Potency Titration of Oral Polio Vaccine by Estimation of Live Virus Content Using Tissue Culture Technique

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Abstract: Expanded program on immunization is one of the strategic universally accepted method for the control of childhood diseases which include poliomyelitis. In Nigeria both monovalent and trivalent oral polio vaccines are routinely used. Thirty-six OPV vaccines representing three different batches of the vaccines in three different levels of health care vaccination centres (federal, state and local government) were monitored and tested. The vaccines were tested using L20B which is mouse cell line genetically engineered with human poliovirus receptors; (CD155). All the 36 samples were found to be potent with a maximum titre of log (8.38) and a minimum titre of log (7.58). The minimum titre was observed only in a primary vaccination centre. In addition, thermal stability was obtained using 16 vials obtained from different storage facilities, had titres which ranged from log 6.5 to 8.4. These values still fell within the normal limits recommended by WHO as minimum accepted values (P1= log 6.0, P2=5.0 and P3= 5.8). It was observed that the storage facilities in all the three tier of vaccination centres had adequate power supply ranging from solar refrigerators, standby generators and the National Electricity supply. Also, polio vaccine vials have vaccine vial monitor (VVM) device which usually indicate change in color when cold-chain is not maintained. This necessitated the change of vaccine carrier when the need arose during the house to house immunization exercise. Adequate potency obtained in this study confirmed ideal storage condition of vaccines in Maiduguri.

Key words:

INTRODUCTION

In May 1988, the 41st World Health Assembly committed the Member States of the World Health Organization (WHO) to the global eradication of poliomyelitis by the year 2000 (resolution WHA41.28). The resolution specified that the polio eradication initiative should be pursued in ways that would strengthen the Expanded Programme on Immunization (EPI).

In 1989, the 42nd World Health Assembly approved a general Plan of Action for Global Polio Eradication. The global effort to eradicate polio is the largest public health initiative in history. Since the initiative was launched in 1988 extraordinary progress has been made to halt transmission of wild poliovirus and achieve global certification of eradication by 2005.

In 1988, polio existed in over 125 countries on five continents, and more than 350,000 children were paralyzed that year. By the end of 2002, the number of polio-infected countries has decreased to seven, polio has been eliminated from three continents, and reported poliomyelitis cases has fallen to around 1900. Poliomyelitis transmission has been interrupted in the American, European, and Western Pacific Regions, and by end 2002 more than 180 countries and territories were polio-free. With the eradication of polio and the eventual cessation of polio immunization, the world will save US$ 1.5 billion per year.

Current eradication strategies recommended by WHO/EPI/GEN/02.1. 2002 have proved successful; these four strategies are:
• high, routine infant immunization coverage with at
least three doses of oral polio vaccine (OPV) plus a dose at birth in polio-endemic countries;
• national immunization days (NIDs) targeting all children <5 years;
• acute flaccid paralysis (AFP) surveillance and laboratory investigations; and
• mop-up immunization campaigns to interrupt final chains of transmission.

MATERIAL AND METHODS

The potency of live oral poliomyelitis vaccine (OPV), both total virus content and individual serotypes separately, is determined in an in vitro assay using L20B cell line. The preparation to be assayed and the reference preparation are diluted in 2% MM. Tenfold dilution steps of the virus suspensions was initially made, but the dilution range selected should encompass at least three dilutions that will infect between 0% and 100% of the cultures inoculated.

The cells are examined for the presence of a specific viral cytopathic effect on days 3–5, with a final reading on days 5–7. The observations are recorded and the titer in CCID₅₀ per human dose calculated on the basis of the final observation.

Materials: microtitre plates (96 wells, flat-bottomed) multichannel pipettes, with tips to deliver 0.05-ml volumes micropipettes to deliver 0.05-ml and 0.1-ml volumes incubator - plate sealer

Reference Preparation: For each assay of trivalent OPV vaccine include a vial of live attenuated poliomyelitis vaccine, the titer of which has been well established, as a working reference preparation to control the accuracy and reproducibility of the testing system (validity).

Medium and Dilutions: Diluent: Eagle’s MEM supplemented with 2% fetal bovine serum. Using the refrigerated diluent, prepare tenfold dilutions. The subsequent dilutions for inoculation into the microtitre plates are prepared in 0.5 log₁₀ steps. The range of dilutions used will depend on the type of virus and the formulation of the vaccine under test. The range chosen should include the expected titre of the vaccine type being tested.

Cells: Use L20B cells line. The passage level of these cells, which should be documented, should be within 15 passages of the tested stock. Watch for any change in growth characteristics such as excess acidity of medium or slowing in the time taken to achieve a complete monolayer.

The number of cells used in the assay is usually about 1–2 x 10⁵ cells per ml of test medium. This concentration should provide a confluent monolayer in microtitre plate wells within two to three days. However the cell concentration should be optimized in each laboratory.

Procedure: The procedure is standard for determining the potency of OPV, both for total virus content (type 1 + type 2 + type 3) and individual serotypes separately using the microneutralization test

- Add 0.1 ml of test medium to all wells of the plates for the test vaccine and reference preparation in which total virus content will be measured.
- Add 0.1 ml of vaccine dilution to a column of 8 wells, in each of the four plates in which the vaccine is to be titrated. Start by transferring the highest dilution into column 10, and use the same dropper pipette for the whole range.
- Add 0.1 ml of the dilutions made of the working reference preparation to a column of 8 wells of the plate for titration of the reference preparation. Again start by transferring the highest dilution into column 10; the same dropper pipette can be used for the whole range.
- Add 0.1 ml of test medium, to equalize the volumes, to each of the cell control wells.
- Remove the plates from the incubator and add 0.1 ml of cell suspension to all wells of all plates. Avoid touching the wells with micropipette tips during this procedure.
- Cover the plates with a sealer.
- Incubate all plates at 36 °C for 5–7 days.
- During this time, monitor the cell control wells to ensure that the cells are forming a monolayer. Also score the positive wells starting at day 4 and keep good records of the data.
- Calculate the titre in CCID₅₀ per human dose using the Kärber formula.

RESULTS AND DISCUSSION

Discussion: Adequate potency of vaccines tested in this study confirms the ideal storage and transport conditions of OPV in FG, St, LG and Field. This study showed 100% potency of all the vaccines tested both at storage and user end. Drawing comparisons with previous studies, Arya et al, 1976 tested 191 samples
in India and found 113 (59%) to be potent. Annual report, ERC, Mumbai reported 48% potency of samples tested in 1986. However, field potency tests done in Maharashtra between 1993-1997 showed an encouraging trend. In 1993, 18.25% samples had loss of potency but by 1996 merely 7.31% of vaccine samples had inadequate potency \cite{3}. In 1997, 45% samples to be potent \cite{5}. Deivanayagam et al obtained a better trend in 1990 with 77% samples being potent \cite{3}. The studies quoted tested field samples of vaccines and potency loss was attributable to breach in the cold chain maintenance. An additional factor in the adequate potency of vaccine samples in this study was the thermo-stabilization with 1M magnesium chloride \cite{3}. In the early 1960s, it was found that the infectivity of enterovirus could be preserved even when they were heated at 50°C if molar MgCl2 was added, a property that is used in the field where stabilised vaccines are used effectively to halt outbreaks of polio. In laboratory, the vaccines show so little loss in virus titre after long term storage at -20°C that the predicted half-life was calculated to be 92 years \cite{5}. It was also noted that vaccine stabilized with MgCl2 suffered no significant loss of potency, after as many as nine cycles of alternate warm and cold conditions \cite{4}.

**Conclusion and Recommendation:** In this study, we did not titrate individual serotype. This would have given information on potency of individual vaccine strain i.e. type-1, type-2 and type-3. But as the total titre is fixed in OPV, composite titer estimation is sufficient to assess the potency. Continuous monitoring of efficiency of cold chain maintenance and vaccine potency testing would contribute towards good vaccine strategy.

This study tested samples at storage and user end and a 100% potency of the vaccines indicated adequate cold chain maintenance and monitoring.

1. Further studies are required in field areas to assess vaccine potency.
2. There should be regular vaccine potency tests both in primary, tertiary health care centres and field units to help in strengthening cold chain monitoring and polio eradication programmes.
3. All vaccine storage facilities should be provided with solar refrigerator in addition to stand by generator to ensure cold chain maintenance.

**Table 1:** Comparism of potency of OPV in different storage facilities and in the field

<table>
<thead>
<tr>
<th>Types of vaccine</th>
<th>FG</th>
<th>St</th>
<th>LG</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monovalent 1</td>
<td>8.30±0.05 (\text{a,b,c} )</td>
<td>8.12±0.08 (\text{a} )</td>
<td>8.05±0.22 (\text{d} )</td>
<td>8.00±0.07 (\text{c} )</td>
</tr>
<tr>
<td>Monovalent 3</td>
<td>8.38±0.03 (\text{a,b} )</td>
<td>8.32±0.08 (\text{c} )</td>
<td>7.98±0.08 (\text{c,b} )</td>
<td>-</td>
</tr>
<tr>
<td>Trivalent</td>
<td>8.38±0.02 (\text{a} )</td>
<td>8.35±0.10 (\text{a} )</td>
<td>8.22±0.06 (\text{d} )</td>
<td>7.58±0.10 (\text{e} )</td>
</tr>
</tbody>
</table>

\(\text{FG}-\text{Federal Government, St}-\text{State Government, LG}-\text{Local Government, SD}-\text{Standard Deviations}\)

Values with the same superscript differ significantly at \(P < 0.05\).

**Fig. 1:** Negative control L20B cell line
4- All vaccine vials should have vaccine vials monitor (VVM) to ensure temperature monitoring during transportation, storage and administration.

5- All storage facilities should be provided with dial thermometers for temperature monitoring.

AKNOWLEDGEMENT

We wish to acknowledge the entire staff of WHO Polio laboratory for providing all necessary materials to carry out the research work.

REFERENCES


