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Original Article

Investigation of the Relationship between the Residual Moisture and Thermal Stability of Lyophilized MMR Vaccine

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Abstract

Background and Aims: The safe, potent and effective live vaccines against Measles, Mumps and Rubella as important childhood diseases have been available for several decades. Several factors can affect the thermal stability of lyophilized vaccine.

Methods: In this study, the effect of residual moisture on thermal stability of 61 batches of MMR vaccine was investigated using an accelerated method that has been recommended by World Health Organization (WHO).

Results: Our results suggest that the best thermal stability for the lyophilized MMR vaccine is in a range of 1.51 to 2.00% of residual moisture with the minimum decrease in the all three components of the vaccines.

Conclusion: It is suggested that the lyophilizators of MMR vaccine production lines should be programmed in a manner that the best range of residual moisture achieves.

Keywords: Thermostability; MMR vaccine; Residual moisture

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Introduction

Measles, Mumps and Rubella are considered to be as important childhood diseases (1). The safe, potent and effective live attenuated vaccines against these diseases have been available since 40-50 years ago (2). Soon after improvement of the quality of these vaccines and confirmation of their satisfying immunogenicity against their mild adverse events which had been shown by post marketing surveillance studies, world health organization (WHO) recommended it and the Measles, Mumps and Rubella vaccines included in expanded program on immunization (EPI) by many countries. Such a decision has made a remarkable decrease in annual measles, mumps and rubella cases worldwide. The MMR vaccine which contains all three live attenuated infectious particles of Measles, Mumps and rubella viruses is as effective as solely prepared Measles, Mumps or Rubella vaccines (3, 4). These live attenuated vaccines which contain different strains of mentioned viruses are manufactured as lyophilized vials worldwide to improve their stability. The lyophilized MMR vaccines could be kept at 2-8ºC or less at least for 3 years with no serious loss of potency (5). Several factors including residual moisture, stabilizer composition and storage condition can affect the thermal stability of the lyophilized vaccine.

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Methods

MMR vaccine

Samples were taken from lyophilized MMR vaccines manufactured at Human Viral Vaccines Department, Razi Vaccine & Serum Research Institute, Iran. In these vaccines AIK-C strain of measles, Hoshino strain of mumps and Takahashi strain of rubella have been used. Samples were taken randomly from 61 batches which all had been produced in the year 1386 (5).

Residual moisture measurement

Carl-Fischer method had been employed (6). Briefly, the original weight of the lyophilized vaccine was determined. The water content of the lyophilized vaccine was evaporated by heating and released vapor was entered to the cell of the Carl-Fischer coloumetric system and the amount of water was determined by the Carl-Fischer reagent. The percentage of water content of the lyophilized vaccine was determination by subtracting this amount of water from the original weight.

Thermal stability test

The accelerated test had been used. Based on this method, samples from each batch of MMR vaccine divided in to two sets. The first set of sampled vials was kept at -20 ºC and the second set at 37 ºC, for 7 days. After this period, a seroneutralization test and CCID50/dose (see below) was used to determine the titer of each component of both sets of MMR

vaccines. The stability of vaccine was determined by the extent of reduction of the titer of viruses in samples that had been kept at 37 ºC in comparison to those one that had been kept at -20 ºC (6).

Seroneutralization test

Two different cell cultures including Vero (GMCK) and RK-13 cells were cultured as monolayer in glass tubes. The former cell line was

used for inoculation of measles and mumps viruses and the latter was used for inoculation of rubella virus. These cells were the best to follow the cytopathic effects of the mentioned viruses.

Neutralization test performed using specific antisera (inactivated for 30 minutes at 56 ºC) against each component of MMR vaccine (measles, mumps and rubella viruses). To determine the titer of each virus, the lyophilized vaccine contents of each vial was dissolved in accompanied sterile solvent, different dilutions were prepared and neutralized with specific antisera against the other two components by incubation of the vaccine antiserum mixture in 4 ºC for one hour and finally inoculated to the appropriate cell monolayer. In the case of rubella, no neutralization of measles was necessary as measles does not grow in RK-13 cells. Final results were read after 10 days from inoculation date using karber method (7-9). In the case of mumps and rubella, a haemadsorption method (using guinea pig and pigeon red blood cells, respectively) also employed for further confirmation of the results (10).

Results

All vaccine samples were categorized in 7 classes of residual moisture. The members of each category subjected to accelerated thermostability test, then the mean of reduction in titer of all the three components of vaccines

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Table 1. Mean of the reduction in titer of measles, mumps and rubella components of 61 batches of lyophilized MMR vaccines in different ranges of residual moisture.

Residual moisture (%)	No. of batches	Mean of reduction in measles titer*	Mean of reduction in mumps titer*	Mean of reduction in rubella titer*
0.00 – 0.50	3	0.43	0.28	0.26
0.51 – 1.00	6	0.24	0.27	0.31
1.01 – 1.50	8	0.29	0.49	0.31
1.51 – 2.00	18	0.25	0.25	0.25
2.01 – 2.50	17	0.30	0.33	0.33
2.51 – 3.00	8	0.48	0.32	0.27
≥ 3.01	1	0.29	0.50	0.50

* all measures that have been placed in the 3rd to 5th columns are based on –log CCID50 per dose. For example the meaning of the first row is that the mean of the extent of reduction in titer of measles, mumps and rubella viruses in the batches of vaccine with ≤0.5% of residual moisture were 100.43, 100.28, 100.26 infectious particles/dose, respectively.

was calculated as log CCID50/dose. The minimum of reduction in titer was determined in each category as a hallmark of the thermal stability. The acquired data from 61 batches of MMR vaccine have been noted in table 1 and have been summarized in table 2 and fig. 1. As an example, of all 61 batches of MMR vaccine, 3 batches had 0.00 to 0.50% of residual moisture. In these 3 batches the mean of reduction in titer of measles, mumps and rubella in accelerated thermostability test were 0.43, 0.28 and 0.26, respectively. It means that the samples of these 3 batches [with mentioned residual moisture] that had kept at 37 ºC in comparison to that ones that have been kept at -20 ºC have lost their measles virus infectivity to the extent of 10 0.43 /dose.

The minimum decrease in titer (the best stability) of measles infectious particles belonged to the vials with 0.51 to 1.00% of residual moisture, whereas for mumps and rubella virus the minimum reduction in titer was for the samples with 1.51 to 2.00% of residual moisture.

Discussion

Residual moisture as an important factor affects the thermal stability of lyophilized MMR vaccine. According to the World Health Organization documents and European pharmacopeia's guideline, the residual moisture in lyophilized MMR vaccines should not be more than 2% and 3%, respectively (7-11). It is noted in the same documents that the vaccine should have at least 1000 (103) infectious particles of each active component and the decrease in the titer of each active component (infectious particles in accelerated stability test) should not be more than 1 log CCID50/dose; otherwise the produced batch will fail and should not be released for vaccination. All 61 batches that tested in this research met these specifications, but were different in the extent of the reduction in titer. Our current research suggests that the best amount of residual moisture in domestic-made MMR vaccines is in the range of 1.51 to 2.00%. The lyophilization cycle is a highly complicated process and should be carefully programmed and performed. A properly lyophilized vaccine could be distinguishable from an inappropriate one by its appearance and stability aspects that both closely relates to the residual moisture. Our lyophilizators should be programmed in a manner that the best range of residual moisture (1.51 to 2.00%) achieves.

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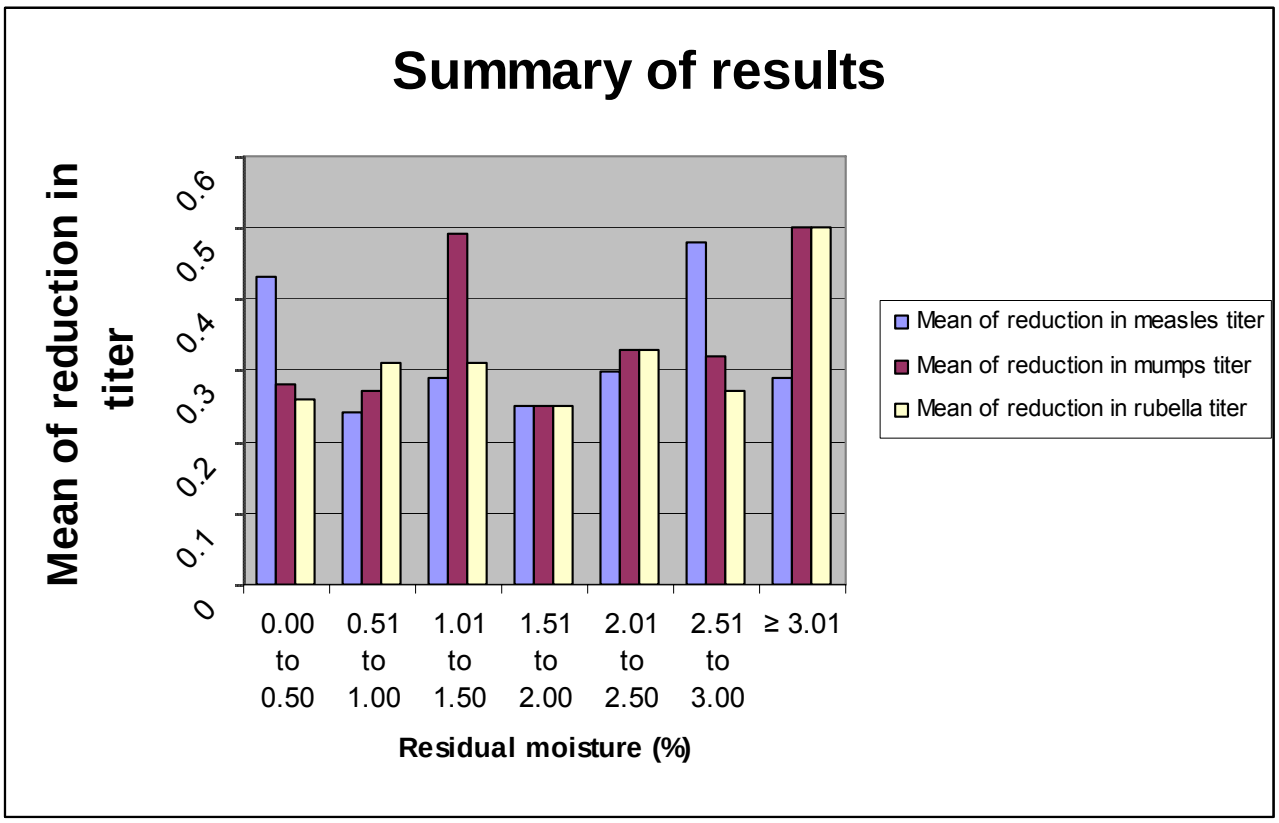


Fig. 1: Mean of the reduction in titer of measles, mumps and rubella components of lyophilized MMR vaccines (during accelerated thermal stability test) in different categories of residual moisture.
Note: all the measures that have been placed on the (x) axis are based on –log CCID50. As an example, in the first category of residual moisture the mean of reduction in the titer of measles, mumps and rubella infectious particles in thermostability test were 10 0.43, 10 0.28 and 10 0.26, respectively. But they converted to 0.43, 0.28 and 0.26 as their –log CCID50/dose

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