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RESEARCH ARTICLE

TREND ANALYSIS OF POTENCY AND STABILITY OF MEASLES VACCINE IN INDIA

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ABSTRACT

Purpose: Efficacy of live viral vaccines is dependent on proper attenuation of vaccine virus. In addition to attenuation, cold chain maintenance also becomes essential to ensure vaccine quality till it reaches the end user. The transport of vaccines with required cold chain in tropical countries like India is comparatively difficult due to its hot and humid environment. The present study was carried out to see the trend analysis of 95 batches of measles vaccine stored at 2-8°C and their thermo stability after exposure at 37°C for 7 days, as such no study is published from India. Methods: All the vaccines included in the study were tested in triplicates against reference vaccine as per the WHO method. The number of wells positive for syncytia formation was counted and titres were calculated using Spearman-Karber Method. The geometric mean titre was calculated for the triplicate readings. The assay is considered valid only if confidence limits (P=0.95) of the logarithm of the virus concentration is greater than ±0.3. WHO criteria state that minimum virus concentration should be $10^{3.0}$ $CCID_{50}$ per human dose for both exposed and non-exposed measles vaccine. Also, after incubation the loss in titre should not be more than $10^{1.0}$. Results: The potency and thermo stability titres of all the batches tested were found to be $10^{3.45}$ to $10^{4.5}$ and $10^{3.11}$ to $10^{3.65}$ respectively and were within prescribed specifications of WHO. Conclusions: Potency and thermo stability of the measles vaccine tested were found in the acceptable range indicating measles vaccine is quite potent, thermostable and suitable for country like India.

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INTRODUCTION

Measles is a highly contagious childhood disease caused by a virus of the genus *Morbillivirus* (family *Paramyxoviridae*). Measles occurs only in humans; transmitted by aerosolized respiratory droplets and by direct contact. The incubation period is 10-14 days from exposure to onset of rash (WHO Weekly Epidemiological Record, 2009). As there is no specific treatment for measles and most people recover within 2-3 weeks after disease, measles can only be prevented by immunization. A number of live attenuated measles vaccines are available, either as monovalent vaccine or in combination with rubella, mumps or varicella. Most live attenuated measles vaccines originated from the Edmonston strain of measles virus, isolated by Enders and Peebles in 1954. Measles vaccine in lyophilized form is stable. Lyophilized vaccine is recommended to be stored in refrigerated conditions, but for long-term preservation of the vaccine with minimum required infectivity titre of measles virus, storage may be done between -70°C and -20°C (WHO Weekly Epidemiological Record, 2009). As per WHO's recommendation, all available measles vaccines are safe, effective and protect equally well against all wild type measles genotypes (Vaidya, 2015) and may be used interchangeably within immunization programs. Since data on trend analysis of potency and thermostability of measles vaccine are important, therefore, present study has been carried out to know the quality and stability of measles vaccine used in India.

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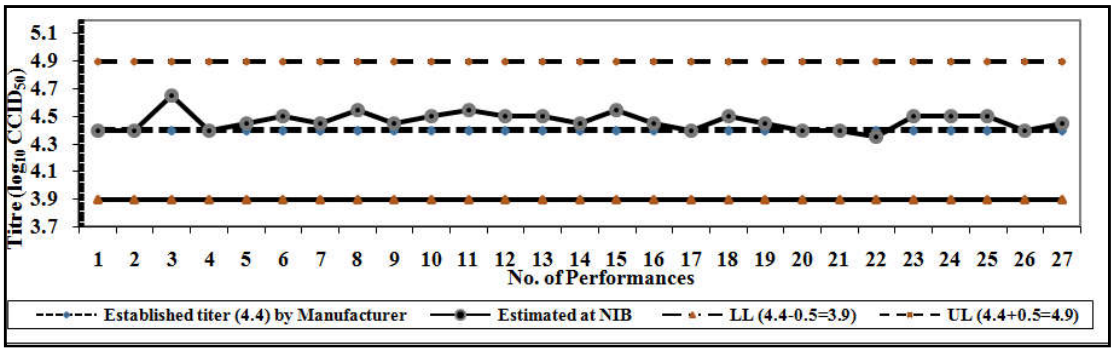
MATERIALS AND METHODS

Ninety five batches of measles Test Vaccine and Reference Vaccine (Edmonston Zagreb strain) along with the sterile water for injection were obtained from the courtesy of M/s Serum Institute of India, Pune, India. The media (M-199), foetal bovine serum, other tissue culture reagents and plastic ware of M/s Sigma, M/s Gibco, M/s Nunc were used. Vero Cell line (CCL-81) used in the study was procured from ATCC, USA. All the measles vaccine batches having sufficient shelf life were tested for the potency and thermostability

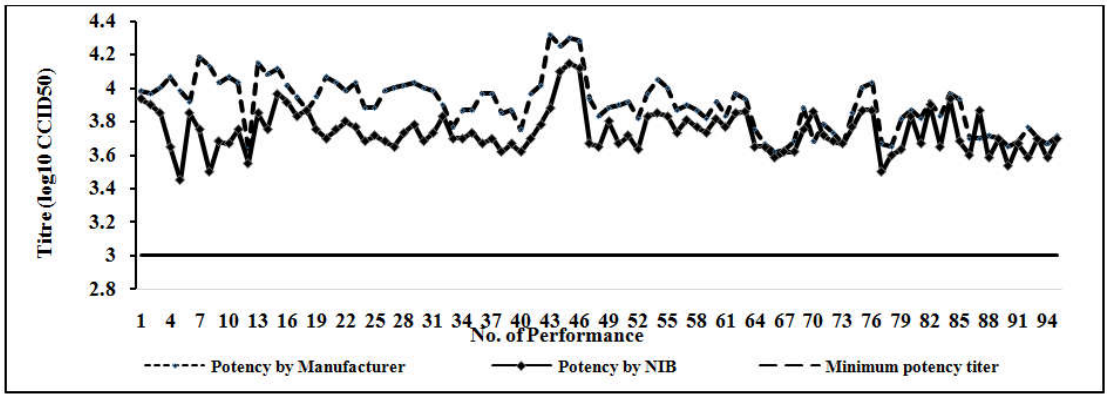
within a period of fifteen months. The potency and thermostability of vaccine thus estimated for each batch were compared to that of the Manufacturer's results given in their respective certificate of analysis.

The test procedure was performed aseptically in the Class IIA2 Biosafety cabinet to ensure product, personnel and environment protection. The virus concentration (potency) was determined in at least three vials (non-exposed) individually against a reference vaccine. Additionally, three vials from the same lot were incubated at 37°C for seven days (exposed). Titration of non-exposed and the exposed vials were carried out in parallel in accordance with the WHO method prescribed for potency of live measles vaccine (Manual of Laboratory Methods; WHO/VSQ/97.04). One reference vaccine for each performance was used irrespective of number of batches of measles vaccine being tested in a particular performance.

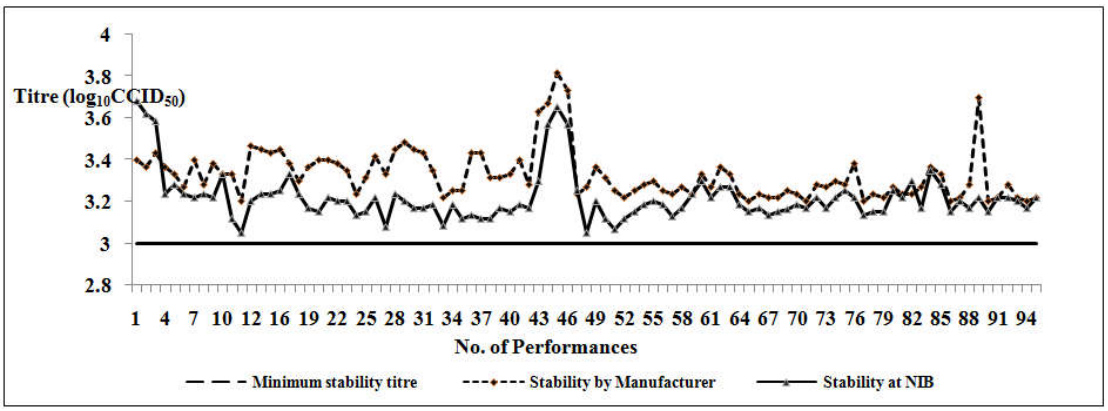
The presence or absence of cytopathic effect in the form of syncytia (multinucleated giant cells) formation was observed microscopically and recorded starting on day 4, day 7 with the final reading at day 10. The titre was calculated using the Spearman-Karber formula and was expressed in terms of $CCID_{50}$ (virus dilution required for causing infection of 50% of the total number of inoculated cell cultures) per human dose (Requirements for Measles, Mumps and Rubella Vaccines, WHO Technical Report Series 840, 1994). The $\log_{10}$ $CCID_{50}$thus obtained reflects the titre of virus in 100µl of the vaccine. Since, one human dose is 0.5ml, its titre would be 5 times. Therefore, a correction factor of 0.7 (since $\log_{10}5$ is 0.7) needs to be added to the $\log_{10}$ $CCID_{50}$obtained by above formula to determine the titre per human dose. The following essential criteria have been established by WHO for calculation of vaccine titre:



Graph 1. Trend analysis of reference vaccine titre estimated by Manufacturer and NIB



Graph 2. Trend analysis of potency titre estimated by Manufacturer and NIB



Graph 3. Trend analysis of stability titres estimated by Manufacturer and NIB

- The titre of the reference vaccine should be within $10^{0.5}$ of the established titre.
- The geometric mean virus concentration of the assayed vials should be at least $10^{3.0}CCID_{50}$ per human dose. Also, the variation between two vials of the same batch should not be more than $10^{0.5}$. The assay is not valid if the confidence limits (P=0.95) of the logarithm of the virus concentration is greater than ±0.3.
- The loss in titre after incubation at 37°C for 7 days should not exceed $10^{1.0}$ and the final titre must be greater than $10^{3.0}CCID_{50}$ per human dose. Both the conditions are essentially to be met.

RESULTS

In the present study, the titre of the reference vaccine used was found between $10^{4.35}CCID_{50}$ and $10^{4.65}CCID_{50}$ per 0.5ml. The titre of the reference measles vaccine established by the Manufacturer was $10^{4.4}CCID_{50}$ per 0.5ml. The acceptable lower and upper limit for the reference vaccine were $10^{3.9}CCID_{50}$ and $10^{4.9}CCID_{50}$ per 0.5ml respectively. The titre of the reference vaccine obtained at NIB was plotted against that of the titre established ($10^{4.4}CCID_{50}$ per 0.5ml) by the Manufacturer (Graph 1). The titres of measles vaccine estimated by Manufacturer and NIB were as per the WHO limits of greater than $10^{3.0}CCID_{50}$. The titres estimated were found to be in concordance with that estimated by the Manufacturer.

Table. Range of potency and stability titres of measles vaccine estimated by Manufacturer and NIB

Potency titre ($CCID_{50}$)		Stability titre ($CCID_{50}$)	
NIB	Manufacturer	NIB	Manufacturer
$10^{3.45}$ to$10^{4.15}$	$10^{3.617}$to $10^{4.317}$	$10^{3.11}$ to$10^{3.65}$	$10^{3.2}$to$10^{3.817}$

The range of potency titres of 95 batches of measles vaccine estimated by NIB was $10^{3.45}$ and $10^{4.15}$ and that of Manufacturer was $10^{3.617}$ and $10^{4.317}$ (Table).The details of comparison between the potency titres of each measles vaccine observed by the Manufacturer and NIB is depicted in Graph 2. The titres of measles vaccine claimed by the Manufacturer and estimated at NIB were found to be similar, and within the WHO specification. The range of estimated thermostability titres of 95 batches of measles vaccine estimated by NIB was $10^{3.11}$ and $10^{3.65}$ and that of Manufacturer was $10^{3.2}$ and $10^{3.817}$ (Table). The details of comparison between the stability titres of each measles vaccine estimated by the Manufacturer and NIB are depicted as Graph 3.

DISCUSSION

The quality of measles vaccine is important not only at the time of release by the Manufacturer but also essential to have retained recommended minimum potency till the time of vaccination for effective immunization of the children. Live attenuated vaccines, in general, are heat sensitive to potency loss during storage and distribution, thus an uninterrupted cold chain is required to maintain the vaccine quality. Globally, by the year 2013, improved vaccine coverage resulted in a 75% reduction in measles related deaths. During the year 2013, about 205 million children were vaccinated against measles during mass vaccination campaigns in 34 countries. All WHO Regions have now established goals to eliminate this preventable killer disease by 2020 (WHO Response Measles Fact sheet).The fourth Millennium Development Goal (MDG 4) aims to reduce the under-five mortality rate by two-thirds between 1990 and 2015. The M&R Initiative, a collaborative effort of WHO, UNICEF, The American Red Cross, The United States Centre for Disease Control and Prevention, and The United Nations Foundation, supports the countries to achieve measles control goals *i.e.* to reduce global measles deaths by at least 95% compared with levels in the year 2000 and to achieve regional measles elimination by 2015. By year 2020, goal is to eliminate measles in at least 5 WHO regions, by ensuring high vaccination coverage with two-dose program of measles and supporting further R&D for cost-effective action and improved vaccination and diagnostic tools.

The benefit of measles vaccination in preventing illness, disability, and death has been well documented. During the year 1999-2004, a strategy led by the World Health Organization and UNICEF led to improvements in measles vaccination coverage that averted an estimated 1.4 million measles deaths worldwide (CDC, MMWR; 2006). The vaccine for measles has led to the near-complete elimination of the disease in the United States and other developed countries (CDC MMWR, 2006). In India almost 80,000 children die each year due to measles and its complications amounting to 4% of deaths under five years of age (Vashishtha *et al*., 2013). A systemic review of data over four decades available from 12 Indian states reported that the median case fatality ratio (CFR) of measles is 1.63% (Sudfeld and Halsey, 2009). Studies have also reported that more than 50% of the global measles associated deaths were reported in India alone (Simons *et al*., 2012; Morris *et al*., 2013).

In the year 1964, as part of study on stability of measles vaccine in field conditions was investigated by MRC, UK. Samples of vaccines used in clinics were returned by post at the end of each session. Vaccines were received from 32 areas throughout England and Wales, where the mean annual temperature at low altitudes varies from about 8.5°C to 11°C. Of the 45 samples tested, only one vial had a serious loss of potency. Remaining all samples "lost in the post" and left out of refrigerator still retained adequate potency to stimulate immunity (Clarke, 1977). However, the chances of the exposure of the vaccine to higher than the recommended storage temperature is more in a tropical country like India, where temperatures typically range from -2°C to 40°C, but can reach 47°C in summer and -4°C in winter.

Therefore, the chances of deterioration of vaccine during lost in the post and left out of the refrigerator are expected more in India. In order to take care of exposure of vaccine to higher than recommended temperature, the WHO (Manual of Laboratory Methods; WHO/VSQ/97.04) and the Indian Pharmacopeia (2014) recommend that the loss in titres after incubation at 37°C for 7 days, should not exceed $10^{1.0}$ and the final titre must be greater than $10^{3.0}$ $CCID_{50}$ per human dose. In the present study, when vaccine stored at the recommended storage temperature within the claimed shelf life, the patterns

of the potency and stability titres of the 95 batches of the measles vaccine tested followed the similar trend as that claimed by the Manufacturer. It is clear that measles vaccine used in India are of Standard Quality but a more heat stable measles vaccine produced by spray drying by using stabilizers like L-arginine, human serum albumin and a combination of divalent cations may prove better in times to come because this has been reported to be stable for 8 weeks at 37°C (Ohtake *et al*., 2013).

Conclusion

The study of 95 batches of measles vaccine revealed that the required potency and thermostability have been retained as per WHO specifications and claims of the Manufacturer suggesting that present measles vaccine is potent, thermostable and suitable for countries like India.

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REFERENCES

Center for Disease Control and Prevention (CDC). "Progress in reducing global measles deaths, 1999-2004". MMWR Morb Mortal Wkly Rep 2006; 55 (9): 247-9.

Clarke, M. 1977. Stability of measles vaccine. *British Medical Journal*., 2(6084): 455. Indian Pharmacopeia 2014.

Manual of laboratory methods for potency testing of vaccines used in WHO expanded program on immunization: WHO/VSQ/97.04.

Morris, S.K., Awasthi, S., Kumar, R., Shet, A., Khera, A., Nakhaee, F., Ram, U., Brandao, J.R., Jha, P. and MDS Collaborators. 2013. Measles mortality in high and low burden districts of India: estimates from a nationally representative study of over 12,000 child deaths. Vaccine., 31: 4655-4661.

Ohtake, S., Martin, R.A., Yee, L., Chen, D., Kristensen, D.D., Lechuga-Ballesteros, D. and Truong-Le, V. 2013. Heat stable measles vaccine produced by spray drying. Vaccine., 28(5): 1275-1284.

Requirements for measles, mumps and rubella vaccines and combined vaccine (live). Requirements for biological substances No. 47. WHO Technical Report series No. 840, 1994: Annex 3.

Simons, E., Ferrari, M., Fricks, J., Wannemuehler, K., Anand, A., Burton, A. and Strebel, P. 2012. Assessment of the 2010 global measles mortality reduction goal: results from a model of surveillance data. Lancet., 379: 2173-2178.

Sudfeld, C.R. and Halsey, N.A. 2009. Measles case fatality ratio in India a review of community based studies. Indian Pediatr., 46: 983-989.

Vaidya, S.R. 2015. Commitment of measles elimination by 2020: Challenges in India. Indian Paediatrics., 52: 103-106.

Vashishtha, V.M., Choudhary, P., Bansal, C.P. and Gupta, S.G. 2013. Measles control strategies in India: position paper of Indian Academy of Pediatrics. Indian Pediatrics., 50: 561-564.

WHO response Measles Fact sheet N°286" (http://www.who.int/mediacentre/factsheets/fs286/en/).

WHO Weekly Epidemiological Record 28Aug 2009, 84th year.

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