IRAQI
STANDARD OPERATING PROCEDURES (SOP)

POLIO LABORATORY SOP

DECEMBER 2014

SOP: NPL
National Polio Laboratory
IRAQ

DISCLAIMER
This guideline has made possible through support provided by the U.S. Agency for International Development (USAID) under Primary Health Care Project in Iraq (PHCPI) implemented by University Research Co., LLC. This guideline has been developed in Iraq in close collaboration with the Ministry of Health (MoH) in December 2014.
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1. ACRONYMS

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<tbody>
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<td>AFP</td>
<td>Acute Flaccid Paralysis</td>
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<td>ASO</td>
<td>AFP Surveillance Officer</td>
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<tr>
<td>CIF</td>
<td>Case investigation form</td>
</tr>
<tr>
<td>CVDPV</td>
<td>Circulating vaccine-derived poliovirus</td>
</tr>
<tr>
<td>Elisa</td>
<td>Enzyme-Linked Immuno Sorbent Assay</td>
</tr>
<tr>
<td>EMR</td>
<td>Eastern Mediterranean Region</td>
</tr>
<tr>
<td>EPI</td>
<td>Expanded Program for Immunization</td>
</tr>
<tr>
<td>EPID</td>
<td>Number &quot;Epidemiological&quot; number (AFP case identification number)</td>
</tr>
<tr>
<td>ITD</td>
<td>Intratypic differentiation</td>
</tr>
<tr>
<td>L20B</td>
<td>Genetically engineered mouse cell line express human polio Virus receptor</td>
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<tr>
<td>NPSU</td>
<td>National Polio Surveillance Unit</td>
</tr>
<tr>
<td>NPEV</td>
<td>Non-Polio Entero Viruses</td>
</tr>
<tr>
<td>NPL</td>
<td>National Polio Lab</td>
</tr>
<tr>
<td>OPV</td>
<td>Oral Polio Vaccine</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>RDA</td>
<td>Cell line derived from human rhabdomyosarcoma</td>
</tr>
<tr>
<td>RRL</td>
<td>Regional Reference Laboratory</td>
</tr>
<tr>
<td>WHO/EMRO</td>
<td>World Health Organization/ Eastern Mediterranean regional Office</td>
</tr>
<tr>
<td>WPV</td>
<td>Wild Poliovirus</td>
</tr>
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</table>
2. Preface

The United States Agency for International Development (USAID) has funded the Primary Health Care Project in Iraq (PHCPI) to assist the Iraqi Ministry of Health (MOH) achieve its strategic goal of quality primary health care (PHC) services in the country. PHCPI supports the MOH in three key components: 1) strengthening health management systems, 2) improving the quality of clinical services, and 3) encouraging community involvement to increase the demand for and use of PHC services. Since March 2011, PHCPI has assisted the MOH to put in place key building blocks to strengthen the health management systems and to support the delivery of quality PHC services at the community and facility levels. PHCPI has worked to build the capacity of the MOH and primary health care centers (PHCCs) to respond to the needs of the Iraqi population and to assist the MOH in achieving its country-level health priorities, such as meeting its Millennium Development Goals (MDGs) by 2015. Interventions have included updating clinical guidelines, training health providers at the PHC level, implementing supportive supervision and quality improvement models and referral systems to improve the quality of primary care services, particularly for maternal and child health.

In October 2013, a modification to PHCPI’s technical scope of work had the project re-focus its efforts to further help the MOH accelerate the achievement of MDGs 4 and 5 and, in March 2014, Iraq confirmed its first case of polio (poliomyelitis) in more than a decade. For PHCPI, awareness and improvement of vaccination coverage has been a key element in addressing MDG 4 and PHCPI specifically addresses this goal with activities providing training to health care providers, traditional birth attendants, and community partners on the importance of proper nutrition and vaccinations in the healthy development of infants and young children.

PHCPI has developed polio-related technical support through the development of an Acute Flaccid Paralysis (AFP) Field Manual. AFP is the most common sign of polio and is used as a surveillance indicator during polio outbreaks. This manual is used by communicable disease surveillance staff to assist primary health care workers to better diagnose, manage and report cases of AFP. To further surveillance and response support, PHCPI, together with the MOH, has developed this standard operating procedure (SOP) to streamline laboratory procedures for the handling and diagnosis of poliomyelitis for the National Polio Laboratory allowing for more accurate detection and response to prevent the re-emergence of polio in Iraq. This document will be updated and approved annually, or as required.
3. Acknowledgements

University Research Co., LLC wishes to thank everyone who has contributed on development, and integration of this guideline. They have given generously of their time and experience. Significant contributions to the development of this guideline were made by USAID/Primary Health Care Project in Iraq (PHCPI) field team, Dr. Hala Jassim, Chief of Party; Dr. Ahlam Khadhim, Immunization Advisor and Saif Ali and URC HQ team, Dr. Neeraj Kak and Taylor Price. Special thanks are due to The Ministry of Health (MoH) Public Health Directorate headed by Dr. Ziad Tariq and the technical working group who contributed time and experience in developing these SOP.

Ministry of Health Technical Working Group:

- Dr. Faisal Ghazi Nasser, PhD Virology, NPL, CPHL, MoH
- Mrs. Rafah Ali Salih, BSc, NPL, CPHL, MoH
- Mr. Jawad Shahrain /Quality Management Section, Directorate of Public Health, MoH
4. Introduction

1) National Polio Laboratory (NPL)

National Polio Laboratory (NPL) is a unit within the virology department at Central Public Health Laboratory (CPHL) that was established during 1982. The laboratory is responsible for testing all Acute Flaccid Paralysis (AFP) cases specimens that forwarded from all Iraqi districts to confirm or deny the presence of polio virus in these cases.

The NPL received World Health Organization (WHO) accreditation in 1999 which has continuously been updated annually. The NPL is one of the WHO/EMRO laboratory networks that participates through technical supervision and assisting laboratory diagnosis of wild polio virus among AFP cases. Additionally, the NPL participates in proficiency testing, done by WHO/EMRO, which is part of quality assurance and is an important step in receiving accreditation.

NPL IRAQ has set a “turn-around time” of 14 days or less as a goal for processing specimens, i.e. the primary isolation result is reported to the surveillance program no more than 14 days from the time the specimen is received at the laboratory. Results of these tests are sent directly to the field through email and to the National Polio Surveillance Unit (NPSU), who in turn follow these results. All positive isolates, which are primarily isolated at IRAQ NPL, and are suspect wild type or a vaccine derived viruses that are accidently isolated from AFP cases after vaccination with live, attenuated Sabine strain polio vaccine. The results of WHO/RRL are delivered directly from the laboratory and emailed to the field and the NPSU, who in turn follow-up these results.

This guideline is prepared for the current needs of the National Polio Laboratory to follow SOPs that are documented, updated and approved annually, or as required. This document is key in streamlining laboratory procedures for any individual working in the lab and those responsible for the collection of samples, transportation and referral of samples to laboratories both inside and outside of Iraq.

The following are the basic indicators of surveillance quality that should be carried out by the ASO on a regular basis.

- Stool specimens reaching NPL IRAQ (WHO accredited laboratory) within 72 hours of stool collection (target >80%).
- Stool specimens reaching laboratory in good condition (target >80%).
- Stool specimens with a turnaround time <14 days (target >80%).
- Stool specimens from which non-polio enteroviruses (NPEV) were isolated (target >10%).
2) Suspected polio case

Any case of Acute Flaccid Paralysis (AFP) in a person under 15 years of age for any reason other than severe trauma or paralytic illness in a person of any age in whom polio is suspected by the physician.

3) Laboratory-confirmed polio virus sample

A sample from an acute flaccid paralysis patient, associated with the isolation of wild poliovirus or circulating vaccine-derived poliovirus (CVDVPV) within a laboratory.

4) NPL responsibilities

1. Conduct specific laboratory tests for acute flaccid paralysis cases suspected to be infected with wild polio virus and also discriminate non-polio enteroviruses (NPEV) and Rota virus which may cause diarrhea in children below 5 years of age.
2. Follow-up to the updated basic methods of work by internationally approved protocols.
4. Conduct technical assessments and officially certify laboratory devices used inside NPL and other laboratory supplies sent from the MoH before distribution to all related laboratories.
5. Collaboration with universities and other governmental sectors to conduct relevant research and laboratory work within the framework of public health.
6. Cooperation with universities in the laboratory practical training for students of the faculties of health sciences and related technical colleges.
7. Hold training workshops especially on acute flaccid paralysis, as well as laboratory investigation of cases of diarrhea and attended by employees of the health institutions of all provinces.
8. Hold regular weekly meeting with the doctor epidemiological specialist responsible for monitoring cases of acute flaccid paralysis.
9. Participate in the annual proficiency test implemented by the World Health Organization / Regional Office for all national laboratories in the region.
5. Specimens Manipulation

Standard Operating Procedures (SOPs) for specimen manipulation, adequate stool sample collection, and collation and transportation inside Iraq:

IRAQ SOP #: NPL STOOL 028:

<table>
<thead>
<tr>
<th>Standard Operating Procedure SOP # : NPL STOOL 028</th>
<th>Version: 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title: Adequate stool sample collection ,collation and transportation inside IRAQ</td>
<td></td>
</tr>
<tr>
<td>Author: WHO Manual 2004.</td>
<td>Approved by:</td>
</tr>
<tr>
<td>Review Date: 30 Oct 2014</td>
<td>Dr. Faisal Ghazi Alhamdani</td>
</tr>
</tbody>
</table>

Objective:

To describe the procedure utilized in the collection of the recommended stool specimens used in the isolation of Polio and Non-polio Entero viruses (NPEVs).

Reference:

WHO Lab Manual 2004

Scope:

This document describes procedure used in all Iraq Health Centers to collect samples in surveying for the presence of wild polio virus among AFP cases.

Responsibility:

This procedure is carried out by a trained health worker under the supervision of neurologist in a hospital and this in coordination with EPI manager at province and national levels.

Materials and equipment:

- Stool collection polio kit
- Water-resistant, indelible pen.
- Stool shipment carrier/fridge (cold box)
- Domestic freezer - 20°C.
- Frozen ice packs

Recommended Specimen for NPL IRAQ Diagnostic Tests:

Stool is recommended in every case of AFP. Virus usually can be found in the feces from onset to up to 8 or more weeks after paralysis, with the highest probability of detection during the first 2 weeks after paralysis onset. Avoid using rectal swabs and enema.
Collection procedure:

The following steps are required:

- Use a clean plastic screw-cap container (stool collection polio kit).
- A label with the name, identification number of the case (the EPID number), the specimen number and the date of collection should be pasted on the side of the container. Use a water-resistant, indelible pen to label the specimen containers.
- If possible, collect fresh stool from the child’s diapers, or get the child to defecate onto a clean paper.
- Collect a volume of stool to a minimum size of 2 gm up to a maximum size of one adult thumb size (2-8 grams). This amount of stool will allow additional testing, if necessary.
- It is not preferred to get rectal swabs or using enema for stool sampling only rarely can rely on when it is impossible to get stool sample.
- Do not fill the container up to the brim. Do not soil the rim of the container.
- After collection, immediately place the container in the stool shipment carrier/fridge. Enema is not a preferred method for stool collection.
- Adequate stool: Two specimens collected within 14 days of paralysis onset and at least 24 hours apart; each specimen must be of adequate volume (2–8 grams) and arrive at a WHO-accredited laboratory in good condition (i.e., no desiccation, no leakage, with adequate documentation and evidence that the cold chain was maintained.
- Filling the case investigation form (CIF)(FORM 2) by trained physician (Appendix 1).
- Filling the contact investigation form (FORM 14) by trained physician (Appendix 3).
- Fill out Specimen Collection Form (FORM 3) (Appendix 2). Collect two stool samples from the AFP case child at a minimum interval of 24 hours apart and only one stool sample from contact.
- All samples should be stored in -20°C (domestic freezer) and should be shipped to the NPL within no more than 72 hours in a cold box supplied with frozen ice packs.
### 6. List of Standard Operating Procedures (SOP's) inside NPL IRAQ

<table>
<thead>
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<th>SOP Code</th>
<th>Description</th>
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<td>NPL SAFETY 1</td>
<td>Safety Precautions for diagnostic Virology</td>
</tr>
<tr>
<td>NPL TC 002</td>
<td>Passaging of the cell culture.</td>
</tr>
<tr>
<td>NPL TC 003</td>
<td>Cell counting.</td>
</tr>
<tr>
<td>NPL TC 034</td>
<td>Cell freezing.</td>
</tr>
<tr>
<td>NPL TC 005</td>
<td>Cell thawing (Recovery of cells).</td>
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<tr>
<td>NPL TC 006</td>
<td>Preparation of laboratory quality – control standard.</td>
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<tr>
<td>NPL TC 007</td>
<td>Titration of quality – control standard.</td>
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<td>NPL TC 008</td>
<td>Adapting newly received cells to local conditions.</td>
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<tr>
<td>NPL TC 009</td>
<td>Interpreting QC standard by Cell Sensitivity Testing and naphthalene stain</td>
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<tr>
<td>NPL TC 010</td>
<td>Establishment of cell banks.</td>
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<tr>
<td>NPL TC 011</td>
<td>Sterility testing.</td>
</tr>
<tr>
<td>NPL TC 012</td>
<td>Washing of glassware for using in tissue culture laboratory</td>
</tr>
<tr>
<td>NPL AFP 013</td>
<td>Receipt and recording of the receipt specimens (Documentation of specimens).</td>
</tr>
<tr>
<td>NPL AFP 014</td>
<td>Preparation of fecal samples for virus isolation (Processing of specimens).</td>
</tr>
<tr>
<td>NPL AFP 015</td>
<td>Cell culture media preparation</td>
</tr>
<tr>
<td>NPL AFP 016</td>
<td>PBS preparation</td>
</tr>
<tr>
<td>NPL AFP 017</td>
<td>Isolation of poliovirus by using cell culture techniques.</td>
</tr>
<tr>
<td>NPL AFP 018</td>
<td>Preparation of RIVM poliovirus typing anti-sera.</td>
</tr>
<tr>
<td>NPL AFP 019</td>
<td>Identification of poliovirus by micro-neutralization method.</td>
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<tr>
<td>NPL AFP 020</td>
<td>Identification of Enterovirus isolates</td>
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<td>NPL AFP 021</td>
<td>Preparation of Enterovirus typing antisera</td>
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<td>NPL MAN 022</td>
<td>AFP Data management</td>
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<td>NPL MAN 023</td>
<td>Reporting of Virus Isolation Results</td>
</tr>
<tr>
<td>NPL MAN 024</td>
<td>Periodic review update and evaluation of Compliance</td>
</tr>
<tr>
<td>NPL ENTERO 025</td>
<td>Storage of Fecal samples and suspensions.</td>
</tr>
<tr>
<td>NPL FTA 026</td>
<td>Sample Application to FTA Micro Cards</td>
</tr>
<tr>
<td>NPL FTA 027</td>
<td>NPL Records for sample registry and reagents with glassware consumption</td>
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<tr>
<td>NPL FTA 028</td>
<td>Adequate stool sample collection, collation and transportation inside IRAQ</td>
</tr>
</tbody>
</table>
1) Safety Precautions for Diagnostic Virology SOP #: NPL SAFETY 001

<table>
<thead>
<tr>
<th>Standard Operating Procedure SOP #: NPL SAFETY 001</th>
<th>Version: 2</th>
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<tbody>
<tr>
<td>Title: Safety Precautions for Diagnostic Virology</td>
<td></td>
</tr>
<tr>
<td>Author: Dr. J. Michael Miller on June 1, 2004.</td>
<td>Approved by: Dr. Faisal Ghazi Alhamdani</td>
</tr>
<tr>
<td>Review Date: 30 Oct 2014</td>
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**Objective:**
To prevent laboratory acquired infections that may occur when microorganisms are inadvertently ingested, inhaled, or introduced into the tissues.

**Reference:**
Laboratory biosafety manual Third edition /WHO - 2004
Dr. J. Michael Miller on June 1, 2004.

**Scope:**
This document describes precautions recommended to be followed inside lab and to record the details of all events that may occur, and to report them.

**Responsibility:**
This procedure is performed by all NPL staff supervisors trained on safety precautions under the supervision of the lab director.

**Safety Precautions:**
- Always be careful. Most of the viruses handled in this laboratory could be pathogenic.
- As a basic rule, do not put anything into your mouth. Smoking, eating, or drinking is not permitted in the laboratory. No mouth pipetting will be allowed on any specimen or reagent of any kind at any time.
- Do not lay personal articles such as handbags, eyeglasses, etc. on the workstations.
- All manipulation of materials which may contain live virus must be performed in a Class II biological safety cabinet.
- Work areas are to be sprayed down at the beginning and end of the day with a mild bleach solution. Biological safety cabinets are to be wiped down immediately after use with 0.5% sodium hypochlorite (10% household bleach) and rinsed with clean water or alcohol.
- Gloves are to be worn at all times when setting up specimens.
- Wash hands well with suitable scrub solution before leaving the laboratory or in case of contamination. Frequent hand washing is one of the most effective procedures available in avoiding laboratory acquired infections. Don't forget to change gloves if they become contaminated.
• Accidents involving cuts or punctures with potentially infected needles, glassware, etc. should be promptly washed with disinfectant soap and water, and all injuries or unusual incidents should be reported immediately to the supervisor. To minimize finger sticks, do not recap needles.

• Techniques that tend to produce aerosols should be avoided. Vortexing and centrifugation should be done in closed containers. Gauze should be used to remove the tops on tubes of bloods, and these specimens should be opened under a hood. Needles should never be cut. Place the whole syringe/needle in a puncture proof container.

• All discarded plates, tubes, clinical samples or other contaminated materials are to be placed in the disposal containers at each bench. These materials are placed into special plastic bags and must be autoclaved prior to disposal. Special disposal boxes must be used for non-contaminated needles, glass, etc. to minimize the risk of injury to housekeeping staff.

• Do not pick up pans, baskets, or other containers, which have been overfilled with contaminated material. Avoid overfilling such containers. If over-filling has occurred, redistribute prior to movement and disposal.

• Containers of contaminated material should be carefully transported to the autoclave room and autoclaved prior to disposal.

• If a culture is dropped or spilled, pour 0.5% sodium hypochlorite over the contaminated area; cover with paper towels and let stand for at least 15 minutes. Using paper towels, dispose of the contaminated material in the autoclave discard.

• In the event of a spill of contaminated material, warn others in the laboratory and notify the supervisor.

• Use care in handling flammable material. Bulk flammable material must be stored in the safety cabinet. Small amounts of these materials, such as ethyl acetate, ethyl alcohol, and methanol, can be stored in safety containers.

• Know the location of fire extinguishers, fire blankets, and showers. Fire safety instructions and evacuation routes are posted. Each employee has the responsibility to become familiar with these instructions.

**Emergency Safety Information**

• Report all accidents and injuries to the Supervisor. Routinely, the Supervisor will arrange for the employee to go to the Employee Health Clinic accordingly.

• If an accident has occurred that may pose a hazard to others in the laboratory or those working within the corridor. Alert coworkers of the accident, use emergency containment equipment located in cabinets in the hallway, notify the supervisor, and the Office of Biosafety committee at CPHL, if necessary.

• In case of critical emergency in Baghdad call 122 for medical emergency help.

• Medical emergency information on laboratory workers is in a packet attached to the inside of each laboratory door.
<table>
<thead>
<tr>
<th>Emergency calls in IRAQ</th>
<th>Contact Number</th>
<th>مكالمات الطوارئ في العراق</th>
</tr>
</thead>
<tbody>
<tr>
<td>Police</td>
<td>104</td>
<td>الشرطة</td>
</tr>
<tr>
<td>Medical ambulance</td>
<td>122</td>
<td>الامناف</td>
</tr>
<tr>
<td>Medical advice</td>
<td>404</td>
<td>المشورة الطبية الطارئة</td>
</tr>
<tr>
<td>Fire</td>
<td>115</td>
<td>الحريق</td>
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2) Routinely passaging cell culture. SOP #: NPL – TC

<table>
<thead>
<tr>
<th>Standard Operating Procedure</th>
<th>SOP #: NPL - TC 002</th>
<th>Version: 1.4</th>
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<tbody>
<tr>
<td>Title: Routinely passaging cell culture.</td>
<td>Author: WHO</td>
<td>Approved by: Dr. Faisal Ghazi Alhamdani</td>
</tr>
<tr>
<td></td>
<td>Review Date: 30 Oct 2014</td>
<td></td>
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</table>

**Objective:**
To describe the procedure utilized in the passaging of the cell culture used in the isolation of Polio and Enterovirus.

**Reference:**
WHO Lab Manual 2004,
Accreditation recommendation 2006.
Laboratory diagnosis techniques for global polio eradication NIID, Japan training course 2008.

**Scope:**
This document describes procedure used in the NPL – Iraq to passaging the cell for weekly splitting and requested cell culture Passaging.

**Responsibility:**
This procedure is carried out by two experts (Supervisor) under the supervision of lab director. Materials and equipment:

**Materials:**
- Culture flasks with confluent monolayers of L20B or RDA cells (NB: cultures that have been confluent for longer than two weeks should not be used);
- Phosphate buffered saline (PBS) without calcium and magnesium;
- Trypsin (or trypsin/Versene);
- Growth medium;
- Trypan blue
- Different sizes of pipettes.

**Equipment:**
- Biological safety cabinet class II (Telstar and Microflow).
- 36°C incubator (Memert/ Germany).
- Inverted Microscope (Olympus).
- Cell counting chamber (Improved Neubauer)
- Cell culture tubes and flasks (Nunc);
- Pipette aid (Acu-Jet)
Good laboratory practice:
Work with one cell line at a time. The seeding levels for various culture vessels are provided as a guide. The optimum seeding level may differ according to cell line, batch of cells (work with one cell line at time). Cell counting should therefore be used to determine the appropriate seeding density or split ratio for new batches of cells or whenever there are changes in major media components. Cell counting should also be used when preparing cell culture tubes for virus isolation to ensure that cell. Procedure:

Routinely weekly cell culture splitting.

- The splitting of the cell line carried out once per week, to ensure that sufficient flasks will be available to meet work load demands for cultures for sample inoculation and preparation of cell suspension.

- The concentration of the splitted flasks for the L20b cell line will be 80,000 cells per flask.

- The concentration of the splitted flasks for the RDA cell line will be 50,000 cells per flask.

- Examine the cells for quality (i.e. an entire monolayer of healthy cells) and absence of contamination as determined by visual examination.

- Decant growth medium from the cell culture flask and gently wash the confluent cell layer twice with Ca and Mg free PBS.

- Add 0.25% trypsin solution (or equal parts of 0.25% trypsin and 1:5000 Versene solutions) in PBS to the monolayer, dispersing it evenly. (A volume of 0.5 ml is adequate for a 25 cm² flask.)

- Place the flask in a 36°C incubator until the cells detach from the surface: this may be assisted by tapping the side of the flask a few times. Check for complete detachment of cells by examining under an inverted microscope.

- Re-suspend the cells in 25 ml growth medium for 75 cm² flask, which halts the action of the trypsin.

- Gently aspirate the suspension a few times through a fine Pasteur pipette to break up cell clumps.

- Dilute 0.2 Trypan blue to 0.2 cell suspension to count the cells.

- Dilute with growth medium to the desired concentration based either upon counting the cells (see above) or upon a pre-determined split ratio (usually 1:3 or greater).

- The optimum split ratio of L20B cells is 100,000 cells per flask, and 80,000 cells per flask for RDA to obtain confluent monolayers of cells in appropriate time must be determined for each new batch of cells received in the laboratory. The split ratio will quickly become apparent as experience is gained with each culture.

- Seed fresh culture flasks, cap tightly, and place in a 36°C incubator to use in the appropriate time according to cells concentration in routinely cell culture Passaging.
<table>
<thead>
<tr>
<th>L20B Routinely weekly cell culture splitting.</th>
<th>L20B Routinely requested cell culture passaging.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunday</td>
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<tr>
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<td>100,000 Cells/flask</td>
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<td>120,000 Cells/flask</td>
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<td>Prepared suspension</td>
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<td>130,000 Cells/flask</td>
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<td>120,000 Cells/flask</td>
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<td></td>
<td>Prepared suspension</td>
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<td>130,000 Cells/flask</td>
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<td>Prepared suspension</td>
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<td></td>
<td>Prepared suspension</td>
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<tr>
<td></td>
<td>120,000 Cells/flask</td>
</tr>
</tbody>
</table>

**Routinely requested cell culture Passaging**

- The L20b and RDA Cell line culture usually needed for inoculation 3 times per week for AFP and Environmental investigation labs.
- According to cells concentration, appropriate concentration used for the passaging.
- Examine the cells for quality (i.e. an entire monolayer of healthy cells) and absence of contamination as determined by visual examination.
- Decant growth medium from the cell culture flask and gently wash the confluent cell layer twice with Ca and Mg free PBS.
- Add 0.25% trypsin solution (or equal parts of 0.25% trypsin and 1:5000 Versene solutions) in PBS to the monolayer, dispersing it evenly. (A volume of 0.5 ml is adequate for a 25 cm² flask.)
- Place the flask in a 36°C incubator until the cells detach from the surface: this may be assisted by tapping the side of the flask a few times. Check for complete detachment of cells by examining under an inverted microscope.
- Re-suspend the cells in 25 ml growth Medium for 75 cm² flask, which halts the action of the trypsin.
- Gently aspirate the suspension a few times through a fine Pasteur pipette to break up cell clumps.
- Dilute 0.2 Trypan blue to 0.2 cell suspension to count the cells.
- Dilute with growth medium to the desired concentration based either upon counting the cells (see below) or upon a pre-determined split ratio (usually 1:3 or greater).
- The optimum split ratio (determined by cell counting) required different concentration of cells (120,000 cells per flask for L20B and 80,000 cells per flask for RDA); to obtain confluent monolayers of cells in appropriate time must be determined for each new batch of cells received in the laboratory. The split ratio will quickly become apparent as experience is gained with each culture.
- Seed fresh culture flasks and tubes, cap tightly, and place in a 36°C incubator.

Reference documents:

- SOP of operation, maintenance & calibration of Biological safety cabinet class II.
- SOP of operation, maintenance & calibration of 36°C incubator
- SOP of operation, maintenance & calibration of Pipette aid.
- SOP of operation, maintenance & calibration of Inverted Microscope.

Reporting:

- Put the data in the cell line passaging record form.
- Record the data in the daily work folder.
- Supervisor must be informed as well as the head of the lab.
3) Cell counting SOP NPL TC 003

<table>
<thead>
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<th>Standard Operating Procedure</th>
<th>SOP #: NPL TC 003</th>
<th>Version: 1.0</th>
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<td>Title: Cell counting</td>
<td></td>
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</tr>
<tr>
<td>Author: WHO Lab Manual 2004</td>
<td>Approved by: Dr. Faisal Ghazi Alhamdani</td>
<td></td>
</tr>
<tr>
<td>Review Date: 30 Oct. 2014</td>
<td></td>
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</tr>
</tbody>
</table>

Objective:
To describe the procedure utilized in the determination of the accurate numbers in a cell suspension.

Reference:

Scope:
This document describes procedure used in the NPL for enumerating cell concentration using “improved Neubauer” haemocytometers.

Responsibility:
This procedure is carried out by two expert supervisors under the overall supervision of the lab director.

Materials:
- Culture flasks with confluent monolayers of L20B or RDA cells (NB: cultures that have been confluent for longer than two weeks should not be used);
- Phosphate buffered saline (PBS) without calcium and magnesium;
- Trypsin (or trypsin/Versene);
- Growth medium;
- Trypan blue
- Different sizes of pipettes.

Equipment:
- Biological safety cabinet class II (Telstar).
- 36°C incubator (Memert).
- Inverted Microscope (OLympus).
- Cell counting chamber (Improved Neubauer)
- Cell culture tubes and flasks (Nunc);
- Pipette aid (accu-jet)

Good laboratory practice: Work with one cell line at a time. Procedure:

- Dilute 0.2 ml of the cell suspension in 0.2 ml of Trypan blue (N.B. use 0.1% w/v Trypan blue in PBS solution); non-viable cells are stained blue.
- Immediately mix well with a fine Pasteur pipette and aspirate sufficient volume to fill both sides of the haemocytometer chamber.
- Count viable cells in each of the four corner squares bordered by triple lines, omitting cells lying on these lines. This is repeated for the second side of the chamber. N.B. cell counts of less than fifty cells are unlikely to be reliable.
- If a marked degree of cell “clumping” (aggregation) is observed, discard and re-suspend the original cell suspension.
- Calculate the mean count of the total viable cells per four corner squares (N.B. viable cells are not stained by Trypan blue).
- Count and calculate the mean count of the other half of the counting chamber. For a valid test, the results of the two counts should be within 20% of the mean value.

**Calculation:**

- Calculate the viable cell concentration per ml using the following formula:
  - \( C_1 = t \times tb \times 1/4 \times 10^4 \)
  - \( t \) = total viable cell count of four corner squares
  - \( tb \) = correction for the Trypan blue dilution (counting dilution was \( 1/tb \))
  - \( 1/4 \) = correction to give mean cells per corner square
  - \( 10^4 \) = conversion factor for counting chamber
  - \( C_1 \) = initial cell concentration per ml
- Example: \( t = 480 \); \( tb = 2 \); \( C_1 = 480 \times 2 \times 1/4 \times 10^4 = 2.4 \times 10^6 \) cells per ml.
- Calculate the dilution factor \( d \) to obtain the working cell concentration per ml \( C_2 \).
  - \( d = C_2 \) (working cell concentration) / \( C_1 \) (initial cell concentration)
- Example: \( C_1 = 2.4 \times 10^6 \); \( C_2 = 2 \times 10^5 \)
- Then: \( d = C_2 / C_1 = (2 \times 10^5) / (2.4 \times 10^6) = 2/24 = 1/12 \)
- The working concentration can be obtained by mixing 1 volume of the original cell suspension with 11 volumes of the growth medium.
- Dispense the cells in growth medium, seed into flasks/tubes and incubate at 36°C. Most continuous cell lines should form confluent monolayers within a few days.
Cell counting using a haemocytometer (based on Freshney)
Important note:
The example given above is only correct for counting chambers of the “improved Neubauer” type. Other counting chambers such as Bürker-Türk may have other specifications. Important variables in these counting chambers are:

a) The depth of the chamber. In the example above this is 0.1 mm; in some counting chamber types, however, this is 0.2 mm.

b) The number of smallest squares per cm². In the example above, there are 25 squares per cm², each 0.2 mm long and 0.2 mm wide; in some counting chambers, however, there are 16 squares per cm², each 0.25 mm long and 0.25 mm wide.

When counting chambers with different specifications are used, different algorithms have to be followed for the correct calculation of the number of cells per ml. It is important to check the specifications of the counting chamber in use and follow the calculation instructions that go with individual counting chambers.

Reference documents:

SOP of operation, maintenance & calibration of Biological safety cabinet class II.
SOP of operation, maintenance & calibration of 36°C incubator
SOP of operation, maintenance & calibration of Pipette aid.
SOP of operation, maintenance & calibration of Inverted Microscope.

Reporting:

Put the data in the cell counting record form.
Record the data in the daily work folder.
Supervisor must be informed as well as the head of the lab.
4) Cell freezing, SOP #: NPL TC 004

<table>
<thead>
<tr>
<th>Standard Operating Procedure</th>
<th>SOP #: NPL TC 004</th>
<th>Version: 1.0</th>
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<tr>
<td>Title: Cell freezing.</td>
<td></td>
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</tr>
<tr>
<td>Author: WHO Lab Manual 2004</td>
<td>Approved by: Dr. Faisal Ghazi Alhamdani</td>
<td></td>
</tr>
<tr>
<td>Review Date: 30 Oct. 2014</td>
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</tbody>
</table>

**Objective:**
To describe the procedure utilized in the preservation of the utilized cell lines.

**Reference:**

**Scope:**
This document describes procedure used in the NPL for maintain stocks of cells in a viable state for long periods at low temperatures.

**Responsibility:**
This procedure is carried out by two Expert supervisors under the overall supervision of the lab director. Materials and equipment:
**Materials:**
- Culture flasks with confluent monolayers of L20B or RDA cells (NB: cultures that have been confluent for longer than two weeks should not be used);
- Phosphate buffered saline (PBS) without calcium and magnesium;
- Trypsin (or trypsin/Versene);
- Growth medium;
- Trypan blue
- Liquid nitrogen
- Different sizes of pipettes.

**Equipment:**
- Biological safety cabinet class II (Microflow).
- Inverted Microscope (Olympus).
- Cell counting chamber (Improved Neubauer)
- Cell culture tubes and flasks (Nunc);
- Pipettes (Acu-jet)
- Liquid Nitrogen Tank

**Good laboratory practice:** Work with one cell line at a time. Procedure:
- Use only cultures of cells that are in a healthy state (i.e. rapidly growing but not completely confluent).
- Detach cells with Trypsin (or Trypsin/Versene). Use sufficient flasks to yield a minimum of 4 x 10^6 cells/ml in the final cryoprotectant solution.
- Re-suspend cells in growth medium; centrifuge at 100 x g for 10 minutes.
- Discard the supernatant and re-suspend thoroughly the cell pellet in pre-chilled growth medium containing 20% fetal calf serum and 10% (v/v) dimethyl sulfoxide.
- Dilute 0.1 ml cell suspension in Trypan blue and count cells in a haemocytometer as described in Section 4.2.5.
- Adjust cell concentration to 4–8 x 10^6 cells/ml (if large flasks will be used for cell revival) or 2 x 10^6 cells/ml (if small flasks will be used for cell revival) in growth medium containing DMSO.
- Dispense in 1 ml or 2 ml volumes in clearly labeled (cell name, laboratory of origin, passage number and date of freezing) screw-capped, external thread vials (caps should be tightly closed), or polypropylene-sealed/glass-sealed ampoules. The former are suitable for storage in gaseous nitrogen, the latter for storage in liquid nitrogen.
- Freeze vials/ampoules slowly. Ideally the temperature should drop at 1°C/minute. Place vials/ampoules in the special container that holds them in the gaseous phase of the liquid nitrogen vessel. Commercial devices are available for which a formula is supplied by the manufacturer for the level vials/ampoules are held; number to be stored and length of time required achieving this temperature drop. Alternatively, place vials/ampoules wrapped in paper towels or cotton wool in a polystyrene container with a wall thickness of ~25 mm and place this in the -70°C freezer overnight.
- Transfer the vials to the gaseous phase (-150°C to -180°C) and polypropylene or glass-sealed ampoules to the liquid nitrogen (-196°C) storage containers. For long-term
storage of cells (i.e. a period of years) liquid nitrogen storage is more reliable. Good laboratory practice: When using gaseous phase or liquid nitrogen containers, closed-toed shoes, visors and heavy-duty gloves must be worn to avoid injuries from nitrogen splashes or explosion of imperfectly-sealed ampoules.

**Reference documents:**
- SOP of operation, maintenance & calibration of Biological safety cabinet class II.
- SOP of operation, maintenance & calibration of 36°C incubator
- SOP of operation, maintenance & calibration of Pipette aid.
- SOP of operation, maintenance & calibration of Inverted Microscope.
- SOP of operation, maintenance & calibration of Liquid nitrogen Tank.

**Reporting:**
- Put the data in the cell freezing record form.
- Record the data in the daily work folder.
- Supervisor must be informed as well as the head of the lab.
Objective:
To describe the procedure utilized in passaging the cells.

Reference:

Scope:
This document describes procedure used in the NPL – IRAQ for passaging the cells maintained freezing. This procedure is carried out by two expert supervisors under the overall supervision of the lab director.

Responsibility:
This procedure is carried out by two expert supervisors under the overall supervision of the lab director. Materials and equipment:

Materials:
- Culture flasks with confluent monolayers of L20B or RDA cells (NB: cultures that have been confluent for longer than two weeks should not be used);
- Trypsin (or trypsin/Versene);
- Growth medium;
- Trypan blue
- Different sizes of pipettes.

Equipment:
- Biological safety cabinet class II (Telstar).
- Inverted Microscope (Olympus).
- Cell counting chamber (Improved Neubauer)
- Cell culture tubes and flasks (Nunc);
- Pipette aid (Accu-jet)

Good laboratory practice: Work with one cell line at a time.

Procedure:
- Remove vial/ampoule from gaseous/liquid nitrogen and transfer immediately to a water bath or preferably a beaker of sterile water at 36°C.
- When contents are completely thawed, wipe outside of vial/ampoule with alcohol to reduce bacterial contamination, transfer cell suspension to culture flask.
● Add, drop-wise, sufficient growth medium for the production of a cell monolayer (N.B. If storage vials contain cells at a concentration of \(4 \times 10^6\) cells/ml, then 1 ml cell suspension should be sufficient for one 75 cm\(^2\) flasks).

● The viability of the thawed cells may be significantly reduced if growth medium is added rapidly at this delicate stage.

● Incubate flask until cells are adherent (6–8 hours) or overnight at 36°C. Carefully decant medium (to get rid of DMSO present) and add fresh growth medium. As an alternative to the above procedure spin thawed cell suspension (made up to 10 ml slowly with growth medium) at 80 x g for 10 minutes; discard supernatant and re-suspend cell pellet in sufficient growth medium for production of a cell monolayer and incubate at 36°C.

Reference documents:

● SOP of operation, maintenance & calibration of Biological safety cabinet class II.
● SOP of operation, maintenance & calibration of 36°C incubator
● SOP of operation, maintenance & calibration of Pipette aid.
● SOP of operation, maintenance & calibration of Inverted Microscope.

Reporting:

● Put the data in the cell thawing record form.
● Record the data in the daily work folder.
● Supervisor must be informed as well as the head of the lab.
6) Preparation of laboratory quality - control standard. SOP #: NPL TC 06

<table>
<thead>
<tr>
<th>Standard Operating Procedure</th>
<th>SOP #: NPL TC 06</th>
<th>Version: 1.0</th>
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<tr>
<td>Title: Preparation of laboratory quality - control standard</td>
<td>Author: WHO Lab Manual 2004</td>
<td>Approved by: Dr. Faisal Ghazi Alhamdani</td>
</tr>
</tbody>
</table>
| Review Date: 30 Oct. 2014 | \n
**Objective:**
To describe the procedure utilized in the preparation of laboratory’s quality-control standards and reference virus stock for the utilized cell lines.

**Reference:**

**Scope:**
This document describes procedure used in the NPL Iraq for growing up virus stocks in RD and L20B cell lines. This procedure is carried out by two expert Supervisors under the supervision of lab director.

**Responsibility:**
This procedure is carried out by two expert Supervisors under the overall supervision of the lab director. Materials and equipment:

**Materials:**
- 1 ml and 10 ml sterile plastic disposable pipettes;
- 50 ml sterile, screw-capped, centrifuge tube (one for each serotype) and each cell line;
- 250 externally threaded, screw-capped, storage vials (1.8–2.0 ml size) for each serotype and each cell line;
- 1 ampoule of NIBSC Sabin Poliovirus Reference Standard of each serotype;
- 75 cm² flasks (one for each serotype) with a confluent layer of healthy L20B cells and RD cells in 25 ml maintenance medium.

**Equipment:**
- Biological safety cabinet class II (Microflow).
- 36°C incubator (Memert).
- Inverted Microscope (Olympus).
- Cell counting chamber (Improved Neubauer)
- Cell culture tubes and flasks (Nunc);
- Pipette Aid (Accu-jet)

**Good laboratory practice:** Work with one cell line at a time.
Procedure:

- Examine the cells for quality (i.e. an entire monolayer of healthy cells) and absence of contamination as determined by visual inspection. A suitable monolayer for use would be one formed within at least two days of seeding.

- Label the flasks that will contain the inoculated culture.

- Working in a BSC with only one serotype at a time, use a sterile plastic disposable pipette to mix the contents of an ampoule of NIBSC Sabin poliovirus reference standard. Transfer half the contents of the ampoule (approximately 0.4 mls) to a 75 cm2 flask of either L20B or RD cells. Retain the other half ampoule of material for inoculating the other cell line at a later time, storing it at 4°C if the other cell line is to be handled on the same day, or at <0°C if it is to be used on another day.

- Incubate the flask, containing the inoculated culture, at 36°C.

- Examine the inoculated culture daily, using an inverted microscope, for the appearance of cytopathic effect (CPE).

- When 75 to 100% of cells show CPE (3+ to 4+ CPE), transfer the flask to < 0°C. Freeze and thaw the contents of the flask, shaking the flask when it is semi-thawed to ensure that all cells are disrupted. Repeat two additional times.

- Working in a BSC, use a sterile 10 ml pipette to mix the contents of the flask. Transfer the contents to a labeled 50 ml centrifug e tube.

- Spin for 20 minutes at 1500g1 in a refrigerated centrifuge after ensuring that centrifuge caps are securely in place and centrifuge buckets are sealed.

- Label each storage vial with the name of the cell line, the name of the virus preparation and the date.

- Working in a BSC, transfer aliquots of the supernatant into labeled storage vials (0.1 ml supernatant per vial to give a total of 250 aliquots).

- Store the aliquots of the virus preparation, which should be used subsequently as the laboratory quality control standard, in a -20°C freezer designated for storage of infectious materials.

Reference documents:

- SOP of operation, maintenance & calibration of Biological safety cabinet class II.
- SOP of operation, maintenance & calibration of 36°C incubator
- SOP of operation, maintenance & calibration of Pipette aid.
- SOP of operation, maintenance & calibration of Inverted Microscope.

Reporting:

- Put the data in the cell counting record form.
- Record the data in the daily work folder.
- Supervisor must be informed as well as the head of the lab.
7) Titration of quality control standard. SOP #: NPL TC 007

<table>
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<tr>
<th>Standard Operating Procedure</th>
<th>SOP #: NPL TC 007</th>
<th>Version: 1.0</th>
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<td>Title: Titration of quality control standard.</td>
<td></td>
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<tr>
<td>Author: WHO Lab Manual 2004</td>
<td>Approved by: Dr. Faisal Ghazi Alhamdani</td>
<td></td>
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<tr>
<td>Review Date: 30 Oct. 2014</td>
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Objective:
To describe the procedure utilized in the Titration of laboratory’s quality-control standards and reference virus stock for the utilized cell lines.

Reference:

Scope:
This document describes procedure used in the NPL – for initial determination and validation of the titer of the laboratory quality control standard and for subsequent routine monitoring of cell-line sensitivity.

Responsibility:
This procedure is carried out by two expert supervisors under the overall supervision of the lab director.

Materials and equipment:

Materials:
- Sterile 96-well flat-bottomed cell-culture microtitre plates with lids;
- 1 ml and 10 ml plastic disposable pipettes;
- 5 ml sterile tubes with caps for dilution;
- Pipettors with aerosol resistant tips (ARTs).
- Different sizes of pipettes.
- Flask with confluent layer of healthy cells of the type for which sensitivity for virus isolation is being evaluated; will need sufficient cells to prepare 10 mls/microliter plate of a cell suspension containing 1–2 x 10⁵ cells/ml.
- Maintenance medium;
- Cell culture tubes (Nunc);
- Low titer Sabin poliovirus reference strain.

Equipment:
- Vortex mixer (Fisher brand L-46)
- Biological safety cabinet class II (Telstar).
- 36°C incubator (Memmert).
- Inverted Microscope (Olympus).
- Pipette aid (Accu-jet)
**Procedure:**

- Label dilution tubes 10-6 to 10-9.
- Dispense 9.0 ml medium to tubes 1–7.
- Rapidly thaw one aliquot of laboratory quality-control standard virus for one serotype. For a calibration experiment all three serotypes need to be tested.
- To the thawed standard virus, add 0.9 ml of medium and mix. This is the 10-1 dilution of the standard virus.
- For a calibration experiment, also thaw and dilute an aliquot of the NIBSC standard virus of the same serotype in an identical manner.
- Add 1.0 ml of virus to the first tube using a sterile pipette or pipettor with ART tip.
- Cover the tube and vortex gently.
- Take another pipette/pipette tip, transfer 1.0 ml to the second tube, and discard pipette/pipette tip.
- Cover the tube and vortex gently.
- Repeat dilution steps, transferring 1.0 ml each time and always changing pipette/pipette tip between dilutions, up until tube 7.
- Add 100 µl of virus dilutions to wells 1 to 10 in rows A to H – that is, 20 wells per dilution.

**To obtain valid test results:**

- Dilutions 10-4 to 10-7 are put on the plate when testing the NIBSC Reference Standard;
- Dilutions 10-5 to 10-8 are put on the plate when testing the laboratory quality control standard prepared in Section 5.2.
- Add 100 µl of maintenance medium to wells A11 to H12 in rows A to H for the cell controls. WHO/IVB/04.10 77
- Prepare a suspension of approximately 1–2 x 10^5 cells /ml, calculating at least 10 ml Per plate.
- Label the edge of the microtitre plate as indicated in Figure 5.2.
- Add 100 µl of cells from a cell suspension containing 1–2 x 10^5 cells /ml to all wells in rows A to H on the plate.
- Cover the plate with non-toxic sealer (if not using a CO2 incubator) and incubate at 36°C.
- Examine for development of CPE, using an inverted microscope, and record daily readings for 5–7 days. For a valid test, the cell control should have a complete monolayer of healthy cells.
- Examine the inoculated culture daily, using an inverted microscope, for the appearance of cytopathic effect (CPE).
Preparation of virus dilution of Sabin poliovirus reference strain

Plate layout for titration of laboratory quality control standard
Example of results of titration of Sabin poliovirus reference standard

Reference documents:

- SOP of operation, maintenance & calibration of Biological safety cabinet class II.
- SOP of operation, maintenance & calibration of 36°C incubator
- SOP of operation, maintenance & calibration of Pipette aid.
- SOP of operation, maintenance & calibration of Inverted Microscope.
- SOP of operation, maintenance & calibration of vortex mixer

Reporting:

- Put the data in the sensitivity record form.
- Record the data in the daily work folder.
- Supervisor must be informed as well as the head of the lab.
8) Adapting newly received cells to local conditions SOP #: NPL TC 008

<table>
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<th>Standard Operating Procedure</th>
<th>SOP #: NPL TC 008</th>
<th>Version: 1.1</th>
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<tr>
<td>Title: Adapting newly received cells to local conditions</td>
<td>Author: WHO Lab Manual 2004</td>
<td>Approved by: Dr. Faisal Ghazi Alhamdani</td>
</tr>
<tr>
<td>Review Date: 30 Oct. 2014</td>
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</tr>
</tbody>
</table>

Objective:
To provide a standard protocol for use by laboratories in adapting cells to the local situation on receipt from reference laboratories.

Reference:
Supplement to WHO Lab Manual of the new algorithm, Dec. 2006

Scope:
This document describes procedure used to adapting newly received cells to local conditions for assuring quality cell cultures occur at the time of the receipt of new cultures in the local laboratory.

Responsibility:
This procedure is carried out by two expert supervisors under the overall supervision of the lab director. Materials and equipment:

Materials:
- Growth medium;
- Alcohol.
- 5 ml, 10 ml and 25 ml plastic disposable pipettes.
- Cell culture flasks (Nunc);

Equipment:
- Biological safety cabinet class II (Telstar).
- 36°C incubator (Memmert).
- Inverted Microscope (Olympus).
- Cell counting chamber (Improved Neubauer)
- Pipette aid (Accu-jet)

Good laboratory practice:
- To minimize the risk of spread of contamination that may be unknowingly present in the newly received cells:
- Schedule work on new cells at the end of the day or after routine processing of all other cells is completed.
- Decontaminate the external surface of the cell culture flask with 70% alcohol before transferring to the cell culture area.
- Microbiological culture in enriched media may be carried out on the original medium to reveal the presence of bacteria and fungi. To minimize the risk of mycoplasma contamination of the newly received cells, strict adherence to aseptic techniques and good laboratory practices should be observed.

- Work with one cell line at a time.

Procedure:

- Check for cell line details, and record it on "Received cell line details checking list" (See attachment # NPL ATT 001), the minimum cell-line details that should be included are:
  - Type of cell line and passage number
  - Date of passage
  - Media type and composition (FCS concentration, L-glutamine, Buffer, Antibiotics)
  - Seeding concentration
  - Percentage confluence at the time of shipment
  - Incubation conditions
  - Sterility testing results
  - Mycoplasma test result and method used
  - Sensitivity test result.

- On arrival, visually examine the package for integrity or any signs of damage or leakage from the flasks. Check the flask labels and ensure that these match the cell line details described in the accompanying documents. Observe for changes in the color of the media that may indicate possible contamination or buildup of toxic waste products. Look for small fungal colonies that may float in the media.

- Microscopic examination for any signs of distress during shipment (i.e., detachment, rouging-up or atypical morphology). Look for any signs of bacterial or fungal contamination. Contaminated cultures should be discarded and a new shipment is requested. Observe the relative cell density and estimate the percentage cell confluence.

- Acclimatize cells to local environment before processing.

- Change medium in which cells were shipped within 24 hrs. – 48 hrs. on receipt to:
  - Replenish depleted nutrients
  - Provide optimal pH, Co2
  - Allow cells to adapt to new culture media

- Media replenishment using 100% new media conditioning:
  - Remove medium from flask and from inside the neck or cap area (Keep the original medium for sterility testing (SOP # 1.9, National Polio Laboratory, and NPL SOP's).
  - Replace medium with new growth medium appropriate to the local incubation conditions for the laboratory.
  - Incubate the cultures at 36°C and observe growth conditions until sub-culture.
• Record observations.

• There are two other options may be used for media replenishment instead of 100% new media:
  
  Option two.  1:1 ratio of Old: New Media.
  
  Option three.  100% original media.

• Subculture cells when 80-90% confluent (SOP # NPL TC 001, National Polio Laboratory, NPL SOP's).

• Observe growth conditions following passage of first flask. Note optimal count, cell variability and seeding density.

• Prepare frozen cell stocks (SOP # NPL TC 003, National Polio Laboratory, NPL SOP's).

• Establish a working cell bank (SOP # NPL TC 008, National Polio Laboratory, NPL SOP's).

Reference documents:
• SOP of operation, maintenance & calibration of Biological safety cabinet class II.
• SOP of operation, maintenance & calibration of 36°C incubator
• SOP of operation, maintenance & calibration of Pipette aid.
• SOP of operation, maintenance & calibration of Inverted Microscope.
  Attachment # NPL ATT 001: Received cell line details checking list
• SOP # NPL TC 001, National Polio Laboratory, NPL SOP's (passaging of the cell cultures).
• SOP # NPL TC 003, National Polio Laboratory, NPL SOP's (Cell freezing).
• SOP # NPL TC 008, National Polio Laboratory, NPL SOP's (Establish a working cell bank).
• SOP # NPL TC 009, National Polio Laboratory, NPL SOP's (Sterility testing).

Reporting:
• Put the attached completed checklist in the newly received cells record form.
• Record the data in the daily work folder.
• Supervisor must be informed as well as the head of the lab.
National Polio Laboratory, NPL, Iraq
ATTACHMENT # NPL ATT 001 / Received cell line details checking list

<table>
<thead>
<tr>
<th>Standard Operating Procedure</th>
<th>Version: 1.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Operating Procedure SOP #: NPL TC 007</td>
<td>Page: 1/1</td>
</tr>
</tbody>
</table>

Cell line receiving date: / / 
Check and record the following details:

<table>
<thead>
<tr>
<th>Source of cells</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of cell line</td>
<td></td>
</tr>
<tr>
<td>Passage number</td>
<td></td>
</tr>
<tr>
<td>Date of passage</td>
<td></td>
</tr>
<tr>
<td>Media type and composition (Fatal Bovine serum concentration, L-glutamine, Buffer, Antibiotics)</td>
<td></td>
</tr>
<tr>
<td>Seeding concentration (seeding levels)</td>
<td></td>
</tr>
<tr>
<td>Percentage confluence at the time of shipment</td>
<td></td>
</tr>
<tr>
<td>Incubation conditions</td>
<td></td>
</tr>
<tr>
<td>Sterility testing results, date tested</td>
<td></td>
</tr>
<tr>
<td>Mycoplasma test result and method used, date tested</td>
<td></td>
</tr>
<tr>
<td>Sensitivity test, date tested</td>
<td></td>
</tr>
</tbody>
</table>

Signature:
9) Cell Sensitivity Testing SOP #: NPL TC 09

<table>
<thead>
<tr>
<th>Standard Operating Procedure SOP #: NPL TC 09</th>
<th>Version: 1.0</th>
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<tbody>
<tr>
<td>Title: Cell Sensitivity Testing</td>
<td></td>
</tr>
<tr>
<td>Author: WHO Lab Manual 2004, ICM meeting</td>
<td>Approved by: Dr. Faisal Ghazi Alhamdani</td>
</tr>
<tr>
<td>Review Date: 30 Oct. 2014</td>
<td></td>
</tr>
</tbody>
</table>

**Objective:**
To describe the procedure utilized in the testing of laboratory’s cell lines in reference to virus stock for the utilized cell lines.

**Reference:**
National Institute for Biological Standards and Control

**Scope:**
This document describes procedure used in the NPL Iraq for growing up virus stocks in RD and L20B cell lines. This procedure is carried out by two expert Supervisors under the supervision of lab director.

**Responsibility:**
This procedure is carried out by two expert supervisors under the overall supervision of the lab director. WHO recommends that both RD and L20B cell lines are periodically tested for sensitivity to virus isolation. As described below, well characterized reference virus preparations of known and reproducible titer are used to evaluate cell line sensitivity.

**Materials:**
- L20B cell suspension near passage # 26
- RD cell suspension near passage # 230
- Sabin virus reference strains (6 viruses) dated 30/7/2006
- Prepared in 0.1ml aliquots and stored at -70°C

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabin 1</td>
<td>Sabin 1</td>
</tr>
<tr>
<td>NIBSC 01/528</td>
<td>NIBSC 01/528</td>
</tr>
<tr>
<td>tcRD2 .1ml</td>
<td>tcRD2 .1ml</td>
</tr>
<tr>
<td>30/7/2006</td>
<td>30/7/2006</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Sabin 2</td>
<td>Sabin 1</td>
</tr>
<tr>
<td>NIBSC 01/530</td>
<td>NIBSC 01/530</td>
</tr>
<tr>
<td>tcRD2 .1ml</td>
<td>tcL20B2 .1ml</td>
</tr>
<tr>
<td>30/7/2006</td>
<td>30/7/2006</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Sabin 3</td>
<td>Sabin 1</td>
</tr>
<tr>
<td>NIBSC 01/532</td>
<td>NIBSC 01/532</td>
</tr>
<tr>
<td>tcRD2 .1ml</td>
<td>tcL20B2 .1ml</td>
</tr>
<tr>
<td>30/7/2006</td>
<td>30/7/2006</td>
</tr>
</tbody>
</table>
Sterile 96-well flat bottomed tissue culture microtitre plates with lids
5 ml sterile tubes for dilutions
Disposable pipet tips 200 ul and 1000 ul
MEM without serum
Growth medium
Cell counting chamber
Trypan blue
Pipettes
Methods
Label 96-well plate for each virus as indicated. (6 plates)
Rows A & B labeled –5, rows C & D labeled –6, rows E & F labeled –7, and rows G & H labeled –8

Titration for cell sensitivity testing:
Thaw viral isolate.
Label dilution tubes 10⁻² to 10⁻⁹ marking each set with Sabin
Dispense 0.9 ml MEM to tubes 2 – 5
Dispense 2.7 ml MEM to tubes 6 – 9
Rapidly thaw one 0.1 ml aliquot of laboratory quality control standard virus for each serotype to be tested
To the thawed standard virus, add 0.9 ml of medium and mix. This is the 10⁻¹ dilution of the standard virus.
Vortex tube and with a new pipet tip, transfer 0.1 ml from the first tube to the second and discard tip
Repeat dilution steps to the 10⁻⁵ tube
Transfer 0.3 ml to tubes 10⁻⁶ through 10⁻⁹

Plate set-up:
Add 100 ul virus to the wells 1 to 10 in rows G and H starting with the 10⁻⁹ dilution (i.e. 20 wells per dilution)
Continue adding virus to all 20 wells of rows 1-10 of each dilution working from highest to lowest dilution
Add 100 ul MEM without serum to wells A11 to H12 for the cell controls
- Add 100 ul of cells from a cell suspension containing 1.75 X 10^5 cells/ml to all wells on the plate. 40 mls of each cell line is enough to test 3 plates
- 14 ml of cell suspension with 5 x 10^5 cells/ml +26 ml of growth medium, 40 ml
- Incubate 5 days
- Check CPE and Calculate CCID_{50}
- Calculation of log CCID_{50}

Log CCID_{50} = L – d (S – 0.5), where: L = -6.0 (lowest dilution used in test), d = 1 (difference between log dilution steps), S = sum of ratio of positive wells.
NAPHTHALENE BLACK (Serial No. 1195)

- Perform this staining at the end of the cell sensitivity test and scan the plate or photocopy the plate and keep the record in cell sensitivity documentation.

- Put stain in a box and dip the plate in it, adding to each well may damage cell sheet.
  (To be applied in future when the item become available)

Materials:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Supplier or equivalent</th>
<th>Cat No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene black</td>
<td>Fisher</td>
<td>N/0211/45</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>Merck</td>
<td>10236</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>Fisher</td>
<td>A/0400/PB17</td>
</tr>
</tbody>
</table>

Record Keeping

Assign reagent batch number. Complete relevant reagent batch record recording lot number and quantities of reagents used.

Method

- Weigh 13.6g ± 0.1g Sodium acetate per litre required on a balance (and add to a suitably sized glass beaker containing 'ultrapure' water, stir until dissolved).

- Using an appropriate sized measuring cylinder, add 60mls acetic acid per liter required, to the Sodium acetate solution. Make up to required total volume with 'ultrapure' water.

- Add 1.0g ± 0.1g naphthalene black per liter, weighed on a balance to the acetic acid and sodium acetate solution and dissolve stain by stirring for at least 30mins.

- Aliquot in 500ml amounts in blood bottles, cap bottles, label, and store for a maximum of 5 years at room temperature. Attach copy of label to reagent batch record.
Objective:
To provide a standard protocol for use by laboratories to establish a master cell bank (MCB) and Working cell banking (WCB) for each cell line.

Reference:

Scope:
This document describes procedure used to establish a master cell bank (MCB) and Working cell banking (WCB) for each cell line, to minimize the cost for transporting of cell lines official sources.

Responsibility:
This procedure is carried out by two expert supervisors under the overall supervision of the lab director.

Materials:
Growth medium;
1 ml, 5 ml, 10 ml and 25 ml plastic disposable pipettes.
Cell culture flasks (Nunc);
Liquid nitrogen

Equipment:
• Biological safety cabinet class II (Telstar).
• 36°C incubator (Memmert).
• Inverted Microscope (Olympus).
• Cell counting chamber (Improved Neubauer)
• Pipette aid (Accujet)
• Liquid Nitrogen Tank (----------)

Good laboratory practice:
• To minimize the risk of spread of contamination that may be unknowingly present in the newly received cells:
• Schedule work on new cells at the end of the day or after routine processing of all other cells is completed.
• Decontaminate the external surface of the cell culture flask with 70% alcohol before transferring to the cell culture area.
Microbiological culture in enriched media may be carried out on the original medium to reveal the presence of bacteria and fungi. To minimize the risk of mycoplasma contamination of the newly received cells, strict adherence to aseptic techniques and good laboratory practices should be observed.

Work with one cell line at a time.

**Procedure:**

- Received cells from an official WHO source to initiate bank as standard procedures.
- Passage one cell culture flask (75 cm²) to two cell culture flasks (150 cm²) then each one re-passage into 4 cell culture flasks (150 cm²).
- Freeze and store in Liquid nitrogen 15 vials each contain 4-8 x 10⁶ cells, to perform Master Cell Bank (MCB).
- Obtain cells from MCB, Passage one cell culture flask (75 cm²) to two cell culture flasks (150 cm²) then each one re-passage into 4 cell culture flasks (150 cm²).
- Freeze and store in Liquid nitrogen 15 vials each contain 4-8 x 10⁶ cells, to perform Working Cell Bank (WCB).
- Use a vial of cells to initiate cultures for routine use.

**Reference documents:**

- SOP of operation, maintenance & calibration of Biological safety cabinet class II.
- SOP of operation, maintenance & calibration of 36°C incubator
- SOP of operation, maintenance & calibration of Pipette aid.
- SOP of operation, maintenance & calibration of Inverted Microscope.
- SOP of operation, maintenance & calibration of Liquid Nitrogen Tank.

**Reporting:**

- Put the data in the cell bank record form.
- Record the data in the daily work folder.
- Supervisor must be informed as well as the head of the lab.
Establishment of a cell bank

<table>
<thead>
<tr>
<th>Cells from official WHO source to initiate bank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passage 1</td>
</tr>
<tr>
<td>Passage 2</td>
</tr>
<tr>
<td>Passage 3</td>
</tr>
</tbody>
</table>

Freeze and store in Liquid nitrogen 15 vials each contain 4-8 x 10⁶ cells

<table>
<thead>
<tr>
<th>Obtain cells from MCB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passage 4</td>
</tr>
<tr>
<td>Passage 5</td>
</tr>
<tr>
<td>Passage 6</td>
</tr>
</tbody>
</table>

Freeze and store in Liquid nitrogen 15 vials each contain 4-8 x 10⁶ cells

Use a vial of cells to initiate cultures for routine use.

| Working cell bank (WCB) at national laboratory |
11) Sterility testing Procedure SOP #: NPL TC 011

Objective:
To provide a standard protocol for use by laboratories to test the sterility of media.

Reference:

Scope:
This document describes procedure used to test the sterility of media, to assure that media free of contamination.

Responsibility:
This procedure is carried out by two expert supervisors under the overall supervision of the lab director. Materials and equipment:

Materials:
Nutrients broths e.g. Soy bean and thioglycolate.
5 ml, 10 ml and 25 ml plastic disposable pipettes.

Equipment:
- Biological safety cabinet class II (Microflow).
- Incubator 36°C (Memmert).
- Incubator 20°C (Binder).
- Pipette aid (Accujet).

Procedure:
- Take representative samples of the filtered media batch and dilute one third of each into nutrient broth e.g. beef heart infusion and thioglycolate.
- Divide each into two, and incubate one at 20°C and the other at 36.5°C for 10 days with uninoculated controls.
- For example: remove 10 ml of filtered medium, add 5 ml of media to 10 ml of nutrient broth and remaining 5 ml to 10 ml of thioglycolate, divide both into two and incubate at the two temperatures.
- If any show signs of cloudiness, the whole batch should be discarded.
- All media should be visually checked before use for correct PH, color, and sign of contamination.
If gentle swirling shows spiral sediment rising from the bottom then the media in the bottle may be contaminated and should be discarded.

Reference documents:

- SOP of operation, maintenance & calibration of Biological safety cabinet class II.
- SOP of operation, maintenance & calibration of 36°C incubator
- SOP of operation, maintenance & calibration of Pipette aid.

Reporting:

- Put the data in the cell counting record form.
- Record the data in the daily work folder.
- Supervisor must be informed as well as the head of the lab.
Objective:
To describe the procedure of washing of glassware for use in tissue culture laboratory.

Reference:

Scope:
This document describes procedure used in the NPL to wash glassware for use in tissue culture laboratory.

Responsibility:
This procedure is carried out by two Experts supervisors under the overall supervision of the lab director.

General Description:
While using glassware it is must that all glassware is meticulously cleaned and sterilized so that cell cultures will not be affected by traces of proteinaceous material, detergents, pyrogens, water deposits and other residual materials which may get deposited on the glassware.

Description:
• Use care in handling glassware as most breakage occur during the cleaning process.
• Rinse all glassware as soon as possible after use
• Store soiled items in water containing a disinfectant or cleanser to avoid drying and making items harder to clean.
• Before cleaning, decontaminate glassware by autoclaving or soaking overnight in chlorine solution (0.5%)
• Pipettes may be decontaminated in a container containing chlorine
• Use 7-X®, DECON® or similar detergent for thorough cleaning of all laboratory glassware. These detergents are easily rinsed out from glassware without leaving residues.

DO NOT use domestic dishwashing liquid detergent under any circumstances

• Clean glass by scrubbing with a brush. Periodically inspect brushes for wear to avoid scratching glass.
• Thoroughly rinse items in tap water, followed by at least 5-7 changes of distilled or deionized water. Even the smallest residual amounts of cleansers, disinfectants or acids can affect the growth of cell cultures.

• Dry glassware on racks or pegboards and inspect after drying. If glassware is hazy has a film or blotches, then additional cleaning is required.

• Sterilize cell culture glassware using a hot air oven at 180°C for 3 hours to destroy pyrogens. Non-glass components which may not withstand 180°C should be sterilized by alternate methods such as autoclaving and re-assembled aseptically

Safety Conditions:
Take extra care during handling of glass ware to avoid injury
Follow all normal safety precautions for using detergents.
Objective:
To describe the procedure of samples received and the procedure of recording the data on the received specimens.

Reference:

Scope:
This document describes procedure used in the National Polio lab, NPL to receive the specimens in lab and to ensure that the Information on specimen labels matches information on the request forms.

Responsibility:
This procedure is performed by trained virology Supervisors under the supervision of the lab director.

Specimens receive:

- On arrival in the laboratory, shipping cartons or carriers must be immediately unpacked in a designated area equipped with a discard container, alcohol swabs and paper towels.

- Safety of laboratory workers is the prime concern and, if available, a Class II Biosafety Cabinet (BSC) should be used to limit exposure of laboratory staff to potential pathogens. If a BSC is not available a clean workbench can be used. This should have a surface covering that can be easily disinfected using common laboratory disinfectants (70% alcohol, sodium hypochlorite solution, 2% glutaraldehyde solution, etc.) and should be located away from areas used for other laboratory activities.

- Enteroviruses are not inactivated by alcohol per se, but a 70% solution is an effective antibacterial and antifungal disinfectant and will inactivate enteroviruses by desiccation if the solution is allowed to completely dry.

- Unpacking and recording of specimens should preferably be carried out by two persons: one records data while the other is gloved and is responsible for opening the package and checking for breakage and leakage of sample containers, and contamination of accompanying documents.

- Any contaminated paperwork should be placed temporarily in the BSC while the information is manually recorded on a clean sheet of paper. Contaminated documents should be handled in the same manner as infectious wastes.
**Recording of the specimens:**

The following minimum information should be included on the laboratory request form accompanying the specimen:

- EPId Number (in an agreed format).
- Patient name.
- Province (or region) of report.
- Town/district of report.
- Province (or region) of residence of the case.
- Town/district of residence of the case.
- Country code.
- Whether the case has been immunized.
- Date of last OPV.
- AFP or contact.
- If a contact, EPID number of the related case with addition of C letter.
- Specimen type (stool, etc.).
- Date of onset of paralysis (exact date, the month is minimum requirement).
- Date of first specimen collection.
- Date of second specimen collection.

The following additional information should be recorded by the laboratory on receipt of a specimen:

- Date specimen received in laboratory.
- Specimen arrived frozen or with ice present (for feedback to EPI) (y/n).
- Specimen arrived in amount large enough for full laboratory analysis (y/n).
- Specimen arrived with no evidence of leakage or desiccation (y/n).

- Whether this is the first or second specimen received from the case. Each specimen should be allocated a specific identification number that is entered in the laboratory “day book”, on the accompanying request form and on the specimen container. This may be an abbreviated version of the EPId number or a sequential in-house number. This number must be used on all containers, centrifuge tubes, cell culture tubes and vials throughout subsequent laboratory procedures.

**Reporting:**

- Put the samples sheets in the fecal samples received record form.
- Record the data in the daily work folder.
- Supervisor must be informed as well as the head of the lab.
Objective:
To describe the procedure utilized in the preparation of fecal samples for virus isolation.

Reference:

Scope:
This document describes procedure used in the NPL – Iraq to treat the samples with chloroform before inoculation.

Responsibility:
This procedure is carried out by two Microbiologist supervisors under the supervision of lab director. Materials and equipment:

Materials:
- 15 ml or 50 ml polyethylene chloroform-resistant centrifuge tubes
- 1 or 5 ml glass pipette
- 5 ml and 10 ml pipettes
- Wooden spatula
- Externally threaded, screw-capped storage vials (5 ml)
- Glass beads, approximately 3 mm diameter
- PBS with antibiotics
- Chloroform (Sigma)
Equipment:
- Biological safety cabinet class II (Microflow).
- -20°C freezer. (Revco)
- +4°C refrigerator. (Sanyo)
- Pipette aid (Accu-jet)

Procedure:
- Label centrifuge tubes with sample numbers.
- Add 10 ml PBS, 1 g of glass beads and 1 ml chloroform to each tube.
- Working in a BSC, transfer approximately 2 g of each fecal sample to a labeled centrifuge tube (ensure that the number of the original sample matches the number on the centrifuge tube).
- Retain the remaining original sample, preferably in its original container, for storage at -20°C.
- Close centrifuge tubes securely and shake vigorously for 20 minutes using a mechanical shaker.
- Spin for 20 minutes at 1500 g in a refrigerated centrifuge ensuring that centrifuge caps are securely in place and centrifuge buckets are sealed. Working in a BSC, transfer supernatant from each sample into two labeled externally threaded screw-capped storage vials (If supernatant is not clear, repeat chloroform treatment).
- Store one fecal specimen at 4 to 8°C to be inoculated in the same day or next day, after inoculation stool suspension is kept at -20°C to 3 months.

Reference documents:
- SOP of operation, maintenance & calibration of Biological safety cabinet class II.
- SOP of operation, maintenance & calibration of -20°C freezer.
- SOP of operation, maintenance & calibration of +4°C refrigerator.
- SOP of operation, maintenance & calibration of Pipette aid.

Reporting:
- Put the data in the fecal samples preparation record form.
- Record the data in the daily work folder.
- Supervisor must be informed as well as the head of the lab.
15) Media preparation for use in cell culture laboratory SOP#: NPL TC 015

Objective:
To describe the procedure of media preparation used in cell culture preparation and propagation

Scope:
This document describes procedure used in the NPL, Iraq, to prepare media for use in cell culture preparation and propagation. This procedure is performed by trained virology expert supervisors under the supervision of the lab director.

General Description:
The medium used in cell culture in a virological laboratory is minimal essential medium (MEM) supplemented with essential vitamins, antibiotics and fetal calf serum (FCS). For composition of medium please refer to 2003 Laboratory Manual.

For all prepared media, batch preparation details should be recorded (including details of the raw materials used) and an expiry date assigned to each batch. Wherever practicable the reagent name, batch number and expiry date should be clearly marked on each aliquot of the batch.

Safety Conditions (Aseptic Technique):
- All steps in media preparation should be performed under strict aseptic conditions.
- All glassware for handling cell cultures and media should be sterilized
- Avoid splashes, spills and aerosols
- Avoid liquid transfer by pouring
- When adding (or replacing) medium, never touch the neck of the culture flasks with the bottle containing the medium or use the same pipette to transfer medium to more than one bottle. Ideally, aliquot the total amount of medium required for each batch of culture bottles being handled and store the remainder at 4-8°C. Dedicate separate medium for each cell line.
- Separate clean and contaminated materials in the BSC II
- Minimize exposure of sterile media and cell cultures to open air (even within the BSC II)
- Perform final preparation of sterile media (i.e. addition of serum or other additives) before dealing with cell cultures
- To avoid the risk of contamination and cross-infection, cell culture in the virus diagnostic laboratory should be best carried out in closed vessels, usually screw-capped tubes and flat-sided bottles.

Sterility testing:
Any aliquots showing cloudiness should be discarded and the whole batch resterilised. If any media in the stored containers show cloudiness then the whole batch should be discarded.
Sterility checking of autoclaved stocks is much less essential, provided proper monitoring of temperature and time of sterilization is carried out.

All media should be visually checked for signs of contamination before use, no matter how it has been sterilized. If a bottle of media has been standing for a long period, low-grade contamination may form fine sediment on the bottom of the bottle. If gentle swirling shows a spiral of sediment rising from the bottom then the media in the bottle may be contaminated and should be discarded.

**Documentation:**
- Maintain records as it helps in crosschecking the procedures. Following are important to note while carrying out this procedure.
  - Media Type
  - Manufacturer
  - Catalogue No.
  - Lot No.
  - Expiry
  - Date received
  - Date prepared
  - Volume prepared
  - Prepared By
  - Quality Control Results
  - Expiry date of prepared Media
  - Storage
  - Date Used
  - Other Comments
Objective:
To describe the procedure of preparation of complete PBS.

Scope:
This document describes procedures used in the NPL Iraq to prepare complete PBS. This procedure is carried out by expert supervisors under the supervision of lab director.

Materials:

- PBS, Phosphate Buffer Saline
- NaCl, Sodium Chloride
- Na2HPO4, Sodium bi-Phosphate
- KCl, Potassium Chloride
- KH2PO4, Potassium Hydro-Phosphate
- CaCl2, Calcium Chloride
- Crystalline Penicillin G
- Streptomycin Sulphate

General Description:

**Phosphate buffered saline, pH 7.2 to 7.4 (PBS)**

PBS is often described in two forms, as incomplete or complete solutions. Both of these forms are available commercially. An incomplete solution of PBS contains no calcium or magnesium ions. A complete solution of PBS contains calcium and magnesium ions and is used for preparation of faecal suspensions and as diluent for viruses. The presence of calcium and magnesium ions stabilizes viruses, particularly poliovirus and other enteroviruses.

Procedure:
Solution A:

<table>
<thead>
<tr>
<th>Salt</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>8.00 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.20 g</td>
</tr>
<tr>
<td>Na₂HPO₄ (anhydrous)</td>
<td>0.91 g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.12 g</td>
</tr>
</tbody>
</table>

- Dissolve the salts in 600 – 800 ml distilled H₂O. Make up to 1000 ml with distilled H₂O and autoclave at 10 psi (70kPa) for 15 minutes. This gives a **working solution of incomplete PBS** (i.e. no calcium or magnesium ions present).

Solution B:

<table>
<thead>
<tr>
<th>Salt</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl₂.6H₂O</td>
<td>0.10 g</td>
</tr>
</tbody>
</table>

- Dissolve in 100 ml distilled H₂O. Autoclave at 121°C and 1 atm, for 15 minutes.

Solution C:

- CaCl₂ 0.10 g

- Dissolve in 100 ml distilled H₂O. Autoclave at 121°C and 1 atm, for 15 minutes.

Working Solution of complete PBS:

- The working solution of complete PBS contains 0.11 M NaCl.
- Add 1 part of Solution B and 1 part of Solution C to 8 parts of Solution A.
- Alternatively use commercially prepared tablets or powder following the manufacturer’s instructions for reconstitution and sterilization.

**PBS with antibiotics**

Dissolve 1x10⁶ units crystalline penicillin G and 1 g streptomycin sulphate in 100 ml sterile complete PBS; distribute into 5 ml volumes and store at -20°C. For use, add 5 ml of this stock solution to 500 ml complete PBS to give a final concentration of 100 units / ml penicillin and 100µg / ml streptomycin. Store refrigerated at 4-8°C for up to one week.
ISOLATION OF POLIOVIRUSES AND OTHER ENTEROVIRUSES BY USING THE NEW CELL CULTURE ALGORITHM

**Objective:**
To describe the procedure of isolation of polioviruses and other enteroviruses by using cell culture technique under the regulation of the new algorithm.

**Reference:**

**Scope:**
This document describes procedure used in the National Polio lab, NPL to isolate polioviruses and other enteroviruses by using cell culture technique under the regulation of the new algorithm...

**Responsibility:**
This procedure is performed by trained virology Expert Supervisors under the supervision of the lab director. Materials and equipment:

**Materials:**
- Culture flasks with confluent monolayers of L20B or RDA cells
- Maintenance medium;
- 01 ml, 5 ml and 10 ml plastic disposable pipettes.

**Equipment:**
- Stationary rack, Sloped 5º position
- Biological safety cabinet class II (Microflow).
- 36°C incubator (Brand).
- Inverted Microscope (Zeiss).
- Pipette aid (Accu-jet)
- Description:

**Procedure:**
- Microscopically examine recently monolayer cultures to be sure that the cells are healthy
- Remove the growth medium and replace with 1ml maintenance medium.
- Label two tubes of RD and two of L20B for each specimen to be inoculated. (Specimen number, date).
- Label one tube of each cell type as a negative control.
NOTE: BOTH CELL LINES MUST BE INOCULATED AT THE SAME TIME

- Inoculate each tube with 0.2ml of specimen extract and incubate in the stationary sloped (5°) position at 36°C
- Examine cultures daily, using a standard or inverted microscope, for the appearance of cytopathic effect (CPE).

**L20B Passage flowchart**

```
Stool extract
  +ve a
  \  /  \
+ve b \  /  \\ CPE +ve a
  |  |  |
  +ve b   Pass into RD
  |  |  |
  +ve b   CPE +ve a
  |  |  |
  +ve b   Pass into L20B
  |  |  |
  +ve b   CPE -ve a
  |  |  |
  +ve b   Report L20B Positive, and refer for ITD
```

**L20B 1st Passage:**

- Record all the observations of the inoculated and control cultures for at least 5 days, recording CPE (+1 to +4) to indicate the percentage of cells affected (1, up to 25%; +2, 25-50%; +3, 50-75% and +4, 75-100%) toxicity, degeneration or contamination.
- If the L20b cell culture shows a +4 CPE effect in the first passage, it re-passages into RD cell culture. If it is +ve L20B on RD cells then it refers to ITD and informing the WHO agent to ship the samples for ITD in RRL, VACSERA /Egypt. If RD cells do not show CPE effect, it reports negative.
- If the L20B cell culture does not show CPE effect within the 5 days, perform a blind passage into L20B cell culture for the second passage.

**L20B 2nd Passage:**

- Record all the observations of the inoculated and control cultures for at least 5 days, recording CPE (+1 to +4) to indicate the percentage of cells affected (1, up to 25%; +2, 25-50%; +3, 50-75% and +4, 75-100%) toxicity, degeneration or contamination.
- If the L20B cell culture shows a +4 CPE effect in the first passage, it re-passages into RD cell culture. If it is +ve L20B on RD cells then it refers to ITD. If RD cells do not show CPE effect, it reports negative.
- If the L20B cell culture does not show CPE effect within the 5 days, it reports negative.

**RDA Passage flowchart**

```
Stool extract
  +ve a
  \  /  \\ CPE +ve a
  |  |  |
  +ve b   Pass into RD
  |  |  |
  +ve b   CPE +ve a
  |  |  |
  +ve b   Pass into L20B
  |  |  |
  +ve b   CPE -ve a
  |  |  |
  +ve b   Report NPEV a
```

- If the RDA cell culture becomes positive, it indicates the need for ITD. If RD cells do not show CPE effect, it reports negative.
- If the RDA cell culture does not show CPE effect within the 5 days, it reports negative.
RD 1st Passage:
- Record all the observations of the inoculated and control cultures for a minimum of 5 days, recording CPE (+1 to +4) to indicate the percentage of cells affected (1, up to 25%; +2, 25-50%; +3, 50-75% and +4, 75-100%). toxicity, degeneration or contamination.
- If the RD cell culture show +4 CPE effect in the first passage, it repassage into L20B cell culture, if it NO CPE it report as Non Polio Enterovirus (NPEV).
- If the RDA+ shows +4 CPE on the L20B cells, it will pass to RD culture, If Positive then refer to ITD, if not report Negative.
- If the RD cell culture does not show CPE effect within the 5 days, perform a blind passage into RD cell culture for the second passage.

RD 2nd Passage:
- Record all the observations of the inoculated and control cultures for for a minimum of 5 days, recording CPE (+1 to +4) to indicate the percentage of cells affected (1, up to 25%; +2, 25-50%; +3, 50-75% and +4, 75-100%). toxicity, degeneration or contamination.
- If the RD cell culture show +4 CPE effect in the first passage, it re-passage into L20B cell culture, if it NO CPE it report as Non Polio Enterovirus (NPEV).
- If the RDA+ shows +4 CPE on the L20B cells, it will pass to RD culture, If Positive then refer to ITD, if not report Negative.
- If the RD cell culture do not show CPE effect within the 5 days, it report negative.
- Pool positive tubes (if both tubes show > 3+ CPE on the same day) before final RD passage.
Safety Conditions:
- Care must be taken to avoid cross-contamination of stool specimens during the transfer of material from the original containers to the storage vials.
- To protect laboratory workers all manipulation of faecal material and faecal suspensions must take place inside a functional Class II biosafety cabinet (BSC). The effectiveness of a BSC is dependent on its position, correct use and regular testing and maintenance. Cabinets should be sited away from doors and through traffic. Movement in the area of a BSC will disturb airflow and so access to the area should be restricted to essential personnel. When working within a BSC it is important to minimize the potential for contamination of the working environment and cross-contamination between specimens. This can be greatly assisted by the following:
  - Cabinets should be switched on 10-20min before use and left on afterwards for a similar period.
  - Do not make rapid movements within the cabinet as this disrupts airflow
  - Manipulate fluids slowly and gently to avoid creating aerosols
  - Do not overcrowd the cabinet and never obstruct the front opening
  - Organize the work area so that sterile reagents and samples do not come into contact with each other (e.g. pots for liquid waste to the left and sterile media to the right with samples handled centrally)
  - Clean and decontaminate the cabinet inner surfaces (both horizontal and vertical) after every working session and periodically (e.g. once per month) decontaminate and clean the tray under the BSC working surface. Replace the BSC front cover when not in use to prevent entry of dust and aerosols

Documentation:
- While carrying out the assignment it is necessary to maintain record as it helps in crosschecking the procedure applied in order to avoid any type of troubleshooting. Following are important to note while carrying out the procedure.
  - Cells (monolayer) Condition
  - No. of Samples inoculated
  - Date of Inoculation
  - Media Type used.
  - Media Bottle No.
  - Media Date
  - Samples inoculated by
  - Worksheets maintenance for CPE
  - Passage Dates for each tube

Reference documents:
- SOP of operation, maintenance & calibration of Biological safety cabinet class II.
- SOP of operation, maintenance & calibration of 36°C incubator
- SOP of operation, maintenance & calibration of Pipette aid.
- SOP of operation, maintenance & calibration of Inverted Microscope.
**Reporting:**
- Put the data in the Daily observation record form.
- Record the data in the daily work folder.
- Supervisor must be informed as well as the head of the lab.
- Record of L20B′RD+ isolates and RD′L20B′RD+ isolates refer to ITD unit.
Poliovirus Isolation New Algorithm

Stool extract

Inoculate in L20B

CPE +ve

Pass into L20B

CPE +ve

CPE -ve

Pass into RD

Inoculate in RD

CPE -ve

CPE +ve

Pass into L20B

CPE +ve

CPE -ve

Pass into L20B

CPE +ve

CPE -ve

Report L20B Positive, and refer for ITD

Report Negative

Record L20B Negative

Record RD Negative

Report Specimen Negative

Report L20B Positive, and refer for ITD

Report negative

Report NPEV

---

a  Observed for a minimum of 5 days
b  Observe until $\geq 3+ \text{ CPE obtained (usually 1-2 days, 5 days maximum; re-inoculate when toxicity or contamination observed)}$

---

c  Total minimum observation time of 10 days ($2 \times 5$ days)

d  Pool positive tubes (if both tubes show $> 3+ \text{ CPE on the same day}$) before final RD passage

e  Isolates can be serotyped by laboratories with an interest in NPEV diagnosis or to confirm proficiency
Objective:
To describe the procedure to be followed for the dilution of RIVM poliovirus typing antisera.

Reference:

Scope:
This document describes the procedure used in NPL -Iraq, to make dilution of RIVM poliovirus typing anti-sera used for identification (serotyping) of polioviruses.

Responsibility:
This procedure is to be carried out by trained lab personnel under the supervision of lab director.

Materials:
- RIVM polio typing kit containing;
- Rabbit antisera with homologous titers of:
  - 40,960 to polio type 1
  - 163,840 to polio type 2
  - 40,960 to polio type 3
- Maintenance medium (MEM with HEPES and 2% FBS)
- Externally threaded screw capped cryovials (1.8ml)

Equipment:
- Biological safety cabinet class II (Microflow).
- -20°C freezer (Revco).
- Pipette aid (Accu-Jet)

Procedure:
- Put 63.5 ml MEM in each sterilized bottles labeled as P1, P2 and P3 anti-sera
- Add 0.5 ml of RIVM poliovirus typing antisera to give 64 ml at working dilution of 1:128.
- Label the bottles and add quantity of monovalent anti-sera to each bottle according to following table:

<table>
<thead>
<tr>
<th>Anti-sera polio 2,3</th>
<th>Anti-sera polio 1,3</th>
<th>Anti-sera polio 1,2</th>
<th>Anti-sera polio 1,2,3</th>
<th>Pool</th>
</tr>
</thead>
<tbody>
<tr>
<td>15ml</td>
<td>15ml</td>
<td>10ml</td>
<td>10ml</td>
<td>Polio1</td>
</tr>
<tr>
<td>15ml</td>
<td>15ml</td>
<td>10ml</td>
<td>10ml</td>
<td>Polio2</td>
</tr>
<tr>
<td>15ml</td>
<td>15ml</td>
<td>10ml</td>
<td>10ml</td>
<td>Polio3</td>
</tr>
<tr>
<td>30ml</td>
<td>30ml</td>
<td>30ml</td>
<td>30ml</td>
<td>Total volume</td>
</tr>
</tbody>
</table>

Volume of each 1/128 dilution antisera required
Aliquot each 1.0 ml of each anti-sera pool into clearly labeled externally threaded screw capped cryovials and store at –20ºC.

Store the remaining monovalent anti-sera in cryovials at –20ºC and use it for confirmation of individual separated isolates or for making further pools.

Reference documents:

- SOP of operation, maintenance & calibration of Biological safety cabinet class II.
- SOP of operation, maintenance & calibration of Pipette aid.
- SOP of operation, maintenance & calibration of -20ºC freezer.

Reporting:

- Put the data in the Typing record form.
- Record the data in the daily work folder.
- Supervisor must be informed as well as the head of the lab.
- Update the typing forms with the new preparation.
Objective:
To describe the procedure to be followed for the dilution of RIVM poliovirus typing antisera.

Reference:

Scope:
This document describes the procedure used in NPL -Iraq, to make dilution of RIVM poliovirus typing anti-sera used for identification (serotyping) of polioviruses.

Responsibility:
This procedure is to be carried out by trained lab personnel under the supervision of lab director. Materials and equipment:

Materials:
- RIVM polio typing kit containing;
- Rabbit antisera with homologous titers of:
  - 40,960 to polio type 1
  - 163,840 to polio type 2
  - 40,960 to polio type 3
- Maintenance medium (MEM with HEPES and 2% FBS)
- Externally threaded screw capped cryovials (1.8ml)
**Equipment:**
- Biological safety cabinet class II (Microflow).
- -20°C freezer (Revco).
- Pipette aid (Accu-Jet).

**Procedure:**
- Put 63.5 ml MEM in each sterilized bottles labeled as P1, P2 and P3 anti-sera.
- Add 0.5 ml of RIVM poliovirus typing antisera to give 64 ml at working dilution of 1:128.
- Label the bottles and add quantity of monovalent anti-sera to each bottle according to the following table:

<table>
<thead>
<tr>
<th>Anti-sera polio 2,3</th>
<th>Anti-sera polio 1,3</th>
<th>Anti-sera polio 1,2</th>
<th>Anti-sera polio 1,2,3</th>
<th>Pool</th>
</tr>
</thead>
<tbody>
<tr>
<td>15ml</td>
<td>15ml</td>
<td>15ml</td>
<td>10ml</td>
<td>Polio1</td>
</tr>
<tr>
<td>15ml</td>
<td>15ml</td>
<td>15ml</td>
<td>10ml</td>
<td>Polio2</td>
</tr>
<tr>
<td>15ml</td>
<td>15ml</td>
<td>-----</td>
<td>10ml</td>
<td>Polio3</td>
</tr>
<tr>
<td>30ml</td>
<td>30ml</td>
<td>30ml</td>
<td>30ml</td>
<td>Total volume</td>
</tr>
</tbody>
</table>

- Aliquot each 1.0 ml of each anti-sera pool into clearly labeled externally threaded screw capped cryovials and store at –20°C.
- Store the remaining monovalent anti-sera in cryovials at –20°C and use it for confirmation of individual separated isolates or for making further pools.

**Reference documents:**
- SOP of operation, maintenance & calibration of Biological safety cabinet class II.
- SOP of operation, maintenance & calibration of Pipette aid.
- SOP of operation, maintenance & calibration of -20°C freezer.

**Reporting:**
- Put the data in the Typing record form.
- Record the data in the daily work folder.
- Supervisor must be informed as well as the head of the lab.
- Update the typing forms with the new preparation.
Identification of polioviruses by neutralization method SOP#: NPL AFP 019

Objective:
Objective is to describe the procedure of identification of polioviruses by micro-neutralization method.

Reference:

Scope:
This document describes procedure used in the National Polio Lab lab (NPL) to identify polioviruses by micro-neutralization method.

Responsibility:
This procedure is performed by trained virology expert supervisors under the supervision of the lab director.

General Description:
For the identification of poliovirus isolates, samples of diluted isolate are mixed with equal volumes of a selected set of polyclonal antisera made in animals against poliovirus types 1, 2 and 3. Using the micro-neutralization (micro titer plate) technique, the serum/virus mixtures are incubated for one hour at 36°C to allow the antibodies to bind to the virus. Subsequently, suspensions of cells are added to the microtiter plates which are examined daily for the presence of CPE. The antiserum that prevents the development of CPE indicates the identity of the virus.

Materials:
- Flask of healthy cells of the type in which the virus was confirmed (usually L20B)
- Maintenance medium
- Polio antiserum pools
- 1 ml and 2 ml disposable plastic pipettes
- Sterile 96-well flat bottomed tissue culture microtitre plates with lids
- Sterile, non-toxic plate sealers
- 5ml sterile tubes for dilution
- Rack for 5ml tubes
- Sterile 50 μl droppers or pipettors with aerosol resistant tips (ARTs)

Equipment:
- Biological safety cabinet class II (Microflow).
- 36°C incubator (Brand).
- Inverted Microscope (Zeiss).
• Pipette aid (Accu-Jet)

Procedure:
• Label the edge of the microtitre plate as indicated in the Figure 1. (For two unknown virus isolates)
• Distribute 50 μl of each of the four antiserum pools in columns 1 to 8, rows A to D, using a different dropper/pipette tip for each pool
• Freeze and thaw Viral isolates 2 times
• Add 50 μl medium to virus control wells, A9 to D10
• Add 50 μl medium to back titration wells E1 to H10
• Add 100 μl medium to cell control wells G11 to H12 and cover plate.
• Label dilution tubes 10^-1-10^-7, marking each set with specimen number (Figure 2).
• Dispense 0.9 ml medium to all tubes.
• Add 0.1 ml virus to first tube (=10^-1 dilution) using sterile pipette or pipettor with ART tip
• Take another pipette/pipette tip, mix thoroughly but gently to avoid aerosols
• Transfer 0.1 ml to the second tube and discard pipette/pipette tip
• Repeat dilution steps.
• Add virus to the back titration wells of the microplate beginning at the 10^-7 dilution in columns 9 and 10, rows E and F.
• The same dropper/pipettor ART tip may be used for one isolate, working from highest to lowest dilution, 10^-7 to 10^-3
• Add 50 μl virus to the test wells as indicated: 10^-3 dilution of isolate 1 to wells A1-A10, 10^-4 dilution to wells B1-B10 etc.
• Repeat the last two steps for the second isolate in rows G and H for the back titration, in wells C1-C10 for the 10^-3 dilution of isolate 2, and in wells D1-D10 for 10^-4 dilution of isolate 2.
• Cover the plate with the lid and incubate between one and three hours at 36°C
• During this incubation period, trypsinize cells and prepare a suspension of approximately 1.5x10^5 cells per ml, calculating at least 10 ml per plate. See Trypsinization protocol.
• Distribute 100 μl of cell suspension into test and control wells
• Seal plate with non-toxic sealers and incubate at 36°C
• Examine and record daily, using an inverted microscope, for development of CPE
• Continue observation and recording until 24 hours after the virus control wells show 100% CPE (usually 3-5 days)
**Interpretation of results:**

Cell control wells should have a complete monolayer of cells. Virus control wells should show complete CPE. The back titration should confirm that the amount of virus used in the test was within the range between $10^{1.5}$ and $10^{2.5}/50\,\mu\text{l}$ corresponding to $10^{4.5}$ to $10^{6.5}$ in the original isolate. The antiserum pools that prevent the development of CPE indicate the identity of the virus isolate or mixture of viruses.

Failure of a virus to replicate in the presence of a pool of antisera is due to the neutralization of infectivity by one of the antisera present in the pool. Figure 3, shows the typical lay-out of a test plate with interpretation of results.

**Table: Interpretation of virus neutralization patterns in the poliovirus identification test**

<table>
<thead>
<tr>
<th>Pool P1+P2+P3</th>
<th>Pool P1+P2</th>
<th>Pool P1+P3</th>
<th>Pool P2+P3</th>
<th>Virus identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>Poliovirus type 1</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>Poliovirus type 2</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>Poliovirus type 3</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>Mixture of poliovirus types 1 &amp; 2</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>Mixture of poliovirus types 1 &amp; 3</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>Mixture of poliovirus types 2 &amp; 3</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Mixture of all three poliovirus types</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>No poliovirus or mixture of poliovirus with other enterovirus</td>
</tr>
</tbody>
</table>

*+ = CPE  0 = No CPE*

**Note:** Where CPE occurs in test wells containing all combinations of polio antisera (last row of figure), there are two possibilities. The first is that the virus is not polio, but some other virus (e.g. an enterovirus or adenovirus). The second is that there may be a mixture of polio and another virus. Since the detection of poliovirus is of prime importance, the L20B isolate should be referred to the NPL as soon as possible for further characterization.

In Table, all possible combinations of CPE and NO CPE, which may be observed in wells containing the various polio antiserum pools, are tabulated with the appropriate interpretation. "Breakthrough" of virus occurring after the final result may be due to too high a dose of virus used in the test; or to the presence of a second virus in a lower concentration. It is therefore recommended that all mixed poliovirus isolates be confirmed using individual antisera. It is not necessary to perform a complete typing test using antisera pools.

**Protocol for confirmation test for virus typing:**

- Collect the virus from the appropriate wells of the plate used for polio typing.
- When the plate is covered with a plate sealer, do not remove the cover as this may cause cross contamination. Puncture the covering of the wells involved with a hot glass pipette. Never try to push a glass pipette through the plastic cover, as the pipette may break and cause serious injuries.
- Make a 1 in 10 dilution of the virus.
- Prepare a plate, containing for each virus to be tested, two wells with 50 µl anti P1 serum, two wells with 50 µl anti P2 serum, two wells with 50 µl anti P3 serum, and two wells with 50 µl growth medium, that serve as virus controls.
- Add 50 µl of the diluted virus to all 8 wells.
- After 1hr incubation at 37 °C, add 100 µl of cells.
- Seal the plate; incubate 24-48 hrs at 36 °C and record CPE.

**Figure 1:** Labeling of Microtiter plate for two unknown Viruses

**Figure 2:** Dilution of Virus Isolates
**Figure 3:** Identification of poliovirus isolates using the Micro-technique

**Reference documents:**
- SOP of operation, maintenance & calibration of Biological safety cabinet class II.
- SOP of operation, maintenance & calibration of 36°C incubator
- SOP of operation, maintenance & calibration of Pipette aid.
- SOP of operation, maintenance & calibration of Inverted Microscope.

**Reporting:**
- Put the data in the Typing record form.
- Record the data in the daily work folder.
- Supervisor must be informed as well as the head of the lab.
Identification of Enterovirus isolates. SOP #: NPL-AFP 020

<table>
<thead>
<tr>
<th>Standard Operating Procedure</th>
<th>SOP #: NPL-AFP 020</th>
<th>Version: 1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title: Identification of Enterovirus isolates</td>
<td>Author: WHO Lab Manual 2004</td>
<td>Approved by: Dr. Faisal Ghazi Alhamdani</td>
</tr>
<tr>
<td>Review Date: 30 Oct. 2014</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Objective:  
Explain the procedure of identification of Enterovirus isolates.

Reference:  
WHO Lab Manual 2004,

Scope:  
This procedure is applied in NPL-Iraq to determine the type of Enterovirus isolates. This procedure is carried out by two virology expert supervisors under the overall supervision of the lab director.

Responsibility:  
This procedure is performed by trained virology expert supervisors under the overall supervision of the lab director.

Materials:  
- Enterovirus serum pools at use dilution.
- Maintenance medium
- Flask of healthy cells of the type in which the virus was confirmed (usually L20B).
- 1 ml and 2 ml disposable plastic pipettes
- Sterile 96-well flat bottomed tissue culture microtitre plates with lids
- Sterile, non-toxic plate sealers
- 5ml sterile tubes for dilution
- Rack for 5ml tubes

Equipment:  
- Biological safety cabinet class II (Microflow).
- 36°C incubator (Brand).
- Inverted Microscope (Zeiss).
- Pipette aid (Accu-Jet)

Good laboratory practice:  
- Cell control wells should have a complete monolayer of cells. Virus control wells should show complete CPE.
- The back titration should confirm that the virus titer between 101.5 and 102.5/50ul corresponding to 104.5 to 106.5 in the original isolate.
- Each unknown virus is tested in duplicate against a trivalent pooled antiserum (PP), a coxsackie virus B1-6 pool (CP), and seven pools against Coxsackie virus A9 and 20 Echovirus (A-G).
• None polioviruses that fail to be identified using these antisera may be in an aggregated form which interferes with the complete neutralization by specific antisera.

• If CPE is seen in all wells containing virus, the isolate should be reported as a non-poliovirus.

Procedure:
• Label the edge of the microtitre plate as indicated in the figure.
• Add 50ul antisera to the appropriate wells in columns 1-9.
• Add 50 ul medium to virus control wells in column 10 rows A to D.
• Add 50 ul medium to back titration wells E1 to H10.
• Add 100 ul medium to cell control wells columns 11 and 12 rows A to D.
• Prepare 10^-2 dilution of virus.
• Add 50 ul virus to all wells in columns 1 to 10 of rows A and B for sample X and rows C and D for sample Y.
• Perform a Back titration of virus X in rows E and F and of virus Y in rows G and H.
• Cover the plate with lid and incubate for one hour at 36ºC.
• During this incubation period, trypsinize RD cells and prepare a suspension of approximately 1.5 x10^5 cells per ml, calculating at least 10 ml per plate.
• Distribute 100 ul of cell suspension into test and control wells.
• Seal plate with nontoxic sealers and incubate at 36ºC.
• Examine and record daily, using an inverted microscope, for development of CPE.
• Continue observation and recording until 24 hours after the virus control wells show 100% CPE.

Reference documents:
• SOP of operation, maintenance & calibration of Biological safety cabinet class II.
• SOP of operation, maintenance & calibration of 36°C incubator
• SOP of operation, maintenance & calibration of Pipette aid.
• SOP of operation, maintenance & calibration of Inverted Microscope.

Reporting:
• Put the data in the Identification of Enterovirus isolates record form.
• Record the data in the daily work folder.
• Supervisor must be informed as well as the head of the lab.
Figure 4: Identification of enterovirus isolates using the micro technique
Objective:
To describe the procedure to be followed for the dilution of RIVM enterovirus typing antisera.

Reference:

Scope:
This document describes the procedure used in National Polio Laboratory -NPL, to make dilution of RIVM enterovirus typing anti-sera used for identification (serotyping) of enteroviruses.

Responsibility:
This procedure is performed by trained virology Supervisor under the overall supervision of the lab director.

Enterovirus antisera:
Antisera have been raised in animals against many echoviruses and coxsackie viruses. Because the large number of viruses make it impractical to perform individual neutralization test, these have been pooled in an overlapping scheme, which allows any viruses to be identified using as few as nine antisera.

Materials:
- RIVM enterovirus typing kit containing anti-enterovirus pools A, B, C, D, E, F, G, and anti-Coxsackie B virus pool and a trivalent anti-poliovirus pool.
- Maintenance medium;
- 01 ml, 5 ml and 10 ml plastic disposable pipettes.
- Externally threaded screw capped cryovials (1.8ml)

Equipment:
- Biological safety cabinet class II (Microflow).
- Pipette aid (Accu-Jet)
- Freezer –20ºC (Revco)
- Procedure:
- Put 9.5 ml MEM in each sterilized tube labeled as A, B, C, D, E, F, G, anti-Cox B and poliovirus pool antisera.
- Aliquot each 1.0 ml of each anti-sera pool into clearly labeled externally threaded screw capped cryovials and store at –20ºC.

Safety conditions:
All procedures should be performed in class II biosafety cabinet.

**Reference documents:**
- SOP of operation, maintenance & calibration of Biological safety cabinet class II.
- SOP of operation, maintenance & calibration of Pipette aid.
- SOP of operation, maintenance & calibration of -20°C freezer.

**Reporting:**
- Put the data in the Preparation of RIVM enterovirus typing anti-sera record form.
- Record the data in the daily work folder.
- Supervisor must be informed as well as the head of the lab.
22) AFP Data management. SOP #: NPL MAN 022

<table>
<thead>
<tr>
<th>Standard Operating Procedure</th>
<th>SOP #: NPL MAN 022</th>
<th>Version: 1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title: AFP Data management</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Objective:**
To provide a standard protocol describes the good laboratory data management in National Polio Laboratory (NPL) Iraq.

**Reference:**
WHO Lab Manual 2004,

**Scope:**
This document describes procedure to record the details of all specimens tested, record the results of testing, and to report the results.

**Responsibility:**
This procedure is performed by all NPL staff supervisors trained on the data management system under the overall supervision of the lab director.

**Good data management:**
- Good data management starts by understanding:
- The meaning of the information generated.
- What you need to tell to people outside of the laboratory.
- Who you need to tell it to.
- How often you need to tell it.

**Every laboratory needs to:**
- Report the results, in an organized format, to the polio eradication program and back to the person who submitted the specimens.
- Produce reports of its work to the director or head of the institute as annual reports or progress reports.
- Produce summary reports to justify why it should continue to receive more funding.
Procedure:

- Recording receipt of specimens
- The following minimum information should be included on the laboratory request form accompanying the specimen:
  - EPId Number (in an agreed format).
  - Patient name.
  - Province (or region) of report.
  - Town/district of report.
  - Province (or region) of residence of the case.
  - Town/district of residence of the case.
  - Country code.
  - Whether the case has been immunized.
  - Date of last OPV.
  - AFP or contact.
  - If a contact, EPId number of the related case.
  - Specimen type (stool, etc.).
  - Date of onset of paralysis (exact date, the month is minimum requirement).
  - Date of first specimen collection.
  - Date of second specimen collection.

The following additional information should be recorded by the laboratory on receipt of a specimen:

- Date specimen received in laboratory.
- Specimen arrived frozen or with ice present (for feedback to EPI) (y/n).
- Specimen arrived in amount large enough for full laboratory analysis (y/n).
- Specimen arrived with no evidence of leakage or desiccation (y/n).
- Recording laboratory results

Virus isolation and characterization

- Record the date when the first cell monolayers are inoculated.
- Information relating to each of the two cell culture passes should be recorded separately.
- Record when CPE is first observed in a culture.
- Record this information in a standard database (specimen-based database), with each line of information relating to one specimen. Thus, for a case with two specimens collected and processed there will be two lines of information.

The recommended minimum information to be collected and recorded on specimen processing and isolation results should include the following:

- Whether the specimen was processed (y/n).
- Date of specimen extraction.
- Temperature at which extract stored;
- Date of first inoculation onto L20B cells.
- Date of L20B+ passed to RD becomes positive.
- Date of first inoculation onto RD cells.
- Date of RD+ passed to L20B becomes positive.
- Date of L20B+ passed to RD becomes positive.
• Date of inoculation of L20B-negative, RD-positive isolate into L20B.
• Result for L20B passage and date of result.
• Date final isolation result available.

**Intratypic differentiation**
The minimum recommended information to be sent with the material for intratypic differentiation includes the following:

• Case identification number (EPId).
• Laboratory specimen number.
• Isolate identification number.
• Passage history of isolate (e.g. L20B second pass).
• Date of sending isolate to RRL.

The minimum recommended information to be recorded by the laboratory carrying out intratypic differentiation includes the following:

• Date referred material received.
• EPId number.
• ITD laboratory sample number.
• Referring laboratory sample number.
• Referring laboratory isolate number.
• Material type.
• Date of ITD processing by method 1.
• Result: polio 1 present (non-Sabin or Sabin-like).
• Result: polio 2 present (non-Sabin or Sabin-like).
• Result: polio 3 present (non-Sabin or Sabin-like).
• Result: NPEV present.
• Result: no virus present.
• Result: not interpretable.
• Date of ITD processing by method 2.
• Result: polio 1 present (non-sabin or sabin-like).
• Result: polio 2 present (non-sabin or sabin-like).
• Result: polio 3 present (non-sabin or sabin-like).
• Result: NPEV present.
• Result: no virus present.
• Result: not interpretable.
• Date final ITD result available.
• Date result sent to National EPI programme.
• Date result sent to national laboratory.
• Date result sent to WHO regional office.

**Reporting laboratory activity and results**

• Laboratory results must be reported in a timely and accurate manner for several reasons. Reporting of laboratory results has a direct effect on the poliomyelitis eradication programme through:
• Feedback to national EPI teams for case follow-up and planning supplementary immunization activities.

• Coordination of the eradication programme through WHO and other international agencies and bodies.

• Monitoring of laboratory results and performance to identify possible problems and constraints.

Feedback to EPI teams
• Results should be made available on individual cases as they become available.

• Summaries of results available (including pending results) should be sent to the EPI managers on a regular basis, either weekly or monthly depending on local requirements.

There are three levels of detail that reports can take:

• Isolation result after two consecutive passages totaling 10 days in the two recommended cell lines. Approximately 75 to 80% of all specimens from AFP cases can be expected to be negative for virus isolation and reported as such. This should be done within 14 days of receipt of the specimens. Local arrangements may require a laboratory to report positive CPE pending typing results, but this should be decided in consultation with the EPI managers.

• Intratypic differentiation results reported from the Regional Reference Laboratory. These should be available within 14 days of receipt of the isolates in the reference laboratory.

• Wild-type poliovirus positives should be reported within 24 hours and, in countries or regions that have been free of wild poliovirus for some time, they should be treated as an emergency.

Details of inadequate specimens and inadequate transport of specimens should be reported to EPI managers as soon as possible so that field staff can be informed and improvements made.

Weekly/monthly reports to WHO
• Provide a weekly or monthly report of results to WHO, the frequency depending upon the WHO Region requirements.

• Laboratories handling more than 100 stool specimens a year provide their routine reports in computer database format. On computer diskettes or sent by e-mail.

• The choice of exactly how to computerize laboratory record keeping depends on a number of factors, including:
  • User preference.
  • Hardware availability and capacity.
  • Software availability and cost.
  • Type of programming required to use the software.
  • Local expertise to develop and maintain the system.

Any computerized laboratory records system should contain the following components:
Data entry.
• Data cleaning (programmes that detect errors in the information entered).
• Routine backup of data.
• Routine analysis and reporting (for decision-making, action, monitoring).
• Feedback (information to be sent back to the case investigators).
• Feed forward (information to be reported to the next level).

Currently use the LABIFA version 4.0 and submit the data to EMERO on a weekly basis.

**In-house reporting**

• Prepare reports on laboratory activities and results to the head of the lab.
• The basic laboratory report format, recommended by WHO, should provide the foundation of these in-house reports.

• Laboratory data for certification

• Reporting of results has a direct effect on certification of polio-free status by providing a continuous record demonstrating that:
  • Wild poliovirus has been absent from the country or region for at least three years.
  • A fully integrated surveillance system exists.
  • Recommended and acceptable procedures have been followed.
  • Laboratory performance has been at an acceptable level.
  • Laboratory accuracy has been at an acceptable level.

• The laboratory is complying with requirements for containment of wild poliovirus stocks.

The following minimum documentation will be required from each national laboratory for as many years as possible, but at least for the three years immediately prior to certification:

• **AFP cases**

  • The total number of stool specimens received, the total number of AFP cases from which stool specimens were received and the total number of stool specimens that were processed each year.
  • The total number of non-polio enteroviruses that were isolated and the non-polio enterovirus isolation rate.
  • The total number of polioviruses that were isolated, the total number of isolates that were sent for intratypic differentiation, and the total number of AFP cases that had results sent for intratypic differentiation.
  • The results of all intratypic differentiation studies, by specimen and AFP case.

• **Non-AFP specimens**

  o The total number of stool specimens received from non-AFP cases, including healthy child surveys and special studies, together with specimens from environmental surveys that were submitted for enterovirus studies and processed each year.
The total number of non-polio enteroviruses that were isolated and the non-polio enterovirus isolation rate.
The total number of polioviruses that were isolated, the total number of isolates that were sent for intratypic differentiation and the total number of specimens that had results sent for intratypic differentiation.
The results of all intratypic differentiation studies, by specimen and specimen type.

- **Missing laboratory data**
  - The reasons for each instance in which a specimen that was received in the laboratory was not processed.
  - The reasons for each failure to send a poliovirus isolate for intratypic differentiation.
  - The reasons for each missing intratypic differentiation result.
  - Detailed laboratory information will be required for certification; the sooner a system is set in place for recording and maintaining this information the easier it will be to provide it when requested.
Figure 5: Polio laboratory data flow: Laboratory results and performance monitoring data flow
Figure 6: Polio laboratory data flow: laboratory data feedback
Objective:
To describe the procedure of reporting virus isolation results of the samples received in the laboratory

Reference:
WHO Lab Manual 2004,

Scope:
This document describes procedure used in National Polio Laboratory (NPL) to report the results of polioviruses and other enteroviruses identification.

Responsibility:
This procedure is performed by trained Data Managers under the overall supervision of the lab director.

Reporting of virus isolation results and follow-up:
- Laboratories without on-site ITD capacity must report preliminary virus isolation results to national authorities and to WHO at this stage.
- The result for an individual specimen is obtained by combining results for duplicate cultures in both L20B and RD cell lines.
- The result for an individual case is obtained by combining the results for all specimens from that case.
- The possible outcomes of virus isolation procedures and suggested wording of reports are provided in Table 1.
- Untyped isolates should be sent as soon as possible to an appropriate ITD laboratory (RRL/Vacsera) (and ideally within 7 days of detection) where virus serotype and intratypic will be determined. Accompanying documentation must include patient and isolate identification information (e.g. EPID and laboratory number) and isolates must be labeled with laboratory number and passage information (e.g. expressed as L+R+ or R+L+R+).
- Laboratories wishing to do so may continue to perform virus serotyping by microneutralization assay on an aliquot of the isolate in their own location. It is emphasized, however, that laboratories should not await serotyping results or separate viruses before shipping isolates to ITD laboratories.
- Report to national authorities and WHO the result of any poliovirus isolate identified as "wild poliovirus of indicated serotype(s)". The report should specify the poliovirus serotype obtained and indicate the intratypic as "non-Sabin-like".
- Reports should be provided within 24 hours of completion of the PCR, and the isolate should be referred to a specialized laboratory for sequencing within 7 days of detection.
- Non-Sabin-like (preliminary) results from any isolate should be reported and isolates referred for sequencing as they become available.
- Final results can be issued at a later time when all virus isolation and ITD results are available for all cultures and specimens of the case.

<table>
<thead>
<tr>
<th>Outcome of virus isolation tests</th>
<th>Comment</th>
<th>Action required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>No viral CPE was observed post-inoculation or post-passage of L20B and RD cell cultures for any of the inoculated cultures or specimens of the case.</td>
<td>Report &quot;Negative&quot; or &quot;No virus isolated&quot;. No further action required.</td>
</tr>
<tr>
<td>L20B positive</td>
<td>Viral CPE was obtained in L20B cells either post-inoculation or post-passage for at least one of the cultures or specimens of the case. CPE was reproducible when the L20B isolate was passaged into RD cells.</td>
<td>Report &quot;L20B positive isolate obtained. Likely to be poliovirus. Isolate will be characterized by ITD tests&quot;. Refer the RD passage of the L20B isolate to an ITD laboratory within 7 days of detection.</td>
</tr>
<tr>
<td>Non-polio Enterovirus detected.</td>
<td>Viral CPE was obtained in RD cells either post-inoculation or post-passage and no CPE was obtained when the RD isolate was passaged into L20B cells.</td>
<td>Report &quot;NPEV positive&quot;. No further action required.</td>
</tr>
<tr>
<td>Both L20B positive and Non-polio Enterovirus detected.</td>
<td>One or more cultures of the specimen was identified as having an &quot;L20B positive isolate&quot; and one or more other cultures of the same specimen was also identified as being &quot;NPEV positive&quot;</td>
<td>Report &quot;L20B and NPEV positive isolates detected&quot;.</td>
</tr>
</tbody>
</table>

**Table 1:** Possible Outcomes and Reporting of virus Isolation results for an Individual Specimen
Periodic review update and evaluation of Compliance SOP #: NPL MAN 024

**Objective:**
To provide a standard protocol describe a procedure to review the improvement of laboratory performance, update and evaluation of compliance. New test algorithms for the cell culture and ITD.

**Reference:**
Recommendation of polio network WHO lab meeting, 2006.

**Scope:**
This document describes procedures applied in national polio laboratory NPL to estimate improvement of Laboratory performance and compliance with reference.

**Responsibility:**
This procedure is performed by the lab Manager, Deputy Manager and supervisors. The meeting recorded in Arabic language.

**Materials:**
- How much last meeting recommendation implemented and evaluation of improvements.
- It includes:-
  - Number of specimens tested.
  - Results of specimens and interpret them.
  - Trained analysis of results.
  - Complains for any problems and how it is solved.
  - What need to tell people what is new recommendation.
- Procedure:
  - It's done weekly by lab people concerned directors sub directors Supervisor, coordinator and technician.
  - List of all people attend this meeting.
  - Evaluation of improvement in objectives, corrective and preventive action of any complains and problem
  - Investigation of any problem happened in the last Meeting and documented it
  - Recommendation for the next meeting signed by lab Manger and people attended.

**Reporting:**
All of the meeting documents collected in the Periodic review update and evaluation of Compliance folder.
**Objective:**
To provide a standard protocol that describes the procedures for the receipt and storage of faecal specimens:
- At hospital lab, if the sentinel hospitals will perform rotavirus identification (EIA)
- At national/central lab, if the lab of the sentinel hospital does not perform EIA

**Reference:**
Recommendation of EMR ROTAVIRUS GASTROENTERITIS SURVEILLANCE NETWORK (EMRSN)

**Scope:**
This document describes procedures applied in national polio laboratory NPL to follow the receipt and storage of faecal samples.

**Responsibility:**
This procedure is performed by the lab Manager, Deputy Manager and supervisors. The meeting should be recorded in Arabic language.

**SUPPLIES and EQUIPMENT**
- Clean bench/work space
- Wooden spatula
- 2 ml cryotubes with screw caps
- Absorbent tissue paper
- Laboratory disinfectants (eg, 70% alcohol, sodium hypochlorite solution)

**Procedure:**
- Recording receipt of specimen in lab logbook (Table 3)
- Information on specimen labels must be carefully checked to ensure that it matches information on the Specimen Transfer, Investigation and feedback form received from hospital/pediatric ward
- Record the following in the lab logbook:
  - Date specimen received in laboratory
  - Patient ID
  - Name of patient
  - Details of receiver (eg., name, position)
- Storage of Samples: (see flow chart of specimen storage and transport, Figure 8)
- Storage at hospitals that do not perform EIA: if specimen analysis (EIA) will be done outside the hospital at the national lab:
- Transfer the information from the Specimen Investigation Request and Feedback form to the Laboratory Logbook
- Store specimens, in their containers, at 2-8°C until transfer for analysis at a national lab. (DO NOT FREEZE)
- Fill in the Off-site Specimen Transfer and Investigation Request Form
- Transfer specimens to the national laboratory using the Standard Transportation Procedure (SOP 3)

**Storage at hospitals that perform EIA and at the national labs:**

- Labs that will conduct EIA (whether the hospital labs or national lab) should divide the specimens when received into aliquots for performing the different investigations as follows:

  - Label 2 externally threaded screw capped storage vials with the storage label (Figure 7), using the same unique patient ID
  - Transfer with wooden spatula one-third of the stool specimen into each pre-labelled storage vial

**Store the aliquots as follows:**

**Short term storage at 2-8°C:**

Specimen in one of the storage cryovials will be used for identifying rotavirus by EIA. Store this vial at 2°C - 8°C until processing

**Long term storage:**

Store the other cryovial at -20°C after adding 3 drops of glycerol and shaking gently. This sample will be used for genotyping. The vial will be stored until 1) transfer from the hospital to the national lab for genotyping, 2) processing at the national lab, or 3) transfer from the national lab to the regional reference lab for genotyping

The remaining original sample in its original labelled container should be stored frozen at -20°C (with added 1-3% glycerol) for at least six months to one year before discarding to allow re-investigation of specimens giving anomalous or queried results. Label the container with the stool label for long term storage (Figure 7)

**USEFUL NOTES**

- Rotavirus is stable at 2-8°C for long periods. However for periods over one month it is recommended that samples are stored at -20°C.

- Adding glycerol to make a final concentration of 1-3%, minimizes the effect of freeze-thawing. This is important for avoiding the deleterious effect of freeze-thawing on genotyping.

- The same patient ID should be used in all laboratory procedures to facilitate feedback of results to the ward and the matching of the results and computerization of data.

- Care must be taken to avoid cross contamination of specimens during transfer of material from the original containers to the storage vials
### Table 3: Laboratory logbook for rotavirus identification

<table>
<thead>
<tr>
<th>Serial</th>
<th>Admission number (Folder Number)</th>
<th>Demographic data</th>
<th>Laboratory data</th>
<th>Lab results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Name</td>
<td>Address (village/City)</td>
<td>Age months</td>
<td>Sex M/F</td>
</tr>
<tr>
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</tr>
</tbody>
</table>

Sample Received by: Name:………………………… Title…………………………………………
Signature………………………… Date (dd/mm/yy)…………………………

Data Entered by: Name:………………………… Title…………………………………………
Signature………………………… Date (dd/mm/yy)…………………………
**Figure 7:** Labels for storage stool specimen in the cryovials (long term storage)

```
Patient ID number

stool collection date (dd/mm/yy)
___/___/___

Storage date (dd/mm/yy)
___/___/___
```

**Figure 8:**

Flow chart of storage and transfer of fecal specimens in the EMRSN

[Flow chart image]
26) Sample *Application to FTA Micro Cards SOP #: NPL FTA 026

<table>
<thead>
<tr>
<th>Standard Operating Procedure</th>
<th>SOP #: NPL FTA 026</th>
<th>Version: 1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author: Whatman FTA Protocol BR01</td>
<td>Approved by: Dr. Faisal Ghazi Alhamdani</td>
<td>Review Date: 30 Oct. 2014</td>
</tr>
</tbody>
</table>

**Objective:**
To assure a safe shipment of samples from NPL IRAQ to the regional WHO reference laboratory to do further testing including Intratypic differentiation and PCR. NPL /IRAQ is a part of Poliovirus Laboratory EMR Network and collaborate with the RRL to receive positive isolates containing viruses observed in tissue culture. The mode of shipment follow IATA regulation by sending samples by using FTA cards containing inactivated viruses within an envelope addressed to WHO/EMRO RRL.

Note: As regarding genetic sequencing RRL will send a proved wild polio virus and or cVDP vaccine derived polio virus to a WHO global reference lab namely CDC / USA

**Reference:** Whatman FTA Protocol BR01, with modifications by the Center for Disease Control and Prevention. Whatman Website:

http://www.whatman.com/FTANucleicAcidCollectionStorageandPurification.aspx

**Scope:**
This document describes procedure used in the NPL – IRAQ for dealing with samples shows a positive cytopathic effect on tissue cultures that rise a suspicion of the presence of polio virus.

**Responsibility:**
This procedure is carried out by two expert supervisors under the supervision of lab director.

**Materials:**
- Indicating FTA Micro cards (VWR 14222-810)
- Lab marker
- Biohazard Waste Container.
- Pipette (200 μl)
- Pipette tips, Filtered (200 μl)
- Lab coat
- Gloves
- Timer or Clock
- Polio isolate to be spotted
- -20°C freezer or
- 4°C Refrigerator
- Zip lock bags or
- Multibarrier Pouch (VWR 89008-898)
- Storage Dessicant Packets (VWR 14222-840)
- Shipping Pouch (FedEx, DHL or Other)
- 70°C heat block
- 1.7ml Micro centrifuge Tubes w/Lid, Nuclease Free (Green Tree Scientific Part #T5050G)
Method:
- Label the FTA card with one Epid#, one Lab ID# and Arm for each card. (2 Cards/1 Case)
- Label (1) 1.7ml Micro centrifuge Tubes w/Lid for each specimen.
- In a biosafety cabinet (class 2B) aliquot 210ul of isolate into the labeled 1.7ml Micro centrifuge Tubes w/Lid.
- Incubate tubes at 70°C for 4 minutes, vortex every minute. (This is to inactivate the virus before spotting on the card.)
- In a biosafety cabinet (class 2B) spot 200ul of tissue culture in each circle in a concentric circular motion. (Make sure to evenly distribute the sample across the circle)
- In the biosafety cabinet allow the sample to dry for about one hour at room temperature. (Do not heat)
- Note: This may take less time or more time depending on the temperature and humidity of the lab.
- Place cards in a clean dry zip lock bag or multi barrier pouch with a desiccant
- Store the cards at -20°C (this is the ideal storage condition) or 4°C.
- Ship cards in regular shipment as a “Category B Biological Substances” at room temperature (FedEx Letter Pak, DHL or other shipper).
- Note: these specimens are non-infectious as long as the cards are dry.
- Please include a line listing in the shipment.
- Notes:
  - Handle cards with the same care as handling isolates.
  - Do not store cards at -20°C or 4°C for longer than 5 business days before shipping.
  - Make sure the biosafety cabinet is left running while drying the cards.
  - Follow WHO’s Standard Operating Procedure for decontamination and discard of infectious materials.
  - Each card should be in a separate bag/pouch with a desiccant in each bag/pouch.
  - Make sure card does not get stuck in the seal of the pouch.
  - Make sure that the cards are completely dry before shipping! We cannot be sure that the cards are non-infectious if they are not dry.

27)

![Diagram of cards with 200ul spots labeled A and B, Epid#, Lab ID, and Passage: L+R+](image)
NPL Records for sample registry and reagents with glassware consumption SOP #: NPL STOOL 027

Objective:
To record the information of AFP case and stool utilized specimens used inside NPL to be utilized in following the flow of specimens and results.

Reference:

Scope:
This document describes procedure used in only NPL to follow the specimens.

Responsibility:
This procedure is carried out by a trained NPL staff under supervision of NPL Director.

Materials and Records:
Only 10 records are maintained and information registered as required in daily work.

- NPL must maintain correct records for each sample
- The epidemiological data from the surveillance system and the laboratory data for each case will be linked by EPID number.
- Filling the line list of all samples using the EPID number to identify each specimen as follows:

<table>
<thead>
<tr>
<th>EPID No.</th>
<th>JOH</th>
<th>WHO</th>
<th>اوامر</th>
<th>سجل</th>
<th>بحث</th>
<th>animation</th>
<th>Onset</th>
<th>Last OPV</th>
<th>Lab code</th>
<th>eğام</th>
<th>هيئة</th>
<th>جنس</th>
<th>عمر</th>
<th>Onset</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>

- Registering the Reagents and glassware consumption of NPL/IRAQ in consumption records as follows:

<table>
<thead>
<tr>
<th>Cryotubes</th>
<th>Gloves</th>
<th>Plates</th>
<th>Tips</th>
<th>Disp. Pipette</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Cell line:
Month: ………/200

<table>
<thead>
<tr>
<th>S. No</th>
<th>Date</th>
<th>Serum Concentration %</th>
<th>Bottle No.</th>
<th>Date of Manufacture</th>
<th>Passage No.</th>
<th>Flasks</th>
<th>T.C tubes</th>
<th>Remarks of worker</th>
</tr>
</thead>
</table>

### Media

**Flow chart of preparation room / materials and samples**
### Working in the tissue culture require filling the following inventory to follow the cytopathic effect in cells through a maximum period of 14 days

- Temperature charts for the instruments are filled daily to keep an eye on the cold chain inside NPL IRAQ unit. (Appendices 5-8)
### 7. Appendices

**Appendix 4: Acute Flaccid Paralysis Case Investigation Form (Form 1)**

<table>
<thead>
<tr>
<th>EPID#</th>
<th>Date of investigation</th>
<th>Day</th>
<th>Month</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

**Patient’s Name**

<table>
<thead>
<tr>
<th>Mother’s name</th>
</tr>
</thead>
</table>

**Address**

<table>
<thead>
<tr>
<th>Province</th>
<th>District</th>
<th>Estate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>House #</th>
<th>Tel. No.</th>
<th>Food ration distributor’s name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tbody>
</table>

**Date of birth**

<table>
<thead>
<tr>
<th>Day</th>
<th>Month</th>
<th>Year</th>
<th>If birth date unknown, age in months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

**Sex**

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

**Date the case was first reported to a government/private health office**

<table>
<thead>
<tr>
<th>Day</th>
<th>Month</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Name of notification site**

<table>
<thead>
<tr>
<th>Name and specialty of treating/reporting doctor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Provisional Diagnosis**

**Date of onset of paralysis**

<table>
<thead>
<tr>
<th>Day</th>
<th>Month</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>

**If the patient died /date of death**

<p>| | | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
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</tbody>
</table>

**How many days from time of paralysis onset to full installation of paralysis**

<p>| | | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

**Is paralysis acute?**

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

**Is paralysis flaccid? (i.e. floppy)?**

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**If paralysis is not acute and flaccid, stop investigation. Specify diagnosis, if known**

<table>
<thead>
<tr>
<th>Yes</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

**Was there fever at onset of paralysis?**

<table>
<thead>
<tr>
<th>Yes</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
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</table>

**Is the paralysis asymmetrical?**

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Site of paralysis**

<table>
<thead>
<tr>
<th>Lt. Leg</th>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
<th>Breathing muscles</th>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rt. Leg</td>
<td>Yes</td>
<td>No</td>
<td>Unknown</td>
<td>Neck muscles</td>
<td>Yes</td>
<td>No</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lft. Arm</td>
<td>Yes</td>
<td>No</td>
<td>Unknown</td>
<td>Facial muscle</td>
<td>Yes</td>
<td>No</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rt. Arm</td>
<td>Yes</td>
<td>No</td>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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</tbody>
</table>

**Where was paralysis in arms**

<table>
<thead>
<tr>
<th>Proximal</th>
<th>Distal</th>
<th>Both</th>
<th>Neither</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Where was paralysis in legs?**

<table>
<thead>
<tr>
<th>Proximal</th>
<th>Distal</th>
<th>Both</th>
<th>Neither</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

**Was there any sensory nerve function loss?**

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
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</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

**History of travel (more than 10 KM 30 days) before onset**

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</table>

**If yes Specify the place governorate Address**

<table>
<thead>
<tr>
<th>Date of visit</th>
<th>dd/mm/yyyy</th>
<th>/</th>
<th>/ 200</th>
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</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

**Number of routine OPV doses received ( exclude zero dose )**

<table>
<thead>
<tr>
<th>Doses</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Number of OPV doses received during campaigns?**

<table>
<thead>
<tr>
<th>doses</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tbody>
</table>

**Date of last OPV dose**

<table>
<thead>
<tr>
<th>Day</th>
<th>Month</th>
<th>Year</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

**History of intramuscular injection before date of onset**

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Site of intramuscular injection**

<table>
<thead>
<tr>
<th>Rt. GluteiL Region</th>
<th>Lt. Gluteal Region</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Date of intramuscular injection**

<table>
<thead>
<tr>
<th>Day</th>
<th>Month</th>
<th>Year</th>
<th>Ink</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Date of 1st stool specimen collection**

<table>
<thead>
<tr>
<th>Day</th>
<th>Month</th>
<th>Year</th>
<th>Ink</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

**Date of 2nd stool specimen collection**

<table>
<thead>
<tr>
<th>Day</th>
<th>Month</th>
<th>Year</th>
<th>Ink</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>
### Appendix 5: Specimen Collection Form (Form 2)

This form must accompany specimens to the central public health laboratory

<table>
<thead>
<tr>
<th>EPID Number</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Patient's name</th>
<th>Sex</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Address</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>District</th>
<th>Province</th>
<th>Day</th>
<th>Month</th>
<th>Year</th>
</tr>
</thead>
</table>

Date of birth

Date of onset of paralysis

Date of first stool specimen collection

Date of second stool specimen collection*

Date stool specimens sent

Date of last OPV dose

Provisional diagnosis of the AFP case

Send results to

* If specimens sent on separate days, complete separate form for each specimen

Section (B) should be completed by a virologist at the laboratory.

<table>
<thead>
<tr>
<th>Specimen Number</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Date specimens received at laboratory</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Condition* of 1st specimen upon receipt at lab</th>
<th>Good</th>
<th>Poor</th>
<th>Unknown</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Condition* of 2nd specimen upon receipt at lab</th>
<th>Good</th>
<th>Poor</th>
<th>Unknown</th>
</tr>
</thead>
</table>

Name of person receiving specimens at laboratory

Signature

* Criteria for good condition = adequate volume, no leakage, no desiccation, reverse cold chain was maintained, and adequate documentation.
### Appendix 6: Contact Stool Collection Form (Form 3)

| EPID number of contact  
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(index AFP EPID number – C #)</td>
</tr>
<tr>
<td>Reason for collection</td>
</tr>
<tr>
<td>Inadequate</td>
</tr>
<tr>
<td>Name of contact</td>
</tr>
<tr>
<td>Address</td>
</tr>
<tr>
<td>Area</td>
</tr>
<tr>
<td>District</td>
</tr>
<tr>
<td>Province</td>
</tr>
<tr>
<td>Country</td>
</tr>
<tr>
<td>Relation to index case</td>
</tr>
<tr>
<td>Household relative</td>
</tr>
<tr>
<td>Period of Exposure to Index AFP cases</td>
</tr>
<tr>
<td>( ) within 30 days prior to onset of paralysis</td>
</tr>
<tr>
<td>( ) within 2 weeks after onset of paralysis</td>
</tr>
<tr>
<td>Date of birth or Age in months</td>
</tr>
<tr>
<td><strong><strong><strong>/</strong></strong><em>/</em></strong>_</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Number of routine OPV doses</td>
</tr>
<tr>
<td>Number of SIA OPV doses</td>
</tr>
<tr>
<td>Date of last OPV</td>
</tr>
<tr>
<td>Specimen number (in case of multiple samples from contact)</td>
</tr>
<tr>
<td>Date of stool collection</td>
</tr>
<tr>
<td>Date stool sent to laboratory</td>
</tr>
<tr>
<td>Date stool received at laboratory</td>
</tr>
<tr>
<td>Laboratory serial number</td>
</tr>
<tr>
<td>Stool condition</td>
</tr>
<tr>
<td>Good</td>
</tr>
<tr>
<td>Results: P1</td>
</tr>
<tr>
<td>Wild</td>
</tr>
<tr>
<td>P2</td>
</tr>
<tr>
<td>Wild</td>
</tr>
<tr>
<td>P3</td>
</tr>
<tr>
<td>Wild</td>
</tr>
<tr>
<td>NPEV</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Date culture results sent from lab to EPI</td>
</tr>
<tr>
<td>Date ITD results sent from lab to EPI</td>
</tr>
<tr>
<td>Comment</td>
</tr>
</tbody>
</table>
تعليمات جمع نماذج حالات الشلل الرخوي الحاد والملامسين:

1. في حالة ظهور أعراض شلل رخوي حاد يتم جمع أول نموذج براز داخل وعاء خاص وتظيف محكم الغلق مجهز ضمن عدة جمع البراز بمقدار (2-8 غم) تقريباً ويكتب عليه ما يأتي:
   - رقم النموذج بـ الرقم الوبائي جـ اسم المريض دـ تاريخ الجمع
   ملاحظة: يفضل عدم جمع نماذج باستخدام المسحات الشرجية أو استخدام الحقن الشرجية.
   1. تحفظ النماذج في المجمدة بدرجة ـ 20 °C.

2. يتم جمع نموذج ثاني بعد 24-48 ساعة ويعامل معاملة النموذج الأول.

3. تتم استمارة المعلومات الخاصة والحالات الشلل الرخوي الحاد من قبل الطبيب المختص وتحفظ بمعدل عن أوعية جمع النماذج (أي في الكيس المخصص لها) أي لا تلف حول الأوعية أو تحفظ في المجمدة مع النموذج تجنبًا للفتث.

4. في حالة جمع نماذج لحالات الشلل يتم جمع عينة واحدة فقط وتعامل معاملة النموذج الأول مع ملء الاستمارة الخاصة بالملامسين من قبل الطبيب المختص.

5. بعد القيام بالإجراءات أعلاه يتم لف نماذج البراز المجمدة بواسطة القطن وتوضع داخل كيس نابلون موجود ضمن العدة ويفلق الكيس جيداً ويشغل المحرار المثبت عليه لمراقبة الحرارة حفاظًا على سلسلة التبريد ويوضع داخل حاوية نقل اللقاحات مع عبوات جليدية مجمدة بدرجة ـ 20 °C.

6. يحرر كتاب رسمي معروفةً إلى قسم مختبرات الصحة العامة / المختبر الوطني لشلل الأطفال، يطلب فيه إجراء فحص التحري وعزل فيروس شلل الأطفال على النماذج المرفقة ويرفق الكتاب مع استمارة المعلومات داخل كيس نابلون ضمن العدة ويفلق جيداً ويوضع فوق كيس النماذج ويفلق الحاوية غلطاً محكمًا وترسل إلى المختبر. يمكن للمختبر الوطني لشلل الأطفال أن يستلم النماذج في اثناء الدوام الرسمي وفي حالة وصول النماذج خارج اوقات الدوام الرسمي يتم استلامها من قبل الخبير المسؤول لمختبر الصحة العامة المركزي ويفحص في التجميد بدرجة ـ 20 °C.

7. 72 ساعة (8) لا يجوز طلباً تأخير إرسال النماذج وينبغي إرسالها خلال ثلاثة أيام من تاريخ جمع النماذج.

8. لا يجوز طلباً تأخير إرسال النماذج وينبغي إرسالها خلال ثلاثة أيام من تاريخ جمع النماذج.

ملاحظة: يجب أن تجمع النماذج خلال (14) يوم من تاريخ ظهور الأعراض حسب تعليمات الرصد الوبائي.

*This document is a summary in Arabic language to those involved in sample collection of AFP cases.
# Daily -80 °C Freezer Temperature Log

|   |  1 |  2 |  3 |  4 |  5 |  6 |  7 |  8 |  9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 |
|+ |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|-80 °C |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

-80 °C ± 9 °C

Asset Number ________________ Month ________________ Year ________________

Acceptable Range -80 °C ± 9 °C

Date issued 9 September 1999       Author DF       Date printed 27 July 2004
Revised by Dr. Faisal G /NPL IRAQ at 30/11/2014
Daily - 20 °C Freezer Temperature Log

Date                 1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16  17  18  19  20  21  22  23  24  25  26  27  28  29  30  31

-20 °C

-  

Initials                 1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16  17  18  19  20  21  22  23  24  25  26  27  28  29  30  31

Asset Number _____________   Month _______________  Year __________

Acceptable Range -20 °C ± 4 °C

- 20 °C Temp. Log. DOC
### Daily 4°C Refrigerator Temperature Log

<table>
<thead>
<tr>
<th>Date</th>
<th>Sun</th>
<th>Mon</th>
<th>Tue</th>
<th>Wed</th>
<th>Thu</th>
<th>Fri</th>
<th>Sat</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4°C</td>
<td></td>
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<td></td>
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<td>-</td>
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</table>

**Acceptable range 4°C ± 2 °C**

Asset Number _____________ Month ______________ Year ______________

Date issued 9 September 1999               Author DF         Date printed 27 July 2004
Revised by Dr. Faisal G /NPL IRAQ at 28/5/2008
# Daily 36°C Incubator Temperature Log

<table>
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<tr>
<th>Date</th>
<th>+</th>
<th>36°C</th>
<th>-</th>
</tr>
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</table>

Asset Number _____________   Month ______________   Year _________

Acceptable range 36°C
8. References

1- Accreditation recommendation WHO Meeting 2006.
2- Dr. J. Michael Miller on June 1, 2004.
3- EMR ROTAVIRUS GASTROENTERITIS SURVEILLANCE NETWORK (EMRSN)
4- Laboratory diagnosis techniques for global polio eradication NIID, Japan training course 2008.
5- Laboratory biosafety manual Third edition /WHO - 2004
6- Recommendation of polio network WHO lab meeting, 2006.
7- Whatman FTA Protocol BR01, with modifications by the Center for Disease Control and Prevention. Whatman Website:
8- WHO Lab Manual 2004,