Determination Of Thermal Stability Of Oral Polio Vaccine (Opv) At Different Temperature Under Laboratory Conditions

Muhammad T[1], SS Baba[2], LT Zaria[2], AD El-Yuguda[2] And IB Thilza[3],

[1]who National Polio Laboratory University Of Maiduguri Teaching Hospital.
[2]department Of Veterinary Microbiology And Parasitology University Of Maiduguri.
[3]department Of Veterinary Medicine, University Of Maiduguri.

muhammadt6@gmail.com

ABSTRACT: Expanded program on immunization is one of the strategic universally accepted methods for the control of childhood diseases which include poliomyelitis. In Nigeria both monovalent and trivalent oral polio vaccines are routinely used. Thermal stability was determined using 16 vials obtained from different storage facilities, had titres which ranged from $\log_{10}$ 6.5 to 8.4. These values still fell within the normal limits recommended by WHO as minimum accepted values (P1= $\log_{10}$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. [Stem Cell. 2010;1(1):69-73] (ISSN 1545-4570).

Keywords: Oral Polio Vaccine; Thermal Stability; Storage Facilities

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 (4). The resolution specified that the polio eradication initiative should be pursued in ways that would strengthen the Expanded Programme on Immunization (EPI). National Immunization Days (NIDS) were initiated in a number of developing countries including Nigeria to accelerate polio eradication strategies. In recent years, Nigeria has contributed over 90% of the polio cases reported globally despite immunization campaign. In developing countries, immunity induced following oral polio vaccine (OPV) is very low, about 30%. Failure of OPV has risen from 5% in 1960s to an alarming 30% currently. The factors for vaccine failure may be due to interference by antibodies in breast milk, presence of nonpolio enteroviruses preventing colonization by the vaccine virus strains, helminthic infestation or presence of non-specific inhibitors in saliva of infants. Decreased potency of the vaccine, break in reverse cold chain and thermal stability could be another probable reason which needs to be investigated (5).

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 $CCID_{50}$ per human dose calculated on the basis of the final observation.

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 dilution. The subsequent dilutions for inoculation into the microtitre plates are prepared in 0.5 $\log_{10}$ 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 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 10^5 cells per ml of test medium. This concentration should provide a confluent monolayer in microtitre plate wells within two to three days.

RESULTS

Table 1. Thermal comparism of Trivalent Oral Polio Vaccine (TOPV) titre at different storage facilities

Temperatures (^{o}C)	Storage facilities		
	FG	St	L.G
25	8.26±0.01	8.20±0.28	8.15±0.00
30	8.24±0.01	8.20±0.14	8.15±0.00
35	8.17±0.03^a	8.00±0.14^a	8.00±0.14^a
40	8.06±0.01^a	7.80±0.00^a	7.09±0.01^a
45	7.85±0.07^a	7.80±0.00	7.65±0.00^a
50	7.65±0.07	7.55±0.07	7.55±0.07
55	7.25±0.07^a	7.20±0.00	7.03±0.05^a
60	7.20±0.00^a	7.05±0.00	6.90±0.00^a

Values with the same superscript differ significantly at $P \leq 0.05$

OPV- Oral Polio Vaccine

FG- Federal Government

St- State Government

LG- Local Government

SD- Standard Deviations

Table 2. Thermal comparism of Monovalent 1 Oral Polio Vaccine (MOPV-1) titre at different storage facilities.

Temperatures (oC)	Storage facilities		
	FG	St	LG
25	8.30±0.42	8.20±0.00	7.75±0.00
30	8.30±0.28[a]	7.75±0.00	7.09±0.01[a]
35	8.10±0.00[a]	8.00±0.00[a]	7.73±0.01[a]
40	7.90±0.00[a, b]	7.09±0.01[a]	7.00±0.14[b]
45	7.80±0.14[a, b]	7.08±0.03[a]	6.80±0.14[b]
50	7.83±0.50	7.08±0.04	6.50±0.14
55	7.28±0.37[a]	7.08±0.03[b]	6.40±0.16[a, b]
60	7.13±0.39[a]	7.04±0.05[b]	6.25±0.30[a, b]

Values with the same superscript differ significantly at $P \leq 0.05$

Table 3. Thermal comparism of Monovalent 3 Oral Polio Vaccine (MOPV-3) titre at different storage facilities.

Temperatures (^oC)	Storage facilities		
	FG	St	L.G
25	8.40±0.00	8.25±0.35	7.90±0.00
30	8.40±0.00[b]	8.25±0.07[a]	7.90±0.00[a, b]
35	8.20±0.00[b]	8.10±0.00[a]	7.75±0.07[a, b]
40	8.05±0.07[b]	8.04±0.07[a]	7.51±0.00[a, b]
45	7.90±0.00[a]	7.86±0.00[b]	7.30±0.14[a, b]
50	7.70±0.14[a]	7.66±0.00[b]	7.10±0.14[, b]
55	7.30±0.14	7.25±0.35	6.90±0.00
60	7.00±0.14	6.80±0.14	6.50±0.00

Values with the same superscript differ significantly at $P \leq 0.05$ across the different storage facilities.

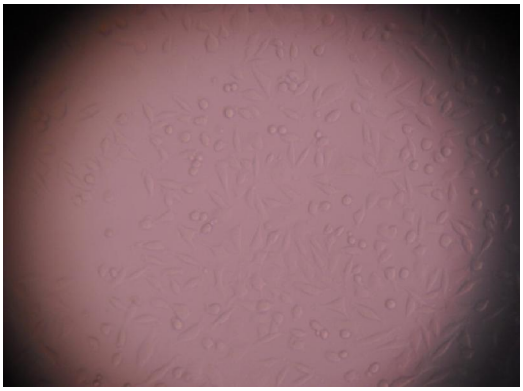

Fig. 1: Negative control L20B cell line

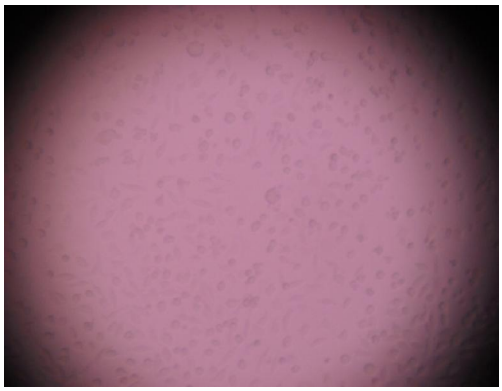

Fig. 2**:** Showing 50% CPE of polio virus on L20B cell line

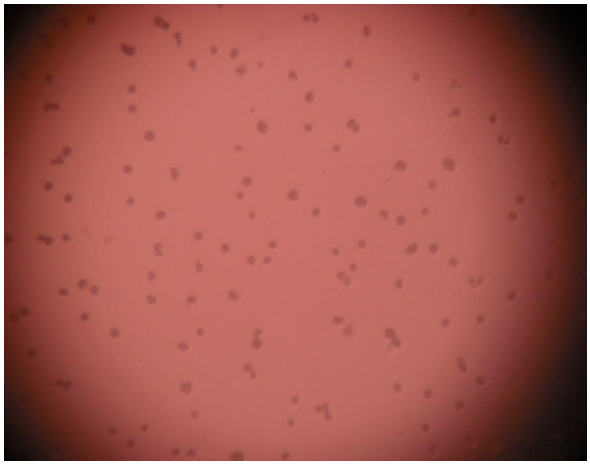

Fig. 3**:** Showing 100% CPE of polio virus on L20B cell line

DISCUSSION

An additional factor in the adequate stability of vaccine samples in this study was the thermo-stabilization with 1M magnesium chloride [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 [3]. 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 [2]. Thermostability requirements were defined by WHO as OPV that loses less than 0.5 log 10 of titre of each of the vaccine strain after exposure to 37°C for 2 days [6]. But current regulations require that for maintenance of potency, the vaccine must be stored and shipped frozen and that after thawing, it must be stored in the refrigerator at not more than 10°C for a period not exceeding 30 days after which time it must be discarded [6].

In a study of stabilizing OPV at high temperature at WHO, there was a general consensus that a vaccine capable of withstanding 45^{o}C for seven days with less than 0.5 $TCID_{50}$ per dose. Reduction of the potency of each of the three serotypes, will offer substantial benefit to global eradication effort (1). In this study, though a significant drop in titer was observed when subjected to varying

temperatures; the minimum titer obtained is still above the minimum stipulated cut-off titer recommended by WHO $\log_{10}(6.0)$. Thus, all the vaccine has been stabilized with 1M magnesium chloride which helps to stabilize the vaccine at high temperature 50^{o}C.

CONCLUSION

In this study, we did not titrate individual serotype in trivalent vaccine. This would have given information on thermal stability 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 thermal stability. Continuous monitoring of efficiency of cold chain maintenance and vaccine potency testing would contribute towards good vaccine strategy.

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3/13/2010