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Original Research Article

Quality Evaluation of Rubella Vaccine used in India

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Abstract

Objectives: Cold chain maintenance is essential to ensure vaccine quality till it reaches the end user. In tropical countries like India with hot and humid environment, the transport of vaccines with required cold chain is comparatively difficult. Also, the efficacy of live viral vaccines is dependent on proper attenuation of vaccine virus. The present study was carried out to analyse the quality of 41 batches of rubella vaccine stored at 2-8°C and their thermostability after exposure at 37°C for 7 days, as such no study is published from India. Accordingly, the trends for the potency and stability titres of these batches of rubella vaccine were analysed during the present study.

Method: All the Rubella vaccines batches were tested in triplicates against reference vaccine as per the WHO formula. The number of wells showing cytopathic effect was counted and titres were calculated using Spearman-Karber formula. 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 rubella vaccine. Also, after incubation the loss in titre should not be more than $10^{1.0}$.

Result: The potency and thermostability titres of all the batches tested were found to be between $10^{3.467}$ to $10^{4.03}$ and $10^{3.276}$ to $10^{3.72}$ respectively and were well within prescribed specifications of WHO. Conclusion: Potency and thermostability of the rubella vaccine tested were found in the acceptable range indicating rubella vaccine used in India is quite potent and thermostable.

Keywords: Quality Control, Rubella vaccine, Cytopathic Effect, $CCID_{50}$ (Cell Culture Infectious Dose), Potency, Thermostability.

1. Introduction

Occurrence of rubella infection in pregnant women is a potentially threatening event posing the risk of developing congenital rubella syndrome or intrauterine death or premature birth of foetus. The majority of girls get protection until they attain adolescence; however not all are protected and therefore, some women remain susceptible to infection during pregnancy [1]. Nevertheless, it is preventable disease and a live attenuated rubella vaccine can induce a strong immune response, provided the effective potency of the vaccine is retained till vaccination [2].

Because rubella is not as highly infectious as measles and because the effectiveness of single dose of a rubella vaccine is ≥ 95% even at age 9 months, only single dose of rubella vaccine is required to achieve rubella elimination and high coverage is to be achieved. However, when combined with measles vaccination, it may be easier to implement a second dose of rubella vaccine using the combined MR or MMR vaccine for both doses [3].

A number of live attenuated rubella vaccines are available, either as monovalent vaccine or in combination with measles, mumps or varicella. Most common strain used for the live attenuated rubella vaccines is RA23/7. Adaptation of rubella virus to human diploid cell strains (HDCS) was achieved in 1964, with attenuation in the same cells accomplished by 1967. Rubella vaccine (RA27/3 strain) produced in HDCS has been reported to be more immunogenic than other strains [4]. The effectiveness of the RA27/3 vaccine has been demonstrated by the elimination of rubella and the CRS from the western hemisphere and by the several European countries that have achieved and maintained high vaccination coverage with vaccine

containing RA 27/3 [5]. Lyophilized Rubella vaccine is stable and is recommended to be stored in refrigerated conditions. Since data on trend analysis of potency and thermostability of rubella vaccine are important, therefore, present study has been carried out to know the quality and stability of rubella vaccine used in India.

2. Materials and Method

Forty one batches of Rubella Test Vaccine and Reference Vaccine (RA27/3 strain) along with the sterile water for injection were obtained from the courtesy of M/s Serum Institute of India, Pune, India. The media (MEM), foetal bovine serum, other tissue culture reagents and plastic ware of M/s Sigma, M/s Gibco, M/s Nunc were used. RK-13 certified cell line (CCL-37) used in the study was procured from ATCC, USA. All the rubella vaccine batches having sufficient shelf life were tested for the potency and thermostability within a period of fifteen months. The shelf life for the rubella vaccine tested in this study is two years from the date of manufacturing, as prescribed on the vaccine vial label. The potency and thermostability of vaccine thus estimated for each batch were compared to that of the Manufacturer's results given in the 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 rubella vaccine [6]. One reference vaccine for each performance was used irrespective of number of batches of rubella vaccine being tested in a particular performance. The presence or absence of cytopathic effect in the form of rounded, bipolar and multipolar cells 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 as $CCID_{50}$ (virus dilution required for causing infection of 50% of the total number of inoculated cell cultures) per human dose [7].

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) has been added to the $\log_{10}$ $CCID_{50}$ obtained by above formula to determine the overall titre per human dose.

The following essential criteria have been established by WHO were followed for calculation of vaccine titre:

a) The titre of the reference vaccine should be within $10^{0.5}$ of the established titre.
b) 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 0.5 $\log_{10}CCID_{50}$. The assay is not valid if the confidence limits (P=0.95) of the logarithm of the virus concentration is greater than ±0.3.
c) 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.

3. Results

In the present study, the titre of the reference vaccine used was found between $10^{4.35}CCID_{50}$ and $10^{4.75}CCID_{50}$ per 0.5ml. The titre of the reference rubella vaccine established by the Manufacturer was $10^{4.28}CCID_{50}$ per 0.5ml. The acceptable lower and upper limits for the reference vaccine were $10^{3.78}CCID_{50}$ and $10^{4.78}CCID_{50}$ per 0.5ml respectively. The titre of the reference vaccine obtained at NIB was plotted against that of the titre established by the Manufacturer (Figure 1).

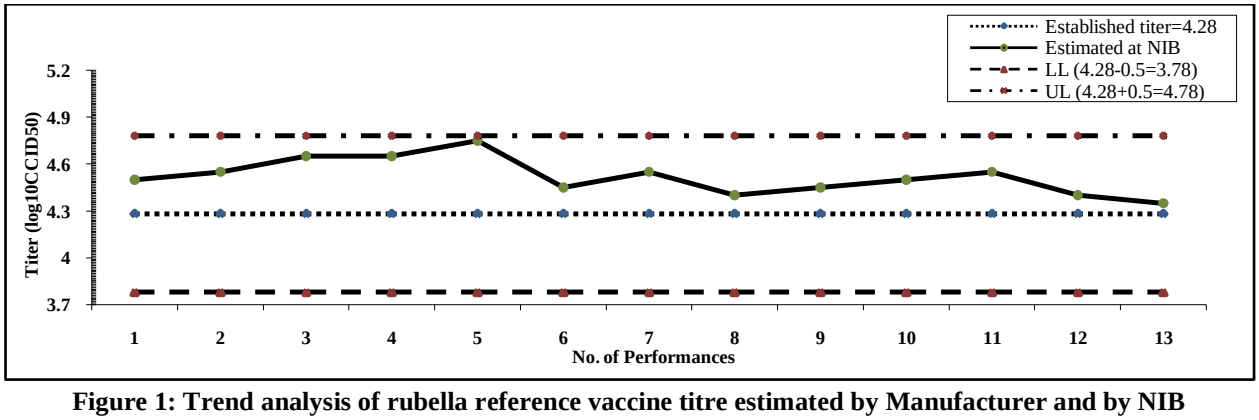

Figure 1: Trend analysis of rubella reference vaccine titre estimated by Manufacturer and by NIB

It is evident that the potency titres of vaccines from Manufacturer and NIB were as per the WHO limits of greater than $10^{3.0}CCID_{50}$. The titres estimated are in concordance with that estimated by the Manufacturer at the time of batch release. The range of potency titres of 41 batches of rubella vaccine estimated by NIB was $10^{3.467}$ to $10^{4.03}$ and that of Manufacturer is $10^{3.6}$ to $10^{4.03}$ (Table 1).The comparison between the potency titres observed by the Manufacturer and NIB is depicted in Figure 2.

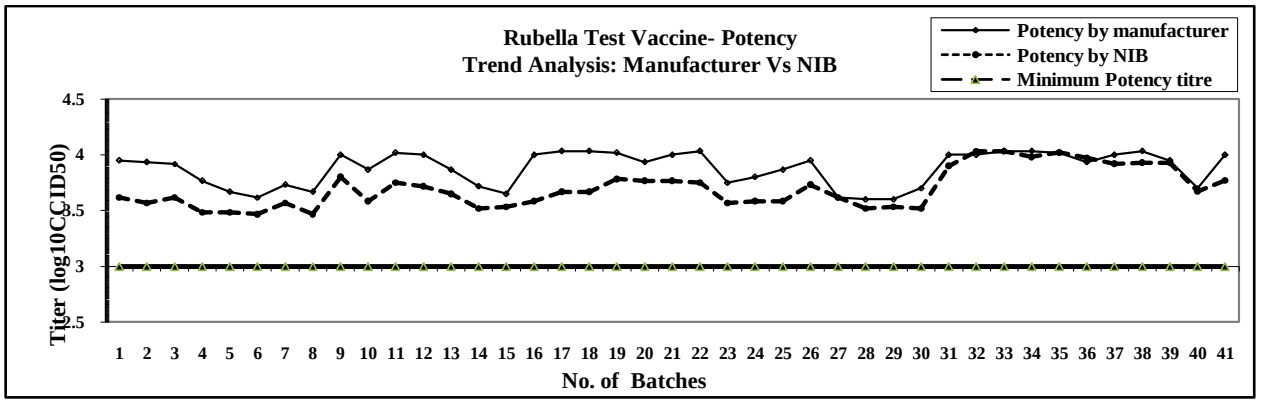

Figure 2: Trend analysis of rubella vaccine potency titre estimated by Manufacturer and by NIB

The thermostability titres claimed by the Manufacturer and estimated at NIB are almost parallel, and lie within WHO specification. The range of estimated thermostability titres of 41 batches of rubella vaccine estimated by NIB was $10^{3.276}$ to $10^{3.72}$ and that of Manufacturer is $10^{3.283}$ to $10^{3.833}$ (Table 1). Similarly, the comparison between the thermostability titres estimated by the Manufacturer and at NIB is depicted as Figure 3.

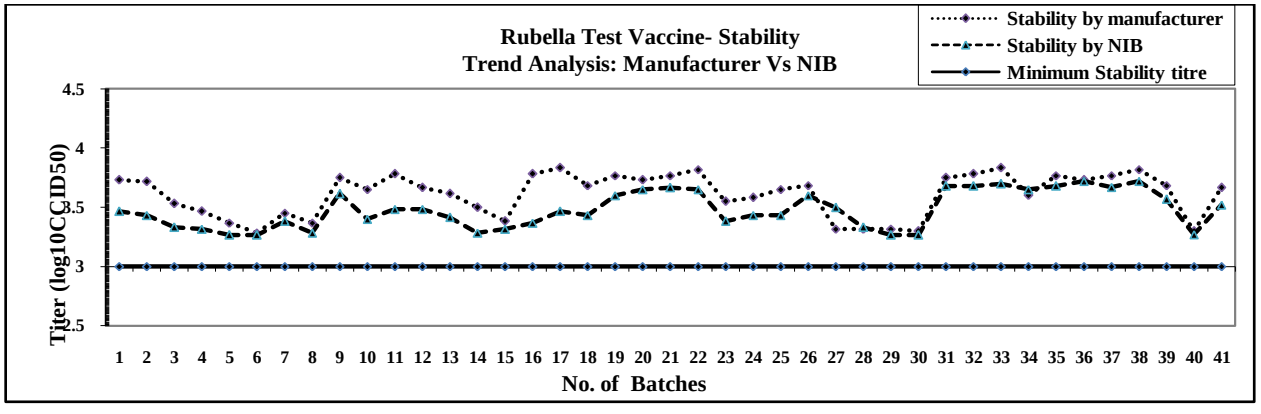

Figure 3: Trend analysis of thermostability titres estimated by Manufacturer and by NIB

Table 1: Range of potency and thermostability titres estimated by Manufacturer and NIB

Range of vaccine potency titre ($CCID_{50}$)		Range of vaccine thermostability titre ($CCID_{50}$)		Recommended titre on storage at 2-8°C and after exposure at 37°C for 7 days	
NIB	**Manufacturer**	**NIB**	**Manufacturer**		
$10^{3.467}$ to $10^{4.03}$	$10^{3.6}$ to $10^{4.03}$	$10^{3.276}$ to $10^{3.72}$	$10^{3.283}$ to $10^{3.833}$	At least $10^{3.0}CCID_{50}$ per human dose	• Loss in titre after incubation should not exceed $10^{1.0}$ and • The final titre must be greater than $10^{3.0}$ $CCID_{50}$. • Both conditions must be met.

4. Discussion

Due to their inherent instability, vaccines require storage, transportation and administration under controlled temperature conditions. Thus it is important that cold chain is maintained properly throughout the supply chain till the vaccine reaches the end user. Loss of vaccine potency during storage and handling leading to administration of sub-potent vaccine is a major public health concern [8]. The quality of rubella vaccine is important not only at the time of release by the manufacturer but also essential to retain the minimum recommended potency till the time of vaccination for effective immunization. Live attenuated vaccines are heat sensitive and may result in potency loss during storage and distribution, thus an uninterrupted cold chain is required to maintain the vaccine quality. Stability of live, freeze-dried vaccines is a major factor for successful vaccinations. The routine use of thermostability test, provides useful information about the stability of vaccine at the recommended storage temperature. Specifications stating that after thermostability (exposure at 37°C for 7 days), a titre equal or higher than the minimum titre, is a guarantee for

sufficient potency at the time of immunization. In order to circumvent the thermolabile problem in tropical countries, more stable vaccines are essential [9].

WHO recommends that all countries that have not yet introduced rubella vaccine should consider doing so using existing, well-established measles immunization programs. To-date, three WHO Regions has established goals to eliminate this preventable cause of birth defects. In April 2012, the Measles Initiative-now known as the Measles & Rubella Initiative-launched a new Global Measles and Rubella Strategic Plan which covers the period 2012-2020. One of the targets is to eliminate regional measles and rubella/congenital rubella syndrome (CRS) by the end of 2015. Also, by the end of 2020, the target is to completely eliminate measles and rubella from at least 5 WHO regions [10].

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 41 batches of the rubella vaccine tested in this study followed the similar trends as that claimed by the Manufacturer in their Certificate of Analysis.

5. Conclusion

The study of 41 batches of rubella vaccine revealed that the potency and thermostability of the batches have been retained as per WHO specifications and claims of the Manufacturer suggesting that present rubella vaccine is potent and thermostable and suitable for countries like India.

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