S2. Supplement to the WHO Polio Laboratory Manual
Adaptation of newly received cells to local conditions

Critical steps in assuring quality cell cultures and reproducibility of cell line stocks occur at the time of receipt of new cultures in the laboratory and establishment of a frozen cell bank. Experiences in the Global Polio Laboratory Network (GPLN) suggest that several factors can affect the adaptation of newly received cells to the local conditions of the laboratory. These include differences in cell culture conditions that exist between the source and the recipient laboratory (e.g. media composition, culture vessels and type of incubator), transportation conditions (e.g. temperature fluctuation, mishandling), presence of contaminants, regimen for medium replenishment, and techniques for cell handling. For successful adaptation of the cells, it is important that the recipient laboratory has information, as specified in Section S2.1 below, about the cells it receives so that there can be a systematic approach to troubleshooting should problems occur. Information should be kept on file for future reference with the date received. The details of observations and procedures carried out during the cell adaptation process should be recorded, as they are generated, and archived for future reference.

S2.1 Shipment of cell cultures

L20B and RD cells should ideally be shipped as low-passage cultures[1] that are in the mid- to late log phase of growth in flasks with cell sheets that are approximately 50% to 70% confluent. The flask is filled almost to the top with growth or maintenance medium depending upon the cell monolayer confluency. Filling the flask with medium ensures that the cells are always bathed in the medium and are not destroyed due to excessive frothing and movement of the package during transport. However, it is critical that the medium does not fill the flask completely and a small air bubble is introduced (approximately 1-2 ml volume) to allow for expansion and contraction of the liquid due to temperature changes. The cap is taped securely with a sealing film wrapped around in a clockwise direction to prevent the cap from loosening during transport as this may lead to leakage of medium. The flask is sealed in a suitably sized watertight plastic bag or leakproof container with sufficient absorbent packing material to absorb all the liquid in the flask in case of breakage, then wrapped in cushioning material (e.g. bubble wrap). The package should be placed in a suitable insulated box with additional cushioning materials to prevent the flasks from moving around during transport. Written details of the cell line (cell line data sheet) and any letters or instructions for special handling on arrival should be sealed in a plastic bag and taped to the inside of the lid of the insulated box.

[1] **Cell passage limits:** It is recommended that L20B and RD cells are shipped at or lower than passage numbers P18 and P228, respectively. In order to maintain cells of consistent quality and sensitivity to poliovirus detection, L20B and RD cells should not be used above passage P35 and P245, respectively. It is therefore important that the sender provides the absolute passage number of the cell line during shipment. If this is not available, the recipient laboratory should request this information from the sender.

The minimum information that the sender should provide includes:

- name of cell line
- passage number (absolute)
- date of last passage
- type of culture media and composition –including the concentration of supplements and additives
- seeding density expressed as the number of cells per flask.
- percentage confluence of the cell sheet at the time of shipment
- incubation conditions at the sending laboratory, e.g. temperature, type of incubator (CO_2 or standard) and culture vessel
- sterility testing results and date tested
- Mycoplasma test result, date tested and method used

Consideration should be given to using a seeding concentration of cells compatible with the expected transit time before arrival in the recipient laboratory. Cells should be shipped at ambient temperature with instructions to handlers and shippers to avoid refrigerating or freezing during transport, and to treat the contents as fragile. Cell cultures distributed in the GPLN are not specifically defined as hazardous, but for shipping purposes, they are categorized as biological products and transported in compliance with national or international regulations and regulations established by individual carriers. In some countries, the distribution and use of genetically modified cells, such as L20B, may be subject to regulation. It is strongly recommended that full details of regulatory requirements are obtained from the recipient laboratory before any shipment is made. Detailed information on general shipping arrangements is described in Chapter 10 of the WHO Polio Laboratory Manual, 4th Edition. Further information concerning packaging and transport can be found in the WHO publication WHOCDS/EPR/ 2007.2 available at http://www.who.int/csr/resources/publications/biosafety.

S2.2 Examination of newly received cells

On arrival, do the following:

- Assess the package for integrity or any signs of damage. Examine the flask for breakage, loose cap or leakage. Check the flask labels and ensure that these match the cell line details described in the accompanying documents.
- Examine the flask of cells macroscopically with the naked eye and look for any fungal colonies that may be floating in the media. Observe for turbidity, detached monolayers and extreme changes in the color (pH) of the media that may indicate possible contamination, build up of toxic waste products, poor cell quality or cell death.
- Examine the cells microscopically for changes in characteristic cell morphology such as sloughing, rounding or retraction as a result of trauma or deterioration during shipment. Observe the cell density and estimate the percent confluence of the cell sheet. Look for floating debris, and any signs of bacterial or fungal contamination. If the culture is contaminated, it should be discarded without opening and a shipment of new cells should be requested.
- Record and archive observations.

- Immediately notify the sender of the condition of the shipment and any problems encountered or detected.

S2.3 Adaptation of newly received cells

Before processing, the cells are acclimatized to the local culture environment to allow them to recover from stress during transport. The medium in which the cells are shipped is replaced with fresh medium after 24 hours of receipt. This will provide the cells with the essential nutrients that may have been depleted or inactivated during transport, and optimal physiological conditions (e.g. pH, CO_2) for growth. The cell lines are then passaged into flasks when they are confluent and further expanded to prepare a master or working cell bank. Following initial expansion, the cell line sensitivity to all poliovirus serotypes is evaluated for all frozen stocks and whenever fresh cells are resuscitated for routine use.

Good Laboratory Practice: Schedule the manipulation of newly received cells only after routine processing of all other cells has been completed. Handle only one cell line at any given time. Use separate bottles of culture medium for each cell line and separate media for new cells and old cells. These measures will reduce the risk of cross-contamination of cell lines and spread of contaminants that may be unknowingly present in the newly received cells.

Do the following:

- Decontaminate the external surface of the cell culture flask by wiping with 70% alcohol before transferring the flask to the cell culture area.
- Aseptically remove most of the medium and leave only the volume that is appropriate to the size of the flask, e.g. 10 ml for a 25 cm^2 flask. Remove any medium from inside the neck or cap area with a sterile pipette. Transfer the removed medium to a sterile container and perform sterility testing using standard microbiological procedures as soon as possible.
- Incubate the cultures for several hours or overnight at 36 °C to acclimatize the cells.
- Examine the cells using a microscope, noting any signs of morphological changes or deterioration, e.g. granularity around the nucleus or cell rounding with detachment from the culture vessel or change in colour (pH) of the medium. These changes may indicate the use of deficient or toxic medium, microbial contamination or that the monolayer is overgrown.
    - ***If the cell sheet is confluent or overgrown***, passage the cell culture (see below)
    - ***If the cell sheet is not confluent***, replace the medium with new growth medium appropriate to the local incubation conditions of the laboratory (see pages 60 to 61 of the WHO Polio Laboratory Manual, 4th Edition). Any new media must have undergone routine quality control procedures to confirm sterility and capability of supporting cell growth. Incubate at 36 °C and continue daily microscopic examinations until a confluent cell sheet is obtained (this usually occurs within 24 to 48 hours), at which time a passage is made of the cell culture (see below).

- ***If contamination is observed at any stage***, immediately discard the culture without opening the flask.

S2.4 Passage of newly received cells and establishment of cell bank

Passage the cell culture when a confluent monolayer is obtained, using the procedures outlined in section 4.2.5 of the WHO Polio Laboratory Manual, 4th Edition. When cultures are passaged:

- Use the cell count or seeding level indicated in the information provided by the sender. The cell count may subsequently be adjusted based on experience, to obtain a confluent cell sheet in the appropriate time.
- Determine the cell count for viable cells using trypan blue.
- Incubate the cultures at 36 °C, and observe after 24 hours, using a microscope, noting efficiency of attachment, growth rate, cell morphology, and any evidence of contamination.
    - **If deficient cell growth and/or unusual appearance** is observed, remove the medium and add fresh growth medium. Continue to incubate and observe the cell condition daily until a confluent cell monolayer is obtained.
    - ***If contamination is observed at any stage***, immediately discard the culture without opening the flask
- Notify the sender of the growth condition of the cells indicating any problems encountered or detected during adaptation of cell lines to local conditions.

Important Note: When cell cultures are proven to be contaminated, immediately remove them from the cell culture area and discard without opening to minimize the chances of the contamination spreading to other cultures or media. Do not attempt to treat the cells to eliminate the contaminant.

- The cell cultures can now be used to establish a master or working cell bank as described in Sections 4.2.6 and 4.2.8, WHO Polio Laboratory Manual, 4th Edition).
- Before accepting the cells for routine use, resuscitate one vial from the working cell bank.
    - Evaluate the cell count and viability of the frozen cells, using trypan blue staining and microscopic examination.
    - Examine microscopically to ensure that the cell suspension is free of bacterial, fungal and Mycoplasma contamination.
    - Evaluate and document the sensitivity of the cells to all poliovirus serotypes using validated in-house authenticated Sabin laboratory quality control standards and the procedure described in Chapter 10, WHO Polio Laboratory Manual, 4th Edition.

S2.5 Evaluation of newly received cells for microbial contamination

Cell cultures obtained from Global or Regional Reference Laboratories are usually assured by the sender to be free of microbial contamination. However, bacterial, fungal or Mycoplasma contamination may occur at the time of dispatch, during transport or at the time of handling in the recipient laboratory. Overt contamination by bacteria and fungi can usually be detected by an increase in the turbidity of the medium and/or a

decrease in pH suggested by yellow color of media containing phenol red indicator. Low level microbial contamination, particularly when antibiotics are added to the medium in which the cells were shipped, may not be revealed by visual examination. Sterility tests should be performed to confirm the absence of microbial contamination when preparing cell banks. Standard microbiological methods can be used for bacterial and fungal detection such as direct observation of gram stained sediments obtained from centrifuged cell culture medium in which the cells were shipped, or by inoculation of the cell culture medium and sediments into enriched broth medium (e.g., thioglycolate, trypticase soy and brain heart infusion broths) or onto agar (e.g., blood and Saboraud dextrose agar plates). It is recommended that these tests are carried out in a microbiology laboratory away from the cell culture area. Refer to Section 4.4.3 of the WHO Polio Laboratory Manual, 4th Edition for *Mycoplasma* detection tests .