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

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Abstract

Representative vaccines from 34 different batches of oral $\log_{10}$ (6.425) and a minimum titre of polio vaccine (OPV) were tested for potency by tissue culture technique. All 34 samples were found to be potent, a maximum titre of $\log_{10}$ (6.425) and a minimum titre of $\log_{10}$ (5.86) was obtained. In addition, 6 vaccines from the immunisation clinic (left over sample after immunisation) were also subjected to potency titration and their potency was found to be within normal limits. Adequate potency confirmed ideal storage condition of the vaccines in Armed Forces set up.

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Key Words : Oral polio vaccine; Potency; Storage

Introduction

In 1988, the World Health Assembly resolved to eradicate polio globally by the year 2000. National Immunisation Days (NIDS) were initiated in a number of developing countries including India to accelerate polio eradication strategies. In recent years, India has contributed one-half of the polio cases reported globally, however 4 NIDS have consistently reached at least 90% of the population aged less than 5 years [1].

In developing countries, immunity induced following OPV is very low, about 30%. Failure of OPV has risen from 5% in 1960s to an alarming 30% currently [2]. The factors for decreased vaccine take rate may be due to interference by antibodies in breast milk, intercurrent nonpolio enteroviruses preventing colonisation by the vaccine virus strains, helminthic infestation or presence of non-specific inhibitors in saliva of infants. Decreased potency of the vaccine could be another probable reason [3]. This study was done with the objective to measure, monitor and document the potency of OPV and adequate vaccine storage conditions in Armed Forces Medical College (AFMC) for a period of one and a half years.

Material and Methods

Total 40 representative OPV samples were tested for potency out of which 34 samples were obtained from the Department of Preventive and Social Medicine and transported in a vaccine carrier to the virology laboratory immediately after collection. 6 samples were obtained from the immunisation clinic. These 6 samples were left over OPV, after immunisation was completed. The OPV is received from health authority of Govt of Maharashtra through Zilla Parishad. The estimation of composite virus content was done by microtitration using HEp2 (Cincinnati) cells,

obtained from Enterovirus Research Centre (ERC), Parel, Mumbai, adopting the standard procedure [4]. The minimum virus content to pass the potency test per one human dose of the vaccine was $\log_{10}$ (5.83) TCID₅₀ [5].

Briefly, vaccine samples and Reference Vaccine (Source : ERC, Parel, Mumbai) were diluted in Minimum Essential Medium (MEM) with Foetal Calf Serum (FCS). Half log dilutions were prepared from 10^{-3} to $10^{-6.5}$. Dilutions from $10^{-4.5}$ to $10^{-6.5}$ were used for titration. HEp2 cell suspension was prepared in MEM with 10% FCS from a confluent monolayer of cells using standard tissue culture techniques. Viable cell count was taken using Neubauer's haemocytometer and trypan blue [6]. Final cell count was adjusted to 10,000 cells/0.1 ml by adding MEM with 10% FCS. Vaccine dilutions of samples and reference vaccine, 0.05 ml of each dilution ($10^{-4.5}$ to $10^{-6.5}$) were dispensed into each of 8 wells of a pre-sterilised, flat-bottomed Nunc microtitre plate with lid, starting from higher dilution to lower dilution. 0.05 ml of plain MEM was added to the cell control row. 0.1 ml of prepared HEp2 cell suspension was added to all the wells. The plate was sealed and incubated at 37°C in carbon dioxide incubator. The plates were read microscopically on day 5 using an inverted microscope for cytopathic effect (CPE), wherein infected cells rounded up, showed shrinkage and marked nuclear pyknosis, became refractile, degenerated and fell off the surface [7]. Kaerber's formula was used to obtain the vaccine titre per dose of 0.1 ml [8]. Potency test was valid if the cell control wells were normal. Following particulars were noted while taking the vaccine samples : date of manufacture and expiry, date of receipt at AFMC, batch number, company manufacturing the vaccine, and whether thermo-stabilised or not with 1M magnesium chloride.

Results

Total 40 representative OPV of various batches were titrated for potency. 34 OPV samples were obtained from

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Table 1

Titre values of OPV samples

Titre value	Number	Percent
$< \log_{10} (5.83)$	0	0
$\log_{10} (5.84-5.94)$	9	26.47
$\log_{10} (5.95-6.05)$	12	35.294
$\log_{10} (6.06-6.16)$	0	0
$\log_{10} (6.17-6.27)$	8	23.53
$\log_{10} (6.28-6.38)$	4	11.76
$\log_{10} (6.39-6.49)$	1	2.94

Table 2

Composite titre of individual sample

Date of manufacture	Date of expiry	Month of collection	Titre No. ($\log_{10}$)
Sep96	Sep98	Dec97	5.86
Sep96	Sep98	Dec97	6.05
Sep96	Sep98	Jan98	6.3
Sep96	Sep98	Jan98	6.175
July97	Aug99	Feb98	5.925
July97	Aug99	Feb98	6.24
July97	Aug99	Mar98	6.05
July97	Aug99	Mar98	6.36
July97	Aug99	Apr98	6.24
July97	Aug99	Apr98	6.24
July97	Aug99	May98	6.05
July97	Aug99	May98	5.925
July97	Aug99	Jun98	6.05
July97	Aug99	Jun98	6.175
July97	Aug99	Jul98	5.925
July97	Aug99	Jul98	6.24
July97	Aug99	Aug98	6.425
July97	Aug99	Aug98	6.05
July97	Aug99	Sep98	6.05
July97	Aug99	Sep98	6.05
July97	Aug99	Oct98	6.3
July97	Aug99	Oct98	5.925
July97	Aug99	Nov98	6.05
July97	Aug99	Nov98	5.925
July97	Aug99	Dec98	5.925
July97	Aug99	Dec98	6.05
July97	Aug99	Jan99	6.175
July97	Aug99	Feb99	6.05
Apr98	Apr2000	Feb99	6.05
Apr98	Apr2000	Mar99	5.925
Apr98	Apr2000	Mar99	5.925
Apr98	Apr2000	Apr99	6.175
Apr98	Apr2000	May99	6.05
Apr98	Apr2000	May99	6.3

storage site (Dept of Preventive and Social Medicine) and 6 samples were obtained from immunisation clinic. All vaccines tested were found to be potent as shown in Table 1.

Reference OPV titre obtained was $\log_{10} 5.8 \pm 0.5$ (5.3-6.3). Maximum titre obtained was $\log_{10} (6.49)$ and minimum titre obtained was $\log_{10} (5.83)$. 35.3% vaccines were found to be within the titres $\log_{10} (5.95)$ - $\log_{10} (6.05)$. The 6 vaccines

Fig. 1 : CPE of poliovirus on Hep2 cell line (20 x)

obtained from the immunisation clinic were also potent with titres above the stipulated cut-off titre of $\log_{10} (5.83)$ and ranged from $\log_{10} (5.86-6.02)$. All vaccines received were thermo-stabilised with 1M magnesium chloride. No marked seasonal fluctuation in vaccine titres was observed, during the period of study. The titre values did not show any correlation with the manufacturing date, nor there was any variation of titre value in the same batch (Table 2).

Discussion

Adequate potency of vaccines tested in this study confirms the ideal storage and transport conditions of OPV in Armed Forces set-up. This study showed 100% potency of all the vaccines tested both at storage and user end. Drawing comparisons with previous studies, Arya et al tested 191 samples from all over the country and found 113 (59%) to be potent [10]. 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 [11]. Khare et al in 1988, found 45% samples to be potent [12]. Deivanayagam et al obtained a better trend in 1990 with 77% samples being potent [5]. 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 [13]. 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 $MgCl_2$ 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 [13]. It was also noted that vaccine stabilised

with MgCl_2 suffered no significant loss of potency, after as many as nine cycles of alternate warm and cold conditions [14]. 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 [15]. 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 no more than 10°C for a period not exceeding 30 days after which time it must be discarded [16].

In our 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 titre 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. Further studies are required in field areas and peripheries to assess vaccine potency. Vaccine take rates in Armed Forces population requires further assessment. Long-term vaccine potency tests both in tertiary care centres and field units would help in strengthening cold chain monitoring and polio vaccination and polio eradication programmes in the Armed Forces population.

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