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# An evaluation of cold chain in Maharashtra & Karnataka states by potency testing of field samples of oral poliovirus vaccine

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**The cold chain for oral poliovirus vaccine was monitored in Maharashtra and Karnataka by potency testing of vaccine vials collected from various stages of the delivery system. Results showed that cold chain maintenance improved in the state of Maharashtra within a period of three years as the monitoring began in 1987. Of the 6289 samples of trivalent OPV collected from all 30 districts of the state during 1990 to 1992, 5834 (92.8%) had retained virus titre of at least $10^{5.81}$ $TCID_{50}$/dose. In comparison, 72 per cent of the 1660 samples collected from the state of Karnataka during the same period were found to contain this minimum required virus titre. Defects in cold chain maintenance in Karnataka could be demonstrated by plotting virus titre of samples of individual batches collected from different outlets. It was concluded that potency retesting of OPV samples for cold chain monitoring will ensure proper storage, transport and use of potent vaccine in the field.**

**Key words** Cold-chain - cold-chain monitoring - polio vaccine

The World Health Organization (WHO) and Member States have committed to eradication of paralytic poliomyelitis by the year 2000[1]. WHO has recommended oral poliovirus vaccine (OPV) for control and ultimately eradication of the disease[2]. The strategy for global eradication efforts is based on the experience gained by the Pan American Health Organization (PAHO) and includes maintaining high levels of OPV coverage, conducting supplemental vaccination activities and improving surveillance of acute flaccid paralysis[3].

Although the currently available OPV is stabilized with magnesium chloride, it is still the most heat labile of the vaccines covered by the Expanded Programme on Immunization (EPI)[4]. Stringent control over the temperature during storage and handling is essential for delivery of potent vaccine to each child. As the vaccine travels from the central storage depot to the end user there is a tendency for the cold storage facilities to become less and less stringent. In warm climates vaccine may not withstand ambient temperature for more than a few hours. Thermal inactivation of the vaccine is cumulative. Cold chain monitor (CCM) cards have been developed to indicate if the vaccine lot has been out of cold storage beyond a certain minimum period of time[5]. The only other way for quality assurance of the vaccine at the user's end is to recheck the virus content of vaccine samples retrieved from the field.

Results of potency tests on vaccine samples obtained from two states, Maharashtra (1987 to 1992) and Karnataka (1990 to 1992), have been analyzed to evaluate efficiency of the cold chain for OPV distribution in these states.

## Material & Methods

*Vaccine samples* : OPV samples were received for potency testing through the State Health Services of

Maharashtra and Karnataka and the Public Health Department of Municipal Corporation of Greater Bombay (BMC). This involved a random selection of samples from various stages of the vaccine distribution channel, by the personnel of EPI. The samples were collected from the main vaccine stores at the State and district levels, hospitals, dispensaries, primary health centres (PHCs), sub-centres and private medical practitioners (PMPs). The samples were transported on wet-ice to the laboratory and were stored at -20°C until tested. A total of 11,787 samples of OPV from Maharashtra obtained during the period 1987 to 1992 and 1660 samples from Karnataka (1990 to 1992) were tested for vaccine potency for evaluating the quality of the cold chain.

*Internal standard* : Internal standard control was prepared by blending predetermined amounts of the three poliovirus types (Sabin). Following multiple titrations it was assigned a mean titre value. Each potency test was validated by simultaneous titration of two vials of the internal standard preparation.

*Cells* : HEp-2 (Cincinnati) cells were originally obtained from the National Institute of Biological Standards and Controls (NIBSC), London. Cells between passage nos. 157 and 170 were used for vaccine potency tests. Cells were cultured in Eagles' minimum essential medium (MEM) with 5 per cent foetal bovine serum (FBS).

*Potency titration* : OPV samples were titrated using the microtitration technique. Vaccine was diluted in MEM containing 2 per cent FBS in three 1.0 $\log_{10}$ and seven 0.5 $\log_{10}$ steps. Dilutions from $10^{-4.5}$ to $10^{-6.5}$ were inoculated onto cells for end point titration. Karber formula was used to compute the titre[6]. A virus titre of at least $10^{5.81}$ $TCID_{50}$/dose was considered as a potent vaccine, thereby inferring that the vial had traveled through an efficient cold chain.

## Results

Results of multiple titrations of internal standard preparations used over a long period of time are presented in Table I. The standard deviation for the four preparations was 0.16. Throughout this work we have used the lower fiducial limit of $10^{6.1}TDIC_{50}$ -0.3 $\log_{10}$ per dose of vaccine as the minimum total virus content for qualifying a sample as satisfactory. Table II shows results of potency tests on 11,787 samples of OPV received from the field to determine performance of the cold chain in the 30 districts of the state of Maharashtra over a period of six years. It is evident that the number of OPV samples retaining the minimum required potency increased from 59.9 per cent in 1987 to 92.5 per cent in 1990. From 1990 onwards more than 90 per cent of the total number of vaccine samples received were found to be satisfactory. Results of three years of cold chain monitoring in Karnataka state are shown in Table III. The proportion of potent samples in 1991 (65.9%) and 1992 (70.0%) were smaller than that observed in 1990 (86.1%). Samples received from Bangalore and Kolar districts appear to have been through the worst cold chain. Only Bellary and Bijapur districts showed improvement in maintenance of the cold chain during 1992.

Lately, WHO has suggested a difference of -0.5 $\log_{10}$ as the lower fiducial limits of virus titration assays[7]. As shown in Table IV, a comparison of cold chain evaluation data using the old and the new fiducial limits of assays determined that about 4 to 6 per cent additional samples would satisfy the potency requirement if the new fiducial limits are applied.

The vaccine distribution channel was broadly divided into two stages *viz.*, 'Upper' and 'Lower' for analysis. The Upper stage included samples collected from main vaccine stores, district stores, hospitals, maternity homes, dispensaries and the private medical practitioners. The Lower stage included samples from primary health centres, sub-centres, rural hospitals, cottage dispensaries *etc.* This distinction was empirically made on the basis of expected availability of equipment and personnel. All samples

**Table I. Results of virus titration of internal standards**

| Internal standard | No. of titrations | Mean titre ($TCID_{50}$/0.1ml) | Standard deviation | 95% Fiducial limits (Mean titre±2SD) |
|---|---|---|---|---|
| A | 100 | 5.87 | 0.16 | 5.55 - 6.18 |
| B | 118 | 6.36 | 0.16 | 5.93 - 6.62 |
| C | 49 | 5.95 | 0.16 | 5.74 - 6.31 |
| D | 110 | 6.28 | 0.16 | 5.93 - 6.56 |

**Table II.** District-wise analysis of OPV potency results for the state of Maharashtra

| Sr. no. | District | Satisfactory OPV potency | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1987 | 1988 | 1989 | 1990 | 1991 | 1992 |
| 1. | Ahmednagar | 50.0 | 45.5 | 96.6 | 97.5 | 95.2 | 98.2 |
| 2. | Akola | 82.5 | 76.6 | 97.6 | 96.5 | 96.7 | 97.1 |
| 3. | Amaravati | 62.5 | 52.6 | 97.8 | 100.0 | 95.1 | 96.1 |
| 4. | Aurangabad | 57.1 | 85.1 | 92.4 | 97.0 | 95.8 | 82.3 |
| 5. | Beed | 53.6 | 33.3 | 79.3 | 89.2 | 92.8 | 90.9 |
| 6. | Bhandara | 83.3 | 47.8 | 92.3 | 98.0 | 90.3 | 88.2 |
| 7. | Bombay | 66.6 | 92.0 | 89.4 | 84.0 | 92.7 | 91.5 |
| 8. | Buldhana | 56.5 | 54.8 | 95.8 | 100.0 | 96.5 | 92.1 |
| 9. | Chandrapur | 36.0 | 69.0 | 83.3 | 91.8 | 96.1 | 89.9 |
| 10. | Dhule | 60.0 | 52.0 | 74.5 | 83.5 | 93.0 | 90.1 |
| 11. | Gadchiroli | — | 76.3 | 76.9 | 100.0 | 97.0 | 93.9 |
| 12. | Jalgaon | 66.6 | 72.4 | 82.9 | 81.4 | 100.0 | 97.1 |
| 13. | Jalna | 71.1 | 70.5 | 93.6 | 97.8 | 86.2 | 86.7 |
| 14. | Kolhapur | 85.7 | 79.3 | 86.6 | 96.9 | 100.0 | 88.9 |
| 15. | Latur | 32.6 | 70.8 | 87.1 | 95.2 | 100.0 | 93.9 |
| 16. | Nagpur | 43.8 | 71.2 | 94.2 | 93.7 | 92.3 | 87.8 |
| 17. | Nanded | 77.4 | 62.0 | 89.6 | 94.2 | 90.7 | 84.8 |
| 18. | Nashik | 65.0 | 80.7 | 91.8 | 88.1 | 84.2 | 91.9 |
| 19. | Osmanabad | 52.9 | 16.6 | 84.5 | 94.1 | 88.7 | 94.0 |
| 20. | Parbhani | 63.6 | 100.0 | 93.6 | 94.1 | 100.0 | 89.0 |
| 21. | Pune | 71.0 | 74.3 | 93.1 | 94.9 | 95.1 | 96.4 |
| 22. | Raigad | 57.6 | 66.6 | 76.3 | 94.4 | 91.0 | 89.2 |
| 23. | Ratnagiri | — | 53.5 | 76.9 | 94.7 | 93.4 | 98.2 |
| 24. | Sangli | 71.4 | 71.4 | 85.3 | 97.8 | 96.6 | 81.6 |
| 25. | Satara | — | 21.4 | 75.3 | 95.8 | 90.7 | 89.7 |
| 26. | Sindhudurg | — | 34.2 | 65.0 | 95.7 | 100.0 | 68.8 |
| 27. | Solapur | 80.0 | 62.8 | 79.0 | 100.0 | 100.0 | 93.9 |
| 28. | Thane | 80.9 | 77.0 | 90.7 | 98.1 | 95.3 | 98.6 |
| 29. | Wardha | 86.6 | 67.5 | 88.4 | 100.0 | 97.2 | 87.2 |
| 30. | Yeotmal | 31.1 | 54.8 | 93.9 | 94.4 | 94.0 | 100.0 |
| All districts | | 59.9 | 70.8 | 87.3 | 92.5 | 94.5 | 91.7 |
| No. of samples | | 1056 | 1612 | 2830 | 1852 | 1841 | 2596 |

Figures indicate percentage of total number of samples tested from each district

collected from Bombay were included in the Upper stage of the cold chain. Efficiency of vaccine outlets at the two stages, to maintain vaccine under good cold chain is shown in Table V. The proportion of satisfactory samples at the two levels were compared by Chi square test and the magnitude of the difference was expressed by determining 95 per cent confidence intervals. In 1990, there was a statistically ($P<0.05$) significant difference in the number of samples scored satisfactory at the Upper and the Lower levels in the state of Maharashtra; the Lower level yielding a better performance. Further break-up of samples received from upper level revealed that of the 636 samples 294 were from Bombay. The efficiency of cold chain in Bombay was 84.0 per cent for the year. When Bombay samples were excluded, the difference was reduced (Chi square 4.95 and C.I. 0.18 to 6.58). In Karnataka also significant

difference in cold chain efficiency at the two levels was observed in 1990. The upper level performed better than the lower level. In 1991 and 1992 there was no difference in efficiency of cold chain maintenance at the two levels in both the states. The cold chain was definitely superior in Maharashtra than in Karnataka.

In Bombay, the Public Health department of the Municipal Corporation and the PMPs carry out vaccinations independently. Approximately 40 per cent of the total vaccinations are provided by the PMPs. In 1992, cold chain maintenance at the PMPs level was also surveyed. Of the 492 (419 of BMC outlets and 73 of PMPs) OPV samples tested, 450 (91.5%) were found satisfactory. Break-up of the results revealed that 95.5 per cent samples of the BMC outlets and 68.5 per cent of PMP origin had retained satisfactory potency. Thus, although only 14.8 per cent of the total samples were collected from PMPs, these represented almost 50 per cent of the total number of cold chain failure incidents.

Virus titres of individual vials of two batches of

**Table III.** District-wise analysis of OPV potency results for the state of Karnataka

| District | 1990 | | 1991 | | 1992 | |
|---|---|---|---|---|---|---|
| | No. tested | Pass | No. tested | Pass | No. tested | Pass |
| Bangalore | - | - | - | - | 12 | 8.3 |
| Belgaum | 78 | 93.6 | 95 | 76.8 | 96 | 77.1 |
| Bellary | 53 | 84.9 | 74 | 63.5 | 68 | 80.9 |
| Bidar | 41 | 97.6 | 54 | 75.5 | 51 | 72.4 |
| Bijapur | 68 | 85.3 | 107 | 68.2 | 96 | 81.3 |
| Dharwad | 38 | 81.6 | 75 | 69.3 | 68 | 67.7 |
| Gulbarga | 45 | 82.2 | 86 | 63.9 | 66 | 66.2 |
| Karwar | 48 | 66.7 | 89 | 46.1 | 51 | 66.7 |
| Kolar | - | - | - | - | 15 | 6.7 |
| Raichur | 45 | 93.9 | 66 | 66.7 | 76 | 65.8 |
| Total | 416 | 86.1 | 645 | 65.9 | 599 | 70.0 |

Pass, per cent samples found satisfactory for potency

**Table IV.** Comparison of potency test results of OPV samples using two cut off levels of virus titre

| Year | Maharashtra | | | Karnataka | | |
|---|---|---|---|---|---|---|
| | A | B | No. of samples | A | B | No. of samples |
| 1990 | 96.6 | 93.1 | 1852 | 91.1 | 86.1 | 416 |
| 1991 | 97.3 | 94.5 | 1841 | 76.4 | 65.9 | 645 |
| 1992 | 95.9 | 91.6 | 2596 | 75.3 | 70.0 | 599 |

A, Lower titre limit of $10^{5.65}$ $TCID_{50}$/dose
B, Lower tire limit of $10^{5.81}$ $TCID_{50}$/dose
Percentage values

**Table V.** Comparison of efficiency of cold chain maintenance at the two levels in the vaccine delivery system

| Year | Cold chain level | | | | |
|---|---|---|---|---|---|
| | Upper | | Lower | | |
| | No. tested | Pass | No. tested | Pass | 95% C.I. |
| *Maharashtra state* : | | | | | |
| 1990 | 636 | 88.0 | 1216 | 94.9* | 4.1 to 9.7 |
| 1991 | 537 | 94.2 | 1271 | 94.6 | -1.9 to 2.7 |
| 1992 | 852 | 91.6 | 1744 | 91.8 | -2.0 to 2.5 |
| *Karnataka state* : | | | | | |
| 1990 | 63 | 93.7 | 353 | 84.7* | 1.9 to 16.1 |
| 1991 | 99 | 65.7 | 546 | 65.9 | -9.9 to 10.4 |
| 1992 | 108 | 71.3 | 491 | 69.7 | -7.8 to 11.0 |

* $P < 0.05$ as compared to upper level
CI, confidence interval, (P1-P2)±1.96 (Standard error)
Pass, per cent samples found satisfactory for potency.
Upper level : main vaccine stores, district-stores, hospitals, maternity homes, dispensaries and private medical practitioners
Lower level : PHCs, sub centres, rural hospitals, cottage dispensaries *etc.*

OPV of a particular manufacturer are presented in Fig. 1. Titre values of 107 samples of batch B/1 used in Maharashtra were distributed around $10^{6.37}$ $TCID_{50}$/dose indicated a good cold chain throughout. Batch B/2 used in Karnataka, however, showed that of the 59 samples tested only 5 had retained minimum required virus titre. The remaining samples had virus titre distributed around $10^{5.3}$ $TCID_{50}$/dose suggesting that a large number of vials remained out of the cold chain at one time and were then distributed further. The data indicated failure to maintain cold chain at initial levels of the vaccine delivery system. Of the 220 vials of batch D/1 received from 22 districts of Maharashtra state, 204 (92.7%) were found satisfactory. A large number of samples had titre between $10^{5.81}$ and $10^{5.93}$ $TCID_{50}$/dose but no serious defect in the cold chain was detected (Fig. 2). Batch D/2 of the same manufacturer used in Karnataka (n=115) showed two sets of titre values; around $10^{5.99}$ (normal distribution) and $10^{5.23}$ $TCID_{50}$/dose (scattered). This revealed failure to maintain cold chain at the middle and lower levels of vaccine handling.

## Discussion

OPV being heat labile has posed enormous problems in maintaining the minimum required potency of the vaccine in most of the developing countries. Considerable efforts are underway to define cold-chain requirements and handling of vaccines in these countries. In recent years the main concern of the EPI is to develop a thermostable OPV able to withstand exposure to 45°C for a period of 7 days[8]. Till such a preparation is available the quality of vaccine needs be assured by monitoring the cold chain for vaccine during its storage and handling. The CCM cards routinely used in cold chain monitoring in many countries are, however, suitable (logistically) for monitoring large lots (about 3000 doses of OPV) of the EPI vaccines. A study carried out in India showed that CCM cards were either lost or data not properly recorded during their travel from the main store to the point of use[9]. Potency testing of samples retrieved from the field was thus, the practical way of monitoring the manner of handling the vaccine.

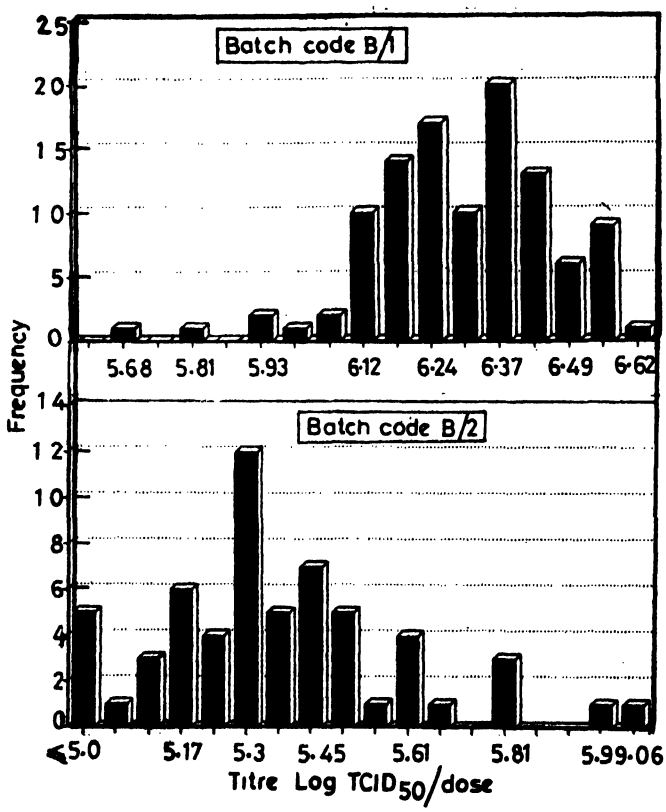


Fig. 1. Frequency distribution of titre of OPV samples received from the field in Maharashtra (batch B/1) and Karnataka (batch B/2).

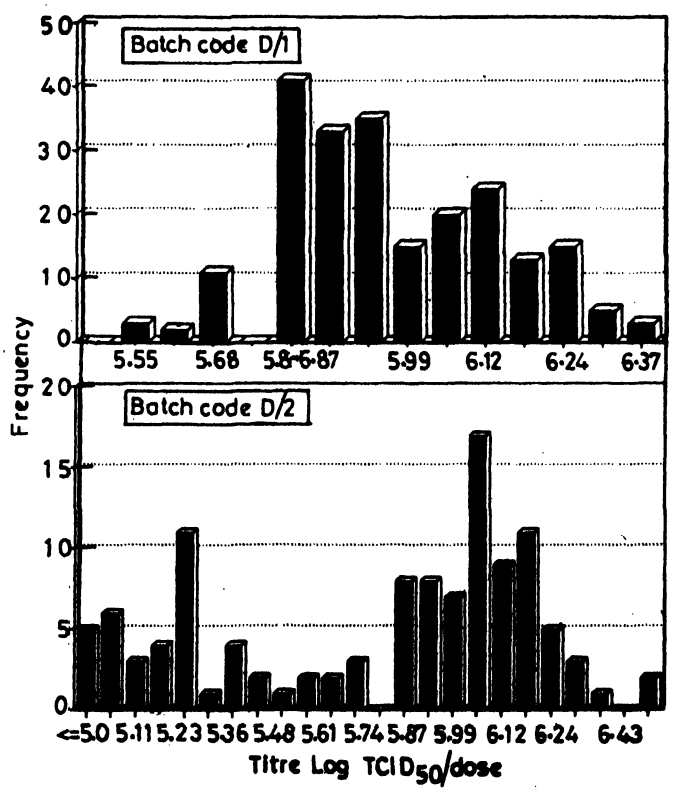


Fig. 2. Frequency distribution of titre of OPV samples received from the field in Maharashtra (batch D/1) and Karnataka (batch D/2).

All OPV batches used through EPI are certified for quality by the National Control Authority, Poliovaccine Testing Laboratory, Central Research Institute, Kasauli. Thus, potency test results of the OPV field samples refer only to the quality/efficiency of the cold chain. By analyzing results of potency tests for vaccine batches from which large number of samples were collected from the cold chain, it was possible to determine cold chain failure incidents at early and middle levels of the vaccine delivery system.

Although WHO has recently suggested a -0.5 $\log_{10}$ difference as the lower fiducial limit of titre for standard trivalent OPV[7], throughout this work we have analyzed the results using the earlier lower fiducial limit (-0.3 $\log_{10}$ per dose) as the minimum total virus content for qualifying a sample as satisfactory. Results of multiple titrations of standard preparations also permitted us to use ± 0.3 $\log_{10}$ as 95 per cent fiducial limits of titration assays. This also enforced stringent control over the cold chain in the two states under study. As shown by our data the difference in the satisfactory samples using the two fiducial limits was about 4 to 6 per cent. The efficiency of cold chain in Karnataka did not improve even after lowering the minimum acceptable titre to $10^{5.65}$ $TCID_{50}$/dose.

Data of the past 6 yr for the state of Maharashtra provided evidence that in 1987 and 1988 the cold chain was not satisfactory in many districts. However, due to strengthening of the activities of EPI a marked improvement in the storage conditions for the vaccine has been achieved. Also monitoring cold chain efficiency by potency testing has ensured accountability and responsibility of the field staff in regard to cold chain maintenance. Since 1990, more than 90 per cent of the vaccine samples had retained the minimum required potency. A definite improvement in cold chain maintenance in most districts was evident. Such improvement has not been observed in Karnataka until 1992.

A random sampling survey of OPV quality carried out in Madras showed that 77 per cent samples collected from vaccine outlets in the city contained the required minimum amount of the virus[10]. Cold chain maintenance is understandably variable from place to place. Maintenance of cold chain is a managerial problem. Field supervision and job responsibilities should be clearly defined to strengthen the cold chain. Our results indicate that this has been achieved in Maharashtra. Evidence suggests that lapse in cold chain maintenance may occur at PMP level. As PMPs provide vaccinations to a large fraction of the population, they should be included in surveillance alongside the EPI outlets.

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