficient to justify eliminating a unit of blood from the supply, additional confirmatory testing is mandated if individuals are to be informed of their serological status. To date, Western blotting has constituted the major means of confirming seropositivity. Unfortunately, Western blotting is difficult to implement on a routine basis in developing countries, since it is costly, poorly standardized, and highly technique dependent. The availability of commercial Western blot kits can be expected to do little to make this technique more accessible, as the kits are too expensive for routine use in the majority of developing-country laboratories.

With this in mind, we subjected the data from our comparative evaluation of five rapid assay kits in Kinshasa, Zaire, to reanalysis from the perspective of using one rapid assay to confirm the results of another. Specifically, we wanted to know if a testing hierarchy which included neither conventional ELISA nor Western blot could ensure that no true-positive specimens were unconfirmed and that no true-negative specimens were erroneously confirmed as positive. Although we looked at multiple possible hierarchies and all possible combinations of the five assays compared in our original study, we report here data for only one possible hierarchy using only one possible pairwise combination of assays.

Had specimens been screened in duplicate by using the HIVCHEK assay and had all repeatably positive specimens been confirmed in duplicate by Serodia-HIV, no specimen would have been confirmed incorrectly, referring to Western blot as the "gold standard." The inverse combination (Serodia-HIV screening plus HIVCHEK confirmation) would have incorrectly identified only a single specimen. It should be noted that this testing hierarchy differs from that employed by blood banks in the United States, not only with respect to the testing method but with respect to two other important variables as well. First, we assumed that the confirmatory assay would be performed in duplicate, whereas in standard blood-banking practices only a single confirmatory assay is routinely done. Second, both screening assays were performed on the same specimen sample rather than on a fresh specimen, as is standard United States blood bank procedure. Therefore, an error in sample identification during screening could have been propagated. We recommend that a fresh portion of specimen be used for retesting whenever a specimen gives an initial positive result.

Another point of interest, from an economic and a public health perspective, concerned the number of specimens which were repeatably positive on duplicate screening but indeterminate on duplicate confirmatory assay, i.e., neither repeatably positive nor repeatably negative. This number is important from an economic standpoint because of the necessity of Western blotting these specimens or testing an additional specimen drawn at a later date in order to confirm the serological status of the donor and to counsel that donor appropriately. To be maximally effective, the confirmatory assay chosen should result in a minimum of indeterminate specimens. Both HIVCHEK and Serodia-HIV seem well suited as confirmatory assays, since their use resulted in identification of only 9 and 10 specimens, respectively, as indeterminate. This number may have public health ramifications as well, since in many developing countries ensuring individual patient follow-up is difficult.

HIVCHEK and Serodia-HIV are distinguished by assay principle as well as source of antigen. HIVCHEK is an immunofiltration assay which incorporates a recombinant

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Attachment 8

DiaTech seeks to develop appropriate diagnostic tests for use in developing countries. Existing technologies will be adapted and/or new technologies developed, including the means for production, distribution and effective use. DiaTech is carried out by the Program for Appropriate Technology in Health (PATH) in collaboration with the United States Agency for International Development (USAID). A Technical Advisory Group (TAG), composed of distinguished scientists, evaluates research and development proposals for funding and monitors work in progress.

DIATECH

Diagnostic Technology for Community Health

DIATECH GOALS

The DiaTech program seeks to improve techniques for diagnosis of major infectious diseases threatening children and adults in the developing world. Diagnostic tests play important roles in health [illegible]:

- Improvement of individual therapy
- Epidemiologic evaluation of disease control strategies
- Disease surveillance
- Identification of chronic carriers.

Available diagnostic methods are often too expensive or otherwise inappropriate for developing world conditions. The goal of DiaTech is to produce rapid, accurate, and inexpensive tests which can be used without extensive training or sophisticated equipment.

TARGET DISEASES

The diseases targeted by the DiaTech program are:

- Malaria
- Diarrheal Diseases
- Acute Respiratory Infections
- Tuberculosis
- Typhoid Fever

DIATECH SOLUTIONS

New biotechnologies and techniques now offer hope for practical clinical tests in developing countries. Developments during the first four years of DiaTech include:

- An immunoassay to monitor the concentration of quinine in the blood of malaria patients.
- A system to detect blood and malaria infection.
- An assay which amplifies malarial DNA in patient specimens by the polymerase chain reaction.
- A portable hemoglobinometer to detect anemia by the rapid measurement of hemoglobin.
- Evaluation of rapid screening assays to detect antibodies to HIV.

During the fifth year, efforts will focus on the optimization, scale-up, and field testing of diagnostic kits, including assays to detect E. histolytica, cholera, and Shigella bacteria in stool specimens, malarial antigens in serum or urine, mycobacterium tuberculosis antigen in sputum and urine.

Future activities include technology transfer of diagnostic manufacturing capability, introduction into health care programs, training in the use of diagnostic kits, distribution, and impact evaluation of tests.

DIATECH PROGRAM PLAN

Research and development has been conducted under subagreements with researchers at universities and companies throughout the United States and abroad. Primary subagreements have been established with The Johns Hopkins University and with the Queen Saovabha Memorial Institute of The Thai Red Cross Society. Additional diagnostic technologies for target diseases are being sought. DiaTech also collaborates with Ministries of Health, research agencies, and companies in the developing world as technologies are field tested and introduced.

PATH

The Program for Appropriate Technology in Health (PATH) is a nonprofit, nongovernmental organization dedicated to improving the availability, effectiveness, acceptance, and safety of health products and technologies in developing countries. PATH identifies new health technologies or improves existing ones by adapting them to the culture and conditions of specific countries.

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