# Stability of oral polio vaccine at different temperatures and its correlation with vaccine vial monitors

Poliomyelitis is a disease caused by poliovirus, which remains a serious health problem throughout the world. In 2006, 531 cases of acute flaccid paralysis (AFP) were reported in India. The 1 M $MgCl_2$ stabilized live attenuated trivalent oral polio vaccine (tOPV) is used predominantly to immunize children up to the age of five years, which provides immunity during the most susceptible period. The vaccine being highly thermo-labile, needs a stringently monitored cold-chain system for its upkeep. The vaccine (OPV) is known to retain its potency over a long period[1] if stored at –20°C. The potency of live oral polio vaccine is determined by an *in vitro* assay using the Hep-2 Cincinnati cell lines[2].

In a study of the stabilizing oral polio vaccine at high ambient temperatures at WHO, there was a general consensus that a vaccine capable of withstanding 45°C for 7 days with less than 0.5 $CCID_{50}$/dose reduction in the potency of each of the three serotypes, would offer substantial benefit to global eradication effort[3].

Addition of 1 M $MgCl_2$ to several enteroviruses, including the three poliovirus strains, has been shown to reduce the loss of infectivity at high ambient temperatures. Nevertheless, a study[1] of the stability of five batches of tOPV containing 1 M $MgCl_2$ from different manufacturers, showed that the average loss in 3 weeks was 1.5 $CCID_{50}$/dose at 22°C and 3.1 $CCID_{50}$/dose at 36°C.

A vaccine vial monitor (VVM) which measures cumulative exposure to heat, is a temperature-sensitive label attached to each vial of the vaccine at the time of manufacture. The VVM is a label made of a heat-sensitive, long-chain polymer with a static, temperature-insensitive reference colour printed as a circle surrounding this heat-sensitive label and is a patent of Lifelines Technology Ltd, UK. This VVM is a circle of diameter 7.0 mm with a square of 2.0 × 2.0 mm positioned at the centre of the circle. The VVM serves primarily to warn health workers when the cumulative heat exposure of a vaccine vial has exceeded a preset limit, beyond which the vaccine should not be used. In addition, changes in the appearance of the VVM before this limit is reached, serve as a guide to health workers on the choice of vials of vaccine to be used first. Studies undertaken by WHO have revealed the concern about the accuracy of the VVM, which in turn may lead to wastage of vaccine which could have been otherwise gainfully utilized[4]. The present study was aimed at a better understanding of VVM and its correlation to the potency retained at these variable temperature-exposed batches of the vaccine, which offer a reason to further validate the VVM in a real-time study to avoid vaccine losses and administration of subpotent vaccine.

Ten batches of trivalent oral polio vaccine were obtained from a leading North Indian manufacturer of OPV for the study. These batches were tested for potency (TOPV) according to the design of the study. The samples were exposed at different temperatures for different time intervals. The temperature range and duration of exposure according to the plan of study are shown in Table 1.

Five samples were also subjected to 10, 20, 30, 40 and 50 cycles of rapid freezing (–80°C) and thawing (22–25°C). The composite virus content was estimated by micro-titration using Hep-2 C-cells for all the batches prior to exposure and after each stage of the prescribed exposure, also recording the VVM at the end of each exposure.

The vaccine samples and National reference standard (1/2001 P; NRS) obtained from CDL CRI Kasauli, were diluted in chilled minimum essential medium (MEM) with 2% foetal calf serum (FCS). Dilutions ranging from $10^{-3}$ to $10^{-7.5}$ were used for the $CCID_{50}$ estimation of the vaccine under study.

Hep-2 C-cell line at passage 157 also received from CDL CRI Kasauli, was propagated in MEM with Earle's salts supplemented with 5% FCS and 1 g/l streptomycin to obtain a juvenile confluent monolayer of cells using standard tissue culture technique. Viable cell count was determined adjusting the cell count to $1–2 \times 10^5$ cells per ml using Neubauer's haemocytometer and trypan blue[5].

The $CCID_{50}$/dose was determined according to the standard WHO protocol by estimating the 50% end-point[6]. 50 µl of each dilution from $10^{-3}$ to $10^{-7.5}$ was dispensed into all wells of a presterilized, flat-bottomed microtitre plate, starting from higher to lower dilution, followed by the addition of 50 µl of MEM with 2% FCS to all wells of vaccine dilutions for potency testing of TOPV. 100 µl of MEM was added to cell control wells, while 100 µl of Hep-2 C ($1–2 \times 10^5$ cells per ml) cell suspension was added to all wells. The plates were incubated at 35 ± 1°C in a carbon dioxide incubator (5% $CO_2$) up to 7 days and the $CCID_{50}$/0.1 ml determined using the Karber's formula[7,8].

Forty samples exposed at different temperatures for different time intervals were titrated for potency (TOPV) according to the plan of study. The VVM status at the end of each exposure was recorded and is shown in Table 2 and Figure 1.

**Table 1.** Study plan

| Temperature (°C) | Duration (days) | | | |
|---|---|---|---|---|
| 2–8 | 0 | 7 | 14 | 28 |
| 22–25 | 5 | 7 | 14 | 28 |
| 30–35 | 2 | 7 | 10 | 28 |
| 42 | 1 | 3 | 7 | 15 |

**Table 2.** VVM status at different temperatures for 14 days

| | Temperature (°C) | | | |
|---|---|---|---|---|
| Days | 2–8 | 22–25 | 30–35 | 42 |
| 0 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 2 | 2 |
| 2 | 1 | 1 | 3 | 3 |
| 3 | 1 | 2 | 3 | 4 |
| 4 | 1 | 2 | 4 | 4 |
| 5 | 1 | 2 | 4 | 4 |
| 6 | 1 | 3 | 4 | 4 |
| 7 | 1 | 3 | 4 | 4 |
| 8 | 1 | 3 | 4 | 4 |
| 9 | 1 | 4 | 4 | 4 |
| 10 | 1 | 4 | 4 | 4 |
| 11 | 1 | 4 | 4 | 4 |
| 12 | 1 | 4 | 4 | 4 |
| 13 | 1 | 4 | 4 | 4 |
| 14 | 1 | 4 | 4 | 4 |

Stages 1 and 2, Vaccine can be used for immunization.
Stages 3 and 4, Vaccine should not be used for immunization.

All vaccine samples scored VVM grade-1 at the time of receipt; these samples were stored at –20°C subsequently pending testing.

At 2–8°C VVM remained grade-1 for all 28 days of observation. At 22–25°C, the VVM remained grade-1 for 2 days, which changed to grade-2 from the third day to fifth day, changing further to grade-3 on to the eighth day and grade-4 thereafter till the 28th day; no further change in colour was observed. At 30–35°C the VVM changed to grade-2 at the end of the first day, remaining grade-3 up to the end of the third day, followed by grade-4 till the 28th day. At 42°C the change in VVM was rapid, showing grade-2 on day one, grade-3 on the second day and grade-4 subsequently.

At 2–8°C, the geometric mean loss in titre, compared to the control samples for the ten batches was 0.09 $CCID_{50}$/dose at the end of the seventh day, 0.21 $CCID_{50}$/dose at the end of 14th day and 0.46 $CCID_{50}$/dose at the end of the 28th day. The loss in potency was marginal on day 7, which further dropped to nearly 0.5 $CCID_{50}$/dose at the end of day 28. These vaccine batches failed to meet the prescription (TOPV) to pass, i.e. $\geq 10^{6.23}$ $CCID_{50}$/dose. The VVM however remained grade-1 throughout the observation period.

At 22–25°C the geometric mean loss of titre, compared to the control samples was 0.24 $CCID_{50}$/dose at the end of the fifth day and 0.54 $CCID_{50}$/dose at the end of the seventh day. This drop in titre accumulated further to 0.88 $CCID_{50}$/dose on 14th day and 1.20 $CCID_{50}$/dose on 28th day. These batches failed to comply with the qualifying prescription on day 5 itself, the VVM remaining grade-2 up to the fifth day of observation.

At 30–35°C, the geometric mean loss of titre, compared to the control samples was 0.42 $CCID_{50}$/dose at the end of the second day, when the VVM changed to grade-3. The vaccine again failed to meet the potency requirements on day 2 only, thus suggesting thermal lability of the vaccine. The titres further dropped drastically at the end of the seventh, tenth and 28th day. The drop was nearly 3.0 $CCID_{50}$/dose on the 28th day.

At 42°C on the first day, the VVM remained well within the usable range grade-2, while the titre deteriorated drastically by 0.43 $CCID_{50}$/dose compared to the control samples. The drop further was marginal 0.49 $CCID_{50}$/dose up to the third day, which further consolidated to 4.10 $CCID_{50}$/dose on the 15th day, while the VVM showed grade-4 on day 3 and remained the same throughout the observation period.

All the ten batches of OPV delivered by the manufacturer were recovered in a frozen state, and were further stored at –20°C. These batches were titrated (TOPV) and the geometric mean titre of these test put up in duplicate was determined. This showed insignificant inter-batch deviation in the $CCID_{50}$/dose values ($P < 0.05$). The titres of the vaccine $CCID_{50}$/dose were determined at the end of each cycle of exposure, which is shown in Table 3 and Figure 2.

Five samples were subjected to rapid freezing (–80°C) and thawing (22–25°C) for 10, 20, 30, 40 and 50 cycles respectively, and their VVM status observed at the end of each cycle. All samples were thawed under running tap water at 22–25°C till the entire contents of the vial thawed. The procedure took 5–8 min to thaw completely. The vaccine was then frozen immediately at –80°C in the Thermo vertical freezing cabinet, which took about 10 min to freeze completely; representative samples at the end of the designated cycles were titrated and the titre compared with the control sample. The results are shown in Table 4 and correlation between titre and VVM grade shown in Figure 3.

Estimation of potency of the OPV has been a prime concern of the various health agencies, which resulted in the incorporation of the VVM in 1996. The VVM has been well validated by WHO[9]. However, certain publications of WHO have raised concern over the VVM and usability of OPV subsequently[6,10]. In the field, where the chance of a serious break in cold chain is high and freezers are less common, the WHO management recommendation is that OPV should not be kept at refrigerated temperatures (0–8°C) at health centres for more than one month, nor transported at these temperatures for more than one week[11,12], a condition grossly not complied for the recommended effective management of OPV in India.

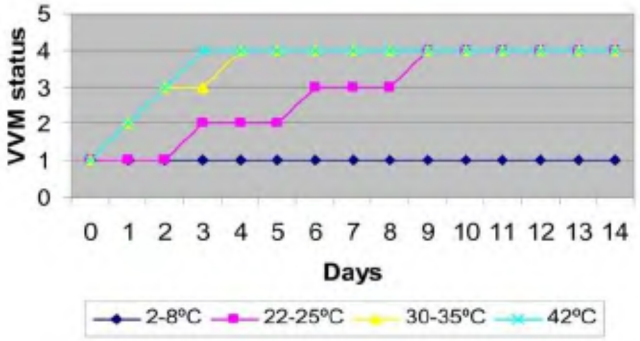


**Figure 1.** VVM status at different temperatures and time intervals.

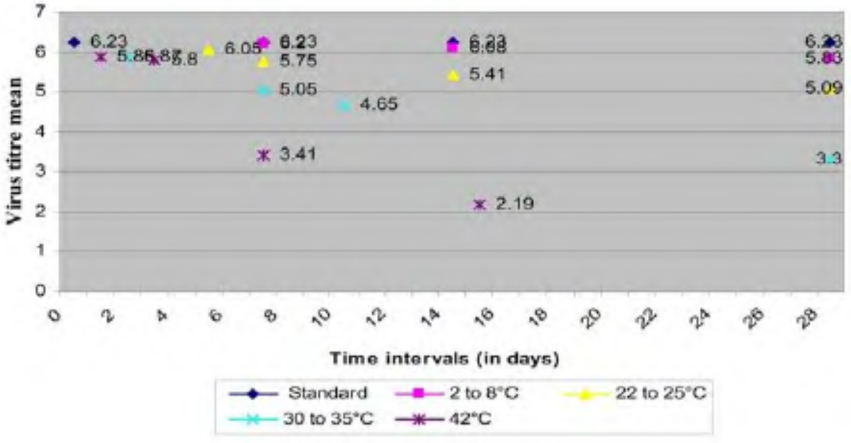


**Figure 2.** Mean virus titre (log $CCID_{50}$/dose) of OPV samples at different temperatures and time intervals.

**Table 3.** Virus titre of tOPV samples held at different temperatures for different time intervals

| Sl. no. | Mean titre of two tests (CS) –20°C | Virus titre (log $CCID_{50}$/dose) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2–8°C | | | 22–25°C | | | | 30–35°C | | | | 42°C | | | |
| | Titre | Day 7 | Day 14 | Day 28 | Day 5 | Day 7 | Day 14 | Day 28 | Day 2 | Day 7 | Day 10 | Day 28 | Day 1 | Day 3 | Day 7 | Day 15 |
| 1 | 6.36 | 6.29 | 6.11 | 5.86 | 6.11 | 5.74 | 5.42 | 5.05 | 5.92 | 5.05 | 4.55 | 3.49 | 5.86 | 5.74 | 3.36 | 2.24 |
| 2 | 6.24 | 6.17 | 6.05 | 5.80 | 6.05 | 5.68 | 5.49 | 4.99 | 5.86 | 5.05 | 4.42 | 3.30 | 5.80 | 5.74 | 3.30 | 2.24 |
| 3 | 6.30 | 6.24 | 6.11 | 5.86 | 5.99 | 5.68 | 5.36 | 5.11 | 5.99 | 4.99 | 4.72 | 3.24 | 5.92 | 5.86 | 3.67 | 2.24 |
| 4 | 6.30 | 6.17 | 6.11 | 5.86 | 6.05 | 5.86 | 5.36 | 5.17 | 5.61 | 5.11 | 4.80 | 3.18 | 5.86 | 5.74 | 3.42 | 2.05 |
| 5 | 6.24 | 6.11 | 6.05 | 5.80 | 5.99 | 5.80 | 5.42 | 5.11 | 5.99 | 5.11 | 4.74 | 3.30 | 5.92 | 5.92 | 3.30 | 2.18 |
| 6 | 6.30 | 6.17 | 6.11 | 5.92 | 6.11 | 5.80 | 5.49 | 5.17 | 5.92 | 5.05 | 4.80 | 3.42 | 5.86 | 5.80 | 3.36 | 2.24 |
| 7 | 6.24 | 6.17 | 6.05 | 5.80 | 6.05 | 5.74 | 5.36 | 5.05 | 5.86 | 4.99 | 4.42 | 3.30 | 5.80 | 5.74 | 3.30 | 2.18 |
| 8 | 6.30 | 6.24 | 6.18 | 5.86 | 6.11 | 5.68 | 5.42 | 5.11 | 5.92 | 5.11 | 4.74 | 3.24 | 5.92 | 5.86 | 3.61 | 2.24 |
| 9 | 6.24 | 6.17 | 6.05 | 5.80 | 5.99 | 5.68 | 5.36 | 4.99 | 5.68 | 4.99 | 4.55 | 3.24 | 5.80 | 5.74 | 3.42 | 2.11 |
| 10 | 6.30 | 6.24 | 6.05 | 5.80 | 6.05 | 5.86 | 5.42 | 5.11 | 5.99 | 5.05 | 4.74 | 3.30 | 5.92 | 5.86 | 3.36 | 2.18 |
| GM | 6.29 | 6.20 | 6.08 | 5.83 | 6.05 | 5.75 | 5.41 | 5.09 | 5.87 | 5.05 | 4.65 | 3.30 | 5.86 | 5.80 | 3.41 | 2.19 |
| LT | 0.00 | 0.09 | 0.21 | 0.46 | 0.24 | 0.54 | 0.88 | 1.20 | 0.42 | 1.24 | 1.64 | 2.99 | 0.43 | 0.49 | 2.88 | 4.10 |

CS, Control sample; GM, Geometric mean; LT, Loss in titre, $CCID_{50}$/dose.

**Table 4.** Virus titres of tOPV batches after 10, 20, 30, 40 and 50 cycles of rapid freezing (–80°C) and thawing (22–25°C)

| Sl. no. | Before freezing and thawing | Virus titre (log $CCID_{50}$ per dose) After rapid freezing and thawing cycles | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VVM Grade | 10 Cycles | VVM Grade | 20 Cycles | VVM Grade | 30 Cycles | VVM Grade | 40 Cycles | VVM Grade | 50 Cycles |
| 1 | 6.36 | 1 | 6.30 | 1 | 6.17 | 1 | 5.98 | 1 | 5.92 | 1 | 5.68 |
| 2 | 6.24 | 1 | 6.17 | 1 | 6.11 | 1 | 6.04 | 1 | 5.98 | 1 | 5.61 |
| 3 | 6.30 | 1 | 6.24 | 1 | 6.11 | 1 | 5.92 | 1 | 5.80 | 1 | 5.55 |
| 4 | 6.30 | 1 | 6.24 | 1 | 6.17 | 1 | 5.98 | 1 | 5.92 | 1 | 5.61 |
| 5 | 6.24 | 1 | 6.17 | 1 | 6.11 | 1 | 5.92 | 1 | 5.86 | 1 | 5.68 |
| GM | 6.29 | | 6.22 | | 6.13 | | 5.96 | | 5.89 | | 5.62 |
| LT | 0.00 | | 0.07 | | 0.16 | | 0.33 | | 0.40 | | 0.67 |

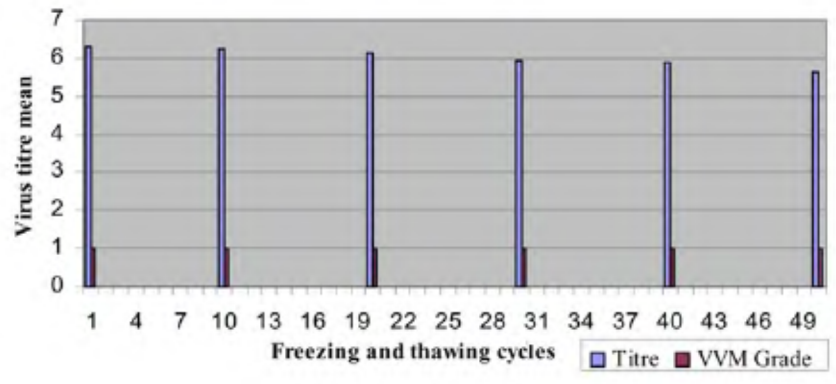


**Figure 3.** Mean virus titre (log $CCID_{50}$) and VVM status of oral polio vaccine after 10, 20, 30, 40 and 50 cycles of rapid freezing (–20°C) and thawing (22–25°C).

The recent resurgence of polio in India has opened the subject of extensive immunization campaign practised during the National Immunization Days (NIDs) and the vaccine efficacy. A recent survey, conducted by Nicholas Grassly and his team from Imperial College, London, attributed this resurgence to overcrowded living condition and poor sanitation in Uttar Pradesh and Bihar in India. The need for an effective post-marketing surveillance of OPV from the endemic pockets in India shall further strengthen the cause of monitoring the efficacy of the vaccine at all transit levels, than just relying on VVM alone.

The current study was thus designed to validate the VVM further at various tem-

peratures simulating the tropical India climate, when the vaccine could be exposed to ambience throughout the year at various transit levels. In order to validate the VVM further and correlate its appearance to the Arrhenus kinetics, the vaccine was subjected to 50 cycles of rapid freezing and thawing, and stored at 2–8°C. At the end of 28 days of exposure, the vaccine failed to comply with the recommended statutory criteria of $\geq 10^{6.23}$ $CCID_{50}$/dose for TOPV content when the titers dropped by 0.46 $CCID_{50}$/dose, compared with the corresponding control samples stored at –20°C, the VVM remaining grade-1 throughout the observation period. The tOPV after distribution at various transit levels remains at 2–8°C for almost 2–4 months pending utilization, suggesting clearly that the VVM would furnish misleading information on the usage of the vaccine, with all possibility of sub-potent vaccine being used for the National eradication campaign, when the titre could further drop to 1.0 $CCID_{50}$/dose or more.

At a constant temperature of 22–25°C, the cumulative heat effect was well correlated with the change in VVM when the titer dropped by 0.24 $CCID_{50}$/dose on day 5 itself, while the VVM was grade-2. This drop in titre further consolidated to 0.54 $CCID_{50}$/dose on day 7 when the VVM was grade-3. The loss in titre further cumulated to 1.20 $CCID_{50}$/dose at the end of day 28, while the VVM showed grade-3 on day 6, which changed to grade-4 on day 9. The vaccine failed to comply with the statutory requirement for TOPV on the day 5 itself, furnishing false information pertaining to vaccine usage.

Enhancing the temperature further to 30–35°C, a reduction in titre of 0.42 $CCID_{50}$/dose was observed on day 2 when the VVM was grade-3, clearly suggestive of vaccine usage and in consonance with the recommended VVM status. This loss further consolidated to 2.99 $CCID_{50}$/dose at the end of day 28, when the VVM changed to grade-4 on day 4 itself.

Increasing the temperature to 42°C simulating a peak tropical summer condition, a 0.43 $CCID_{50}$/dose drop was recorded on day 1, while the VVM was grade-2. This drop in titre further consolidated to 4.10 $CCID_{50}$/dose at the end of day 15, while the VVM changed to grade-4 on day 3 itself and the drop in titre was 0.49 $CCID_{50}$/dose. The drop in titre was significant at the end of day 1, while the VVM was grade-2, suggesting that the vaccine could be used safely.

In order to validate the VVM further, the vaccine was subjected to rapid freeze-thaw cycles when a 0.07 $CCID_{50}$/dose drop was recorded at the end of ten cycles, 0.16 $CCID_{50}$/dose drop at the end of 20 cycles which further consolidated to 0.33 $CCID_{50}$/dose at the end of 30 cycles, increasing to 0.40 and 0.67 $CCID_{50}$/dose at the end of the 40 and 50 cycles respectively. The VVM remained grade-1 throughout, suggesting that this intermittent heat exposure could not be recorded by the VVM.

Our study suggests that vaccine misuse could be prevented to a large extent, particularly when the VVM transits from grade-2 to grade-3 in situations of long duration transit of vaccine to remote endemic locations. The VVM is capable of recording the constant cumulative heat exposure, while the intermittent exposure (freeze and thaw) has clearly no effect of the VVM, suggesting the need for further validation of the VVM under all conditions of variable heat exposure. In conjunction with our study we would recommend enhancement of the current statutory limit from $10^{6.23}$ $CCID_{50}$/dose (TOPV) a batch to pass the test, in the most endemic area with diverse demography and geographical terrains, when the bare minimum is blended by the manufacturers to meet the statutory requirements. An enhanced titre of the OPV would ensure administration of a potent vaccine even in a remote tropical location under adverse conditions reported hitherto.


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