

Original Article

Stability Study of Measles AIK-C Strain, Mumps RS-12 Strain and Rubella Takahashi Strain in MMR Vaccine

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ABSTRACT

Stability studies play a critical role in assuring product quality at all points in the vaccine life cycle and have a major impact on the success of immunization programs worldwide. The purpose of stability study is determination of the vaccine quality under the variety of environmental factors and to establish a re-test period or a shelf life and recommended storage conditions. In this study three batches of MMR vaccine of Razi institute in Iran with AIK-C strain for measles, RS-12 strain for mumps and Takahashi strain for rubella were tested for accelerated stability, after seven days incubation at 37 °C and for long-term stability the samples were stored at 2-8 °C and tested intervals in three months until 36 months after production. All of quantitative and qualitative control tests including Potency and Identity, Safety, Sterility, Mycoplasma and Physicochemical tests were done in each period. In accelerated stability the mean loss of activity was 0.375, 0.373 and 0.210 log₁₀ and in long-term stability the mean loss of activity was 0.626, 0.50 and 0.46 log₁₀ for measles, mumps and rubella components of MMR vaccine respectively. In residual moisture test the mean increase of moisture content in the vaccines was 1.084 %. In qualitative tests the vaccine met the WHO specifications too. Results of this research indicated the MMR vaccines with these strains are stable at least 36 months if the cold chain considered properly.

Keywords: MMR, Stability study, Vaccine, Potency test, Quality control

INTRODUCTION

Measles is an almost invariable clinical experience of childhood and is often a severe disease, frequently complicated by middle-ear infection or bronchopneumonia. Mumps is an acute disease of children and young adults. In this infection glandular and nerve tissue are most often affected. Rubella (German measles) give rise to a mild exanthematous illness, accompanied by few constitutional symptoms and occurs most commonly in childhood. This infection

in woman in early pregnancy can induce birth serious and permanent defects (congenital rubella syndrome). Immunization against measles, mumps and rubella has been of interest WHO for many years and live combined vaccine from suitable attenuated strains of these viruses is produced (WHO TRS 840). Efficacy and safety of vaccines is related to the vaccine components contains, proteins, carbohydrates, lipids, inactive or attenuated microorganisms with stabilizers, adjuvants and preservatives. Noticing the sensitivity of measles, mumps and rubella virus to temperature and

light (Plotkin et al., 2008), Knowledge about the stability of a vaccine and especially the rate at which it loses its potency at a given temperature, can be helpful in deciding whether the vaccine should be destroyed, sent for retesting or used. So, Stability of vaccines has major impact on the success of immunization programs worldwide and may responsible for vaccine failures and clear definition of the stability characteristics of a vaccine is of critical importance (Knezevic, 2009). In stability studies accelerated stability study was designed to determine the rate of change of vaccine properties over time as a consequence of the exposure to temperatures higher than those recommended for storage. These studies may provide useful support data for establishing the shelf life or release specifications. Long-term stability study (real time/ real condition) was performed on the physical, chemical, biological and microbiological characteristics of vaccine during and up to the expected handling and storage conditions. The results are used to recommend storage conditions and to establish the shelf life and /or the release specifications (Pfleiderer, 2009). Totally, the purpose of the stability study is preparation of document for the quality of vaccine ingredients, vaccine changes by different effective factors such as temperature, humidity and light, determination of vaccine shelf life or vaccine expiry date, estimation of vaccine retest time and recommendation of suitable maintenance condition (Egan and Schofield, 2009). Validated analytical procedures in the stability study that can detect the changes with time in the chemical, physical or biological properties of the vaccine, and that are specific. So, in this study, accelerated and long-term stability study performed for Razi MMR vaccine with AIK-C, RS-12 (Razi Sasani- 12 that established in Razi institute of Iran) and Takahashi strains for measles, mumps and rubella respectively.

MATERIALS AND METHODS

Sampling. Three hundred vials from each three consecutive batches of the MMR vaccine were sampled randomly (260 vials for long-term stability study, 20

vials for accelerated stability study and 20 vials as archive). Then, the samples stored in the refrigerator at a temperature 2-8 °C similar to packing and condition that recommended in the vaccine leaflet by manufacturer. The accuracy of refrigerator temperature checked by temperature observation, three times in a day and rechecked by cool vision system that recorded and controlled the temperature everyone hour.

Accelerated stability study. In accelerated stability the samples incubated at 37 °C for 7 days. Then, the exposed and unexposed vaccines tested for potency concurrently by the same method as mentioned (WHO, 1995).

Long-term stability study. The vaccines were tested 13 times every three months in three years in 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, and 36 months after production for long-term stability. In each period all of quality control tests were performed includes:

Potency test. In each periods of the study, according to the standard WHO protocol (WHO, 1995) after preparation of 2×10^5 cells /ml Vero (Verda Reno) cells (ATCC, CCL-81) and RK13 (Rabbit Kidney) cells (ATCC, CCL-37) cell lines (Freshney, 2005), titration of viruses was performed as duplicate by two vials of each vaccine. After 4-7 days, the cells in micro plate were observed by invert microscope for detection of cytopathic effects. The CCID₅₀/dose of vaccines was calculated by Spearman-Kärber method with estimation of the 50% end point. Then, the geometric mean titer (GMT) was calculated duplicate data.

Safety test. Three and one doses of vaccine samples were injected to two Guinea pigs and five mice intra peritoneal respectively. Negative and positive control groups were injected by distilled water and working reference preparation respectively. Animals were observed for any local and general reaction and weighted for seven days.

Sterility and mycoplasma test. The samples were cultured in tryptic soy broth (TSB), thioglycolate broth, brain heart infusion agar (BHA) and blood agar culture medium for detection of aerobic and anaerobic bacteria and fungal contamination. For mycoplasma detection,

the vaccines were cultured in PPLO broth and sub cultured in PPLO agar (Pharmacopoeia, 2012).

Physicochemical tests. In each period physicochemical tests including appearance, air tightness, label, vacuum, solubility grade and residual moisture content tests were performed for all of the samples. Color, consistency, form of lyophilized vaccine and any visible particle after reconstitution were considered in appearance test. In air tightness and labeling inspection, the vials air tightness and stability of label were inspected. The existence of vacuum and the solubility grade in water were tested in vacuum and solubility tests (Pharmacopoeia, 2012). For moisture content, the content of residual water in vaccine was determined on the base of Carl Fischer coulometric method (Pharmacopoeia, 2012).

Test Validation. For validation of the tests, system suitability was checked and all of the quality control tests of a working reference preparation (WRP) were determined in parallel of test samples. To determine the corrected potency of the working reference preparation, the following formula was used:

Corrected potency of the working reference preparation = The established titre of the international reference reagent as the result of an international collaborative study / The GMT of the international reference reagent × The GMT of the working reference preparation (ICH Q2 R1 2005).

Statistical Analysis. Appropriate statistical evaluation of vaccine stability data promote strategic stability study design, in order to reduce the uncertainty associated with the determination of the degradation rate, and the associated risk to the customer. Use of statistical tools such a least squares regression analysis was employed to model potency decay (Egan and Schofield, 2009). In the case of measuring the degradation rate, testing at the beginning and the end of the study improves the precision of this estimation. So, in this study a linear regression model was used for analysis of stability data.

RESULTS

Accelerated stability study. According to the requirements, titer of the vaccines should not be less

than 10^3 CCID50/dose for each viruses after seven days incubation at 37 °C and the difference between exposed and unexposed samples should not be more than 10^{-1} CCID50/dose (WHO, 1994; Pharmacopoeia, 2012). All of the vaccines passed the thermo stability test. As shown in table 1, the reduction titer after incubation at 37 °C for all of the samples was not more than 1(-Log₁₀CCID50/dose) and the mean loss of activity was 0.375, 0.373 log₁₀ and 0.210 respectively for measles, mumps and rubella components.

Long-term stability study results

Potency test. According to WHO and Pharmacopoeia requirements, titer of MMR vaccine should not be less than 10^3 CCID50/dose for each virus as minimal protective titer (WHO, 1994; Pharmacopoeia, 2012). All of the three batches of vaccines met the specification during the 36 months after production in potency test. The measles, mumps and rubella components of MMR vaccine showed a mean loss of activity of 0.626, 0.50 and 0.46 log₁₀ respectively after 36 months storage. The potency results of three components of MMR vaccines in long-term stability study, has been shown in figure 1, 2 and 3.

Safety test. The animals (mouse and guinea pig in control and test groups) that injected with the vaccines were healthy and without any general and local reaction and any weight losses (met the specification of this test).

Table 1. Accelerated stability study in MMR vaccines

Vaccine	Potency	Accelerated Stability titre	Reduction titre
	(Mean Titer (-Log ₁₀ CCID50/dose))		
1	Measles	4.00	0.25
	Mumps	4.37	0.37
	Rubella	4.00	0.13
2	Measles	4.00	0.25
	Mumps	4.25	0.25
	Rubella	3.87	0.25
3	Measles	4.00	0.50
	Mumps	4.25	0.50
	Rubella	3.87	0.25

Table 2. Moisture content (% W /V) of MMR vaccines in long term stability study

Vaccine	0 th	3 th month	6 th month	9 th month	12 th month	15 th month	18 th month	21 th month	24 th month	27 th month	30 th month	33 th month	36 th month
1	1.384 ± 0.156	1.531 ± 0.148	1.690 ± 0.158	1.759 ± 0.162	1.820 ± 0.156	1.960 ± 0.159	2.038 ± 0.158	2.064 ± 0.149	2.065 ± 0.165	2.359 ± 0.164	2.563 ± 0.154	2.610 ± 0.151	2.694 ± 0.148
2	1.583 ± 0.158	1.623 ± 0.162	1.682 ± 0.168	1.802 ± 0.167	1.890 ± 0.156	1.945 ± 0.154	2.034 ± 0.168	2.048 ± 0.148	2.048 ± 0.151	2.198 ± 0.163	2.520 ± 0.168	2.650 ± 0.153	2.721 ± 0.156
3	1.419 ± 0.147	1.588 ± 0.148	1.612 ± 0.153	1.680 ± 0.149	1.720 ± 0.159	1.780 ± 0.159	1.890 ± 0.160	1.950 ± 0.163	1.990 ± 0.170	2.010 ± 0.168	2.221 ± 0.166	2.386 ± 0.169	2.521 ± 0.171

A=mean±SD

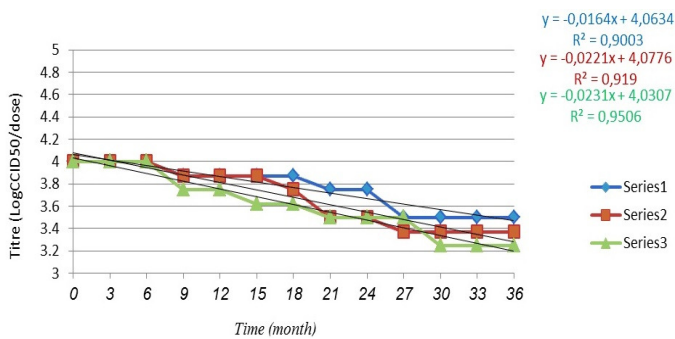


Figure 1. Linear regression fit of data for Measles Potency

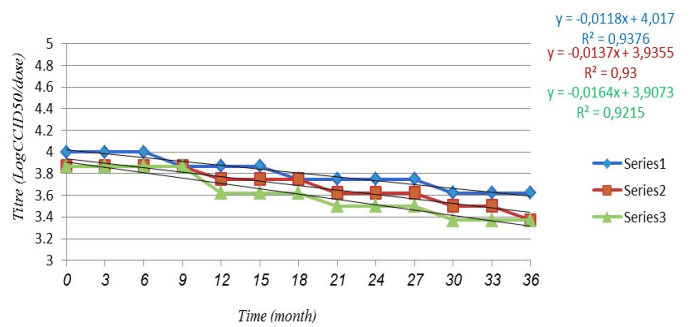


Figure 3. Linear regression fit of data for Rubella Potency

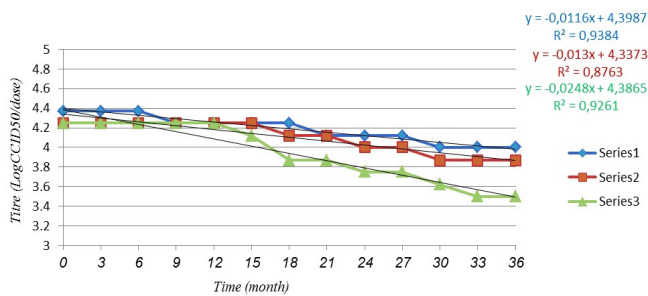


Figure 2. Linear regression fit of data for Mumps Potency

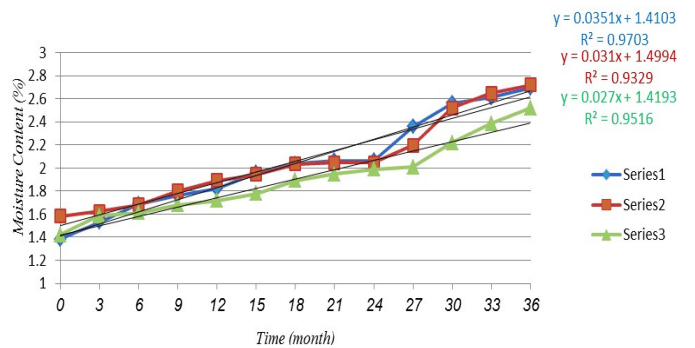


Figure 4. Linear regression fit of data from table 2 (Moisture Content)

Sterility and mycoplasma tests. All of the samples were free from bacterial (aerobic and anaerobic), fungal and mycoplasma agents during the study.

Physicochemical tests. All of the vaccines met the specification in each period of long-term study (The vaccines were lyophilized cream color and free of any visible particle after reconstitution, airtight, the labeling was readable and the vials had vacuum and soluble in water). In residual moisture content test, the mean increase of moisture content in the vaccines was 1.084 %. (The residual moisture of the vial should be less than 3 %) (Table 2 and figure 4).

Test Validation. All of the tests in this study were valid. In potency test (as the most important test) the difference between the geometric mean titer (GMT) and the corrected potency of the working reference preparation was less than 2SD (In limit of $\pm 2SD$) (SD was 0.136, 0.135 and 0.111 for measles, mumps and rubella respectively). Coefficient of variation (CV) for working reference preparation in this study was 4.01%, 3.71 and 2.98 for measles, mumps and rubella respectively (CV for standard WRP was 4.28%, 4.12 and 3.70).

DISCUSSION

Vaccines are combinations of components that are sensitive to environmental factors. In addition to changes in non biological ingredients of vaccines by circumferential factors, biological changes especially in live vaccine may be occurred. So, stability study of biological products plays an important role for determination of product changes in maintenance period, ensuring of safety and efficacy of vaccines and is the ability of a vaccine to retain its chemical, physical, microbiological and biological properties within specified limits throughout its shelf life. The purpose of stability study is to provide evidence on how the quality of a vaccine varies with time under the influence of a variety of environmental factors and to establish a re-test period or a shelf life and recommended storage conditions. During development, stability studies are done to assure product quality and

to obtain the data needed to support licensure. Stability studies may also be performed after licensure to ensure that product continues to perform as it did pre-licensure, as well as to evaluate the effect on product quality of deliberately introduced manufacturing changes (Schofield, 2009a). At each phase in the product life cycle, it is important to consider the goals of stability evaluation and to perform appropriate statistical analyses in order to assure and reach appropriate conclusions about product quality (Krause, 2009). Evaluation of stability is an essential part of the assessment of the vaccine quality. Indeed, the stability studies are aimed at verifying that the vaccines maintain their original quality criteria throughout their shelf lives (Socarras and Magari, 2009). Several factors effect stability of vaccines such as, stabilizer, heavy water (D₂O) (Der Yuan et al., 2000), lyophilization process, vials or tubes of vaccines, freeze thawing cycles, process and equipment used in production and the cold chain used for maintenance and transportation of vaccines. MMR vaccine is extremely stable between -70 °C and -20 °C. Reconstituted vaccine loses 50% potency in one hour at 20 to 25 °C and almost all its potency when it is held at 37 °C for 1 hour (Galazka et al., 1998). A set of data for the combined MMR vaccine was published in 1992 (Bishai et al., 1992), as seen it has over 2000 days shelf life at 10 °C, which is the same as that for measles alone. The vaccine is also sensitive to sun light however colored glass vials further minimize loss of potency (Plotkin et al., 2008; Schofield, 2009b). WHO considers measles to be the most sensitive vaccine to environmental situation specially temperature. Results of a study showed that cycling between 8 °C and 37 °C caused significant loss in potency of measles (Mann et al., 1983). In comparison of the data based on that reported by Allison et al. (1981), the first generation of measles vaccine only had a shelf life of 100 days at 10 °C, while the improved (Freeze drying techniques and additives) has a shelf life of 2000 to 3000 days at 10 °C (loss of 20% of the initial dose). According Shaayestehpour et al study (Shayestehpour et al., 2012) when the

reconstituted measles vaccine was incubated at 4 °C, 25 °C, and 37 °C, the titer loss per hour was equal to 0.05, 0.1 and 0.2 Log₁₀ CCID₅₀, respectively and the half-life of this vaccine at these temperatures was 5.31, 2.26, and 1.36 hours, respectively. Also the loss of potency for measles vaccine produced by AIK-C strain is 0.33 Log after storage at 37 °C for one week, while the reported amounts for commercial vaccines such as Mevilin-L, Attenuvax, Edmonston-Zagreb, and Rimevax are 0.7, 0.7, 1 and 0.78, respectively. Lyophilized and reconstituted vaccine containing AIK-C strain is more stable in comparison with Edmonston B, Schwartz, Biken-CAM, and Leningrad strains and the stability of the reconstituted AIK-C strain vaccine is similar to Moraten strain at 37 °C (Shayestehpour et al., 2012). McAleer et al study (McAleer et al., 1980) for mumps alone shows a shelf life of 700 days at 10 °C so it has a shorter shelf life, but lower temperature sensitivity. A study of the stability of the jerl lynn strain of mumps vaccine showed that the lyophilized vaccine could be stored for at least three years at -20 °C without significant loss of infectivity (Plotkin et al., 2008). Colinet et al. (1982) showed the calculated loss of activities at 4 °C were 0.000027 log₁₀ and 0.0004 log₁₀ per day for the measles and mumps components respectively. Expected falls in titer after storage at 4°C for 9 and 15 months were calculated from these values. The measles component would be expected to show a loss of activity of 0.07 log₁₀ after nine months storage and of 0.12 log₁₀ after 15 months. Over the same time period the titers of the mumps component, would be 0.11 log₁₀ and 0.18 log₁₀ lower than the original values (Colinet et al., 1982). Freeze dried monovalent rubella vaccine as well as the rubella component of bivalent vaccines (measles-rubella or mumps-rubella) or of trivalent MMR vaccine shows low degradation rates (Galazka et al., 1998). The results of McAleer et al. (1980) for Rubella vaccine indicate a shelf life of 2000 days at 10 °C, essentially the same as the improved measles vaccine, but rubella is less temperature sensitive and the rubella component seems to be more stable than the other components of combined virus

vaccines. The viability of rubella virus and the potency of rubella vaccine at 4 °C are also maintained for at least five years. At room temperature, there is significant loss after three months and at 37 °C, three week period is sufficient to damage vaccine potency (Plotkin et al., 2008). Long term stability is used to recommend storage conditions and to establish the shelf life and the release specifications (Philip, 2009). In this study, stability study performed for MMR vaccine with AIK-C, RS-12 and Takahashi strains for measles, mumps and rubella respectively. The result of potency test of the MMR vaccine in duration of the long-term stability study indicated, all of the three components of vaccines had titer more than 10³ CCID₅₀/dose (WHO vaccine specification) until the end of 36 month after production. The result of our study showed the comparison of three viruses in MMR vaccine. Determination of stability parameters should result in quantitative values with the detectable rate of change. The results indicated measles component had the most and the rubella component had the lowest titer reduction. The results of vaccines potency tests were conformed, by the raise rate of moisture content in vaccines at the end of study. Consideration of qualitative parameters was another point of this study. Results showed in qualitative parameters include safety, sterility, mycoplasma and physicochemical tests, all of the vaccines met WHO specifications. In addition to supporting release potency determination, accelerated stability studies may be used to support a strategy to recalculate product expiry after an unintended temperature excursion such as a cold storage unit failure or mishandling during transporting (Schofield, 2009a). As WHO mentioned, freeze dried vaccine such as MMR should retain at least 1000 live virus particles in each dose at the end of incubation at 37 °C for seven days and if during such process titer is decreased, then it will have done so by not more than 1 log₁₀. The result of accelerated stability in this study indicated all of vaccines had less than 1 log₁₀ reduction titer after incubation and met WHO accelerated stability specifications (WHO TRS 840). The use of

regression analysis provides incentive to properly design vaccine stability studies, while holding stability measurements to specification presents a disincentive from collecting valuable data. As shown in Figures 1, 2, and 3, there is suitable and logical degradation in titre of the MMR vaccine and there is not any significant difference between all of the three batches of the MMR in three components and all of the samples had similar linear regression. The result of moisture content confirmed potency of the vaccine in the stability study (Figure 4). This result indicated there is consistency and good stability in production of this MMR vaccine. So in comparison of previous studies, in this study all of the vaccine efficiency parameters include quantitative and qualitative parameters were evaluated in stability study periods in parallel, accelerated stability study was done, the stability of three routine vaccinal strains of MMR vaccine was compared and analyzed and the moisture content of vaccine was determined and compared with vaccine potency in all of the intervals test periods. Totally the results indicated MMR vaccines with AIK-C, RS-12 and Takahashi strains met WHO requirements for biological for at least three years in 2-8 °C and seven days at 37 °C.

All vaccines should be routinely stored at the temperatures recommended by manufacturers and national immunization programs. The cold chain remains a highly vulnerable point for these programs in developing countries with tropical climates. Developed countries with temperate climates can have similar problems. In all countries, systems of refrigeration, temperature-monitoring and record-keeping are required to make sure that each vial of vaccine is maintained under appropriate conditions and that it is used before the expiry date shown on the label.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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