Archives of Razi Institute, Vol. 76, No. 5 (2021) 1203-1211


DOI: 10.22092/ari.2021.352200.1550

***Original Article***

# Correlation Study of the Most Important Environmental Influencing Factors on the Razi MMR Vaccine

**Soleimani, S[1 *], Rashid, S[1]**

*1. Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), P.O. Box 31975-148, Karaj, Iran*

Received 12 October 2020 Accepted 16 March 2021
Corresponding Author: s.soleimani@rvsri.ac.ir

**Abstract**

Measles, mumps, and rubella (MMR) are among the most important viral infectious diseases in Iran and neighboring countries. After using a trivalent vaccine for these three diseases for a long time, in recent years, these diseases have been significantly controlled in Iran. One of the important points of storing the vaccine is that the vaccine strains are highly temperature-sensitive viruses. Due to tropical climatic conditions in Iran, the cold chain may not be achievable during the storage and transmission of the MMR vaccine. Therefore, the efficacy of the vaccine may be affected. This study aimed to evaluate the MMR vaccine potency at different temperatures (stress tests) and frequent light exposures. All quality control tests in the form of stability studies were performed on the samples from three consecutive batches produced during a full-scale Razi production. The samples were stored at 2-8, 22-25, 35-37, and 42-45°C in specific time intervals, exposed to frequent light, and underwent freezing/thawing conditions. According to the results, the storage of the vaccine at high temperatures caused a decrease in potency and increased moisture content in the vaccine vials. The best temperature for maintenance and transportation of MMR is 2-8°C. The time and frequency of light exposure may affect the vaccine potency. Based on the sensitivity of the vaccine strains to environmental conditions, the development of plans for storage and transportation of vaccines in different situations and training the vaccine injection staff seem necessary.

**Keywords:** Cold chain, MMR, Potency, Stability study, Vaccine

## 1. Introduction

Vaccines are very effective public health tools that save millions of lives annually, especially against major infectious diseases around the world. Measles, including childhood illnesses with invariable clinical symptoms, often occurs with middle-ear infection or bronchopneumonia. Mumps shows its effects by involving nerve tissues in children and young adults. Rubella is one of the childhood diseases the effects of which are often accompanied by some contingent symptoms, such as cutaneous rashes, as well as severe and persistent congenital defects.

Human immunization against measles, mumps, and rubella (MMR) is one of the programs pursued by the World Health Organization. In addition to the attenuated strains of these viruses, the live combined vaccine is made. Measles and rubella have been eliminated in Iran by Razi vaccines.

The vaccines are sensitive biological products that slowly lose their effectiveness over time (1, 2); otherwise, the loss of vaccine potency may be accelerated during the time (3). The affecting factors are storage and transportation temperatures, specific pH, light, stabilizer, and other factors which influence

their protective titers (4). Therefore, it is very important to store and transport them in proper conditions. Some vaccines (e.g., attenuated viral vaccines) have more sensitivity to the storage, transportation, and changes in conditions (5). Due to the difficulties in preserving standard storage conditions in developing countries, loss of vaccine potency occurs as a consequence of exposure to undesirable temperatures (6). Vaccine potency depends on a prescribed temperature range during distribution from manufacturing to using that is named "cold chain" which should be 2-8°C during transport and storage (7, 8). Therefore, changes over the standard temperature range affect the potency of vaccines (9).

Because of the lack of ice packs, freezers, and efficient transport subtractions, the cold chain under field situations is frequently challenging and is a critical point for the vaccination managers (10)

Since kinetics is used for vaccine degradation rate estimations, particularly through accelerated stability studies in which vaccines are not stored and transported according to the recommended conditions (11), the samples in this study were stored at 2-8, 22-25, 35-37, and 42-45°C. The overall objective of this study was the measurement and monitoring of the potency of vaccines at different storage conditions. Furthermore, the relationships between the vaccine potency and cold chain were determined in this study. It is worth mentioning that the vaccine distribution network observations at different transit levels, and the correlation of vaccine potency with the exposed temperature, freezing/thawing, and light were discussed in this study which would be of help to the vaccine program managers.

## 2. Materials and Methods

### 2.1. Sampling and Placing in Different Conditions

A total of 270 vials from three Razi MMR vaccine consecutive batches were sampled randomly. Subsequently, the first series of samples (30 vials from each batch) was stored at 2-8°C (the temperature of the refrigerator and the optimum temperature that was recommended by the manufacturer), 22-25°C (room temperature), 35-37°C (temperature of the semi-tropical regions, such as some regions in Iran), and 42-45°C (temperature of the tropical regions, such as many regions in Iran) for 2-60 days (2, 4, 7, 10, 14, 21, 30, and 60 days after exposure at 2-8, 22-25, 35-37, and 42-45°C, as well as 60 days at -20°C). The second series of samples was stored at 2-8°C and exposed to direct light (because of the sensitivity of these viruses to ambient light) for 1, 2, 3, 5, and 10 times (each turn for five min). The third series of samples was freezing/thawing three times. The validity of these temperatures was controlled by two-time determination in a day and rechecked by recording and controlling the temperature every one hour by a cool vision system.

### 2.2. Sterility and Mycoplasma Test

At the beginning and after each trial, for the detection of fungal contamination, as well as aerobic and anaerobic bacteria, each sample was cultured in brain heart infusion agar, tryptic soy broth, thioglycolate broth, and blood agar culture medium. Furthermore, the samples were cultured and subcultured in PPLO broth and PPLO agar, respectively, for mycoplasma detection.

### 2.3. Physicochemical Test

Physicochemical tests, including airtightness, appearance, labeling, and vacuum, as well as solubility grade, were conducted for the samples at the beginning and after each trial of the study. Consistency, color, lyophilized form, and visible particle after reconstitution were controlled in appearance.

### 2.4. Residual Moisture Content Test

Evaluation and estimation of residual moisture content in the vaccine were performed by the Carl Fischer method which is more accurate and faster than other methods.

### 2.5. Safety Test

To evaluate the side effects of vaccine samples, three guinea pigs and five mice were selected from each group. Following that, one and three doses of the sample, distilled water, and working reference preparation were injected intraperitoneally to samples, as well as negative

and positive control groups, respectively. During the next seven days, all of them were observed in terms of any local and general reactions with their weight.

### 2.6. Vaccine Potency Test

All of the samples at the beginning of the study and 2, 4, 7, 10, 14, 21, 30, and 60 days after exposure to 2-8, 22-25, 35-37, and 42-45°C, as well as 60 days at -20°C, after exposure to light for 1, 2, 3, 5, and 10 times were tested for potency using the standard protocol provided by WHO (12) on the cell culture. The cell culture concentration was equal to $2\times10^5$ cells/ml of Vero (Verda Reno) (ATCC, CCL-81) and RK13 (Rabbit Kidney) (ATCC, CCL-37) cell lines (13). The virus was inoculated by $10^{-2}$ to $10^{-5}$. Observations by an inverted microscope for the detection of cytopathic effects on cells in microplate were performed after 4-7 days.

The vaccine CCID50 was determined with an estimation of 50% endpoint by the Spearman-Karber method per dose (14). The geometric mean titer was also calculated in triplicate (13, 15).

### 2.7. Test Validation and Statistical Analysis

System suitability was controlled, and the potency of a vaccine standard was determined in parallel to test vaccines for test validation. The difference was $10^{0.27}$ CCID50/dose (less than $10^{0.5}$ on the base of requirements). Therefore, the assay validation was accepted (16). Promoting strategic sustainability studies based on appropriate statistical analysis reduces the uncertainty associated with estimating degradation. Statistical tools include the least square regression analysis that can be used to model potency decay (17). What reinforces the accuracy of this estimate is the testing at the beginning and end of this study. Therefore, in this study, the analysis of vaccine stress test data was performed using a linear regression model.

## 3. Results

### 3.1. Sterility and Mycoplasma Test

The absence of any fungal, bacterial (aerobic and anaerobic), and mycoplasma agents in all of the MMR vaccine samples at the beginning and after any stress situation was observed in this study.

### 3.2. Physicochemical Tests

The samples in this study met the specifications in each test section that included a lyophilized cream color, free from any visible particle after reconstitution, airtight, readable labeling, as well as positive vacuum and soluble in each vaccine challenge situation.

### 3.3. Residual Moisture Content Test

In the residual moisture content test, the mean increase values in moisture content in the vaccines at 22-25, 35-37, and 42-45°C were 1.135%, 1.907%, and 2.109%, respectively (Table 1). The linear regression fit of the data from table 1 was shown in figure 1.

**Table 1.** Moisture content tests of MMR vaccines in a long-term stability study

| Sample | Temp. | Times | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2 Days | 5 Days | 7 Days | 10 Days | 14 Days | 21 Days | 30 Days | 60 Days |
| A | 2-8 | 1.375±1.139 | 1.376±1.133 | 1.380±1.155 | 1.380±1.149 | 1.382±1.144 | 1.390±1.139 | 1.390±1.142 | 1.397±1.156 |
| | 22-25 | 1.382±1.141 | 1.385±1.139 | 1.385±1.137 | 1.391±1.127 | 1.390±1.158 | 1.394±1.152 | 2.221±1.156 | 2.480±1.156 |
| | 35-37 | 1.384±1.149 | 1.395±1.140 | 1.395±1.168 | 2.185±1.171 | 2.315±1.149 | 2.675±1.142 | 2.966±1.176 | 3.221±1.171 |
| | 42-45 | 1.391±1.144 | 1.411±1.139 | 1.525±1.138 | 2.386±1.166 | 2.544±1.171 | 2.881±1.144 | 3.283±1.176 | 3.462±1.68 |
| B | 2-8 | 1.395±1.142 | 1.395±1.131 | 1.390±1.149 | 1.389±1.169 | 1.403±1.129 | 1.403±1.151 | 1.459±1.171 | 1.612±1.168 |
| | 22-25 | 1.397±1.146 | 1.398±1.141 | 2.029±1.178 | 2.088±1.166 | 2.191±1.167 | 2.198±1.166 | 2.345±1.147 | 2.575±1.156 |
| | 35-37 | 1.396±1.131 | 1.399±1.148 | 2.115±1.148 | 2.385±1.148 | 2.474±1.148 | 2.688±1.148 | 2.997±1.148 | 3.373±1.148 |
| | 42-45 | 1.402±1.139 | 1.659±1.141 | 2.325±1.154 | 2.588±1.161 | 2.704±1.159 | 2.911±1.151 | 3.322±1.171 | 3.509.±1.177 |
| C | 2-8 | 1.377±1.146 | 1.377±1.140 | 1.379±1.146 | 1.380±1.164 | 1.383±1.177 | 1.382±1.117 | 1.390±1.147 | 1.396±1.148 |
| | 22-25 | 1.394±1.141 | 1.400±1.141 | 1.988±1.139 | 1.988±1.138 | 2.114±1.171 | 2.289±1.166 | 2.380±1.152 | 2.525±1.152 |
| | 35-37 | 1.394±1.129 | 1.419±1.140 | 1.881±1.139 | 2.419±1.133 | 2.575±1.114 | 2.681±1.121 | 3.000±1.162 | 3.301±1.160 |
| | 42-45 | 1.400±1.129 | 1.601±1.139 | 2.011±1.161 | 2.633±1.159 | 2.845±1.123 | 2.955±1.163 | 3.288±1.149 | 3.505±1.156 |

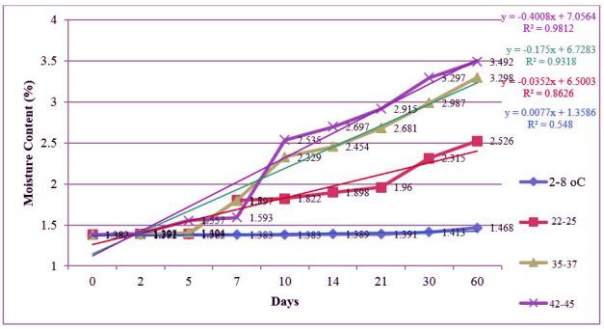


**Figure 1.** Linear regression fit of data for moisture content at different temperatures and time intervals

### 3.4. Safety Test

The animals in the control and experimental groups were injected with the vaccines, and they were healthy without any local and general reactions or weight loss.

### 3.5. Vaccine Potency Test

The result of the MMR vaccine potency test in different situations and times was shown in table 2 and 3. As shown in the table 2, at -20˚C, there was no reduction in the potency of the vaccine in test periods. The measles potency of the samples that were exposed to 22-25˚C until 14 days met the specifications, and after 60 days, it decreased to 2.37, 2.12, and <2.00 (log CCID50/dose) for each batch, respectively. The mumps potency of the samples exposed to 22-25˚C until 30 days met the specifications, and after 60 days, it decreased to 2.87 (log CCID50/dose ) for all three batches.

The rubella potency of the samples that were exposed to 22-25˚C until 21 days met the specifications, and after 60 days, it decreased to 2.73, 2.73, and 2.32 (log CCID50/dose) for each batch, respectively.

For the samples at 35-37˚C, the potency was decreased lower than that mentioned in the standard specification after 10 days for measles and rubella, and after 21 days for mumps. For the samples at 42-45˚C, the potency was decreased lower than that in the specification after 5, 7, and 14 days for measles, rubella, and mumps, respectively.

The linear regression fit of data from table 2 was shown in figures 2, 3, and 4. In addition, after 1, 2, 3, 5, and 10 times exposure to light, there was no change in the potency of the vaccines. Moreover, the potency of the samples that were frozen/thawed remained unchanged.

**Table 2.** Potency (log CCID50/dose) of MMR vaccine exposed to different temperatures in the study

| Sample | Initial Titer[a] | | | Temp. | Times | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 2 Days | | | 5 Days | | | 7 Days | | | 10 Days | | |
| | Me | Mu | Ru | | Measles | Mumps | Rubella | Measles | Mumps | Rubella | Measles | Mumps | Rubella | Measles | Mumps | Rubella |
| A | 3.45 | 4.70 | 3.70 | 2-8 | 3.45 | 4.70 | 3.70 | 3.45 | 4.70 | 3.70 | 3.45 | 4.70 | 3.70 | 3.45 | 4.70 | 3.70 |
| | | | | 22-25 | 3.32 | 4.45 | 3.45 | 3.32 | 4.45 | 3.45 | 3.32 | 4.45 | 3.45 | 3.32 | 4.45 | 3.45 |
| | | | | 35-37 | 3.20 | 4.20 | 3.20 | 3.00 | 4.00 | 3.00 | 3.00 | 4.00 | 3.00 | 2.87 | 3.70 | 2.87 |
| | | | | 42-45 | 3.00 | 4.20 | 3.00 | 2.87 | 3.87 | 2.87 | 2.50 | 3.45 | 2.87 | <2.00 | 3.32 | 2.50 |
| B | 3.45 | 4.45 | 3.70 | 2-8 | 3.45 | 4.45 | 3.70 | 3.45 | 4.45 | 3.70 | 3.45 | 4.45 | 3.70 | 3.45 | 4.45 | 3.70 |
| | | | | 22-25 | 3.32 | 4.45 | 3.45 | 3.32 | 4.32 | 3.32 | 3.32 | 4.20 | 3.32 | 3.20 | 4.20 | 3.12 |
| | | | | 35-37 | 3.20 | 4.20 | 3.20 | 3.20 | 4.00 | 3.00 | 3.00 | 4.00 | 3.00 | 2.87 | 3.45 | 2.87 |
| | | | | 42-45 | 3.20 | 4.20 | 3.20 | 3.00 | 3.70 | 3.00 | 2.50 | 3.45 | 2.87 | <2.00 | 3.20 | 2.32 |
| C | 3.45 | 4.45 | 4.00 | 2-8 | 3.45 | 4.45 | 4.00 | 3.45 | 4.45 | 4.00 | 3.45 | 4.45 | 4.00 | 3.45 | 4.45 | 4.00 |
| | | | | 22-25 | 3.32 | 4.45 | 4.00 | 3.32 | 4.45 | 3.70 | 3.20 | 4.20 | 3.45 | 3.20 | 4.20 | 3.12 |
| | | | | 35-37 | 3.20 | 4.20 | 3.70 | 3.20 | 4.00 | 3.32 | 3.00 | 4.00 | 3.00 | 2.87 | 3.87 | 2.87 |
| | | | | 42-45 | 3.00 | 4.20 | 3.70 | 2.87 | 3.70 | 3.20 | 2.32 | 3.45 | 3.00 | <2.00 | 3.20 | 2.70 |

a- Titer (Log 10)

**Table 3.** Potency (log CCID50/dose) of MMR vaccine exposed to light in the study (Continued)

| Sample | 5 Times | | | | 10 Times | | | |
|---|---|---|---|---|---|---|---|---|
| | **Measles** | **Mumps** | **Rubella** | **Moisture** | **Measles** | **Mumps** | **Rubella** | **Moisture** |
| **A** | 3.32[a] | 4.70 | 3.70 | 1.829±1.168 | 3.32 | 4.70 | 3.70 | 2.019±1.151 |
| **B** | 3.20 | 4.45 | 3.70 | 1.741±1.129 | 3.20 | 4.32 | 3.70 | 2.008±1.144 |
| **C** | 3.32 | 4.32 | 4.00 | 1.812±1.166 | 3.20 | 4.32 | 3.45 | 2.123<br>1.159 |

a- Titer (Log 10)

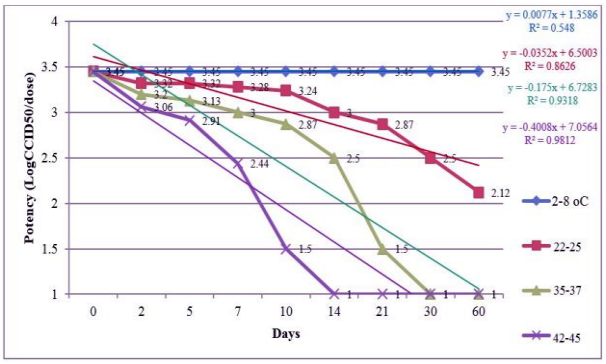


**Figure 2.** Linear regression fit of data for mean virus titer (log CCID50/dose) of Measles at different temperatures and time intervals.

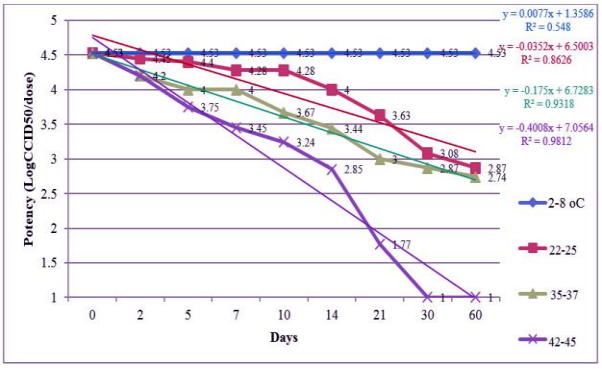


**Figure 3.** Linear regression fit of data for mean virus titer (log CCID50/dose) of Mumps at different temperatures and time intervals

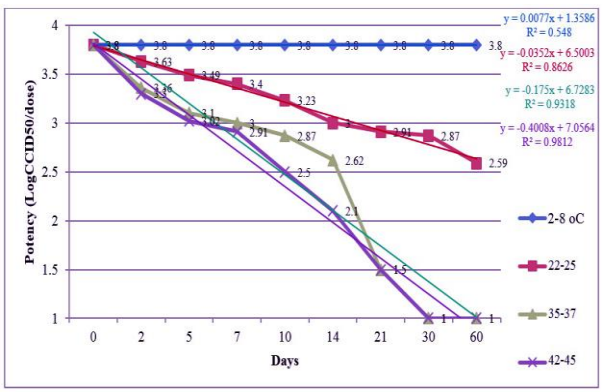


**Figure 4.** Linear regression fit of data for mean virus titer (log CCID50/dose) of Rubella at different temperatures and time intervals

## 4. Discussion

Due to the high sensitivity of vaccines, especially live viruses, to environmental factors, the vaccine storage and transport conditions must be considered to reserve the appropriate effects of the vaccine in the target population. Since environmental agents can affect vaccine components, variations in non-biological and even biological components, especially in live attenuated vaccines, can be possible. Accordingly, most vaccines must be kept at a low recommended temperature from production to use due to their sensitivity to high temperature (11). The proper definition of stability is the ability of a biological product to preserve biological, physical, microbiological, and chemical specifications in storage and transport that shows at the appointed time. Several effective factors, such as vaccine preservative, lyophilization process, vials or tubes, monitoring of the process, vaccine production, maintenance equipment, maintenance, transportation cold chain, temperature, freezing/thawing, pH out of physiological limits, organic solvents, some antiseptics and inactivating agents, and light affect vaccine stability (18, 19). A reduction in the potency of vaccines occurs during long-term maintenance, transit to health centers, and the period immediately before injection (5). Vaccines stability is of concern to the vaccine manufacturers, programmers, and health centers because of unreliable transportation and storage facilities (11). Better immunization and implementation of programs are related to the awareness of vaccine stability, which is possible by examining the properties that are related to safety, effectiveness, and measurement of time exposure to temperatures out of 2-8°C (20).

According to the guidelines provided by the WHO (21), the appropriate temperature throughout the in-country distribution chain for most vaccines is 2-8°C. Therefore, monitoring of the fragile cold chain and the methods used to protect vaccine quality from vaccine producer to the recipient was recommended (21). Preserving of vaccine potency by cold chain creation and maintenance during transportation seems to be more critical in developing countries, especially tropical countries, such as Iran, where the cold chain is usually not available and exposure of vaccine to unhealthy environmental conditions, including high temperatures, can be expected. Therefore, the cold chain can be a critical factor limiting the ability of the immunization program (22). A successful immunization program requires vaccines to be stored and transported properly along with good continuous monitoring of temperature that helps to detect the cold chain failure (22, 23).

A significant portion of vaccination failures results from the thermal (20) instability of many of the currently used vaccines (24).

It should be noted that during the storage process, loss of viral antigen integrity plays an important role in the stability of viral vaccines. Stability in live viral vaccines, such as MMR, is recognizable by investigating their infectious titers (19). As a result, the evaluation of factors affecting biological product stability is an important factor in the identification of vaccine changes in storage and transportation to ensure that they have a safe and effective vaccine.

The lyophilized measles vaccine is extremely potent at temperatures below zero. The lyophilized vaccine stays stable in the cold chain, and it does not hurt by freezing/thawing. Many MMR vaccine formulations currently fail to meet the minimum time requirement established by the WHO in a week at 37°C (25). The improved vaccines retain their minimum potency for more than two years when staying at an optimal temperature of 2-8°C. All three vaccines (measles, mumps, and rubella) have similar thermostability requirements according to what has been offered by the WHO. Additionally, in compound viral vaccines, rubella components are more stable than other components (20, 26). Analysis from the exposure of more than 30 measles vaccine samples to 37°C for one week showed that their stability, in this case, anticipated their life span for more than one year at 8°C (27).

MMR vaccine stability and thermostability have been the subject of many types of research over the past few decades (28-30). Recent studies have shown a failure to comply with recommended guidelines on the handling of vaccines in general and pediatrics population, as well as community clinics (7).

In a study of two second-generation measles vaccines to evaluate their potency, both of them were placed at 37°C, one for 14 days, and the lyophilized form for 21 days. The first one can induce seroconversion in seronegative children. Evidence has shown that the un-reconstituted Merck measles vaccine can maintain its potency for eight months at room temperatures and four weeks at 37°C (29). The vaccine is inactivated rapidly at 54-56°C, losing more than 0.65 and 1.3 log10 during one- and three-day exposures, respectively (20). The stability of both components of the lyophilized measles-mumps vaccine at 4, 23, 37, and 45°C are similar. For both components at 37°C, the degradation rate is about 0.01 log10 per day. Half-lives are also similar for the measles and mumps components (4.7 and 5.4 days at 45°C; 12 and 13 days at 37°C; as well as 71 and 65 days at 23°C, respectively).

The mumps component in MMR vaccines from one manufacturer shows good stability at 37°C for up to 21 days. During a 30-day exposure to 37°C, the half-lives of mumps component were about 10 days and lost 0.9 log10 (i.e., about 0.03 log10) per day. The lyophilized monovalent rubella vaccine and the rubella component of measles-rubella, mumps-rubella, and MMR vaccines show low degradation rates. The average loss ranges from 0.046 to 0.109 log10 CCID50 per week at 37°C (20). Samant et al. investigated the cold chain integrity in India's rural districts (10). The aforementioned study indicated that defective stabilizers and electricity plugs

and sockets were the reasons for the failure in many cases (31).

In this study, the effects of the most influential factors on the measles, mumps, and rubella viruses in MMR vaccine stability were evaluated. The study also evaluated the stress tests on the vaccine potency and assessed the testing of the vaccine for measles, mumps, and rubella virus potency separately based on the standard WHO (12) protocol. In addition to quantitative parameters, the qualitative parameter includes physicochemical tests, sterility, and mycoplasma that could also be considered.

The results of this study and the usable recommendations can be utilized in transportation and storage conditions to establish the shelf life and release specifications in an inappropriate and stressful situation. Moreover, the findings can be used to assess the product suitability throughout the shelf life and establish the quantitative values by the detectable grade of change. The cold chain and its failure at different levels of the vaccine distribution networks, as well as the correlation between light and MMR potency, were discussed in this study which would be of help to the vaccine program teams at different levels of storage, transport, and health center.

The geometric mean infectious virus titer must equal or exceed the required minimum of infective units per human dose (3 log10) and must not be decreased by more than 1 log10 infective units (20). The results of the MMR vaccine potency test at the recommended temperature (2-8˚C) (refrigerator) and -20˚C showed that all of the samples met the WHO measles, mumps, and rubella vaccine specifications, and the composite titer estimation was sufficient to assess the thermal stability. This has been proven in previous studies until 36 months. In addition, the results indicated that this vaccine does not need to be stored at -20˚C. The situation of the vaccines kept at room temperature (22-25˚C) is different; accordingly, the potency drops below the specification after two weeks, three weeks, and 30 days for measles, mumps, and rubella, respectively.

At 35-37˚C, after 10 days for measles and rubella and 21 days for mumps, the result of potency was not acceptable. At 42-45˚C, only after five days for measles and rubella, and after two weeks for mumps, the potency was not acceptable. Furthermore, the residual moisture of the samples stored at 22-25, 35-37, and 42-45˚C increased over time; however, after 60 days, it was still in the specification range.

The results of this study indicated that this vaccine was stable for 14 days at 22-25˚C, <10 days at 33-37˚C, and <5 days at 42-45˚C. In another part of the study that examined the effect of light on the vaccine, there were not any major changes in the vaccine after exposure to light 10 times. Despite the susceptibility of these viruses to light, the vaccine's effectiveness in light is because the vaccine is packed in dark vials.

The potency of the samples in freezing/thawing remained unchanged; accordingly, it appears that this item does not affect the vaccine's effectiveness due to the lyophilization process on vaccine vials.

The results of the other tests of the MMR vaccine three batches showed that all of the vaccines in sterility, mycoplasma, and physicochemical tests met the WHO (12) specifications at the beginning and the end of this study. Therefore, this result indicated the good condition in the production of this vaccine in the Iran Razi Institute.

Statistical tools prepared an incentive to properly design the vaccine stability investigations. The attainment of knowledge on the degradation principles and the statistical tools for evaluating vaccine stability is essential for product quality management. As shown in the tables, there is no significant variation among the three MMR batches, and the vaccines had similar regressions. As a result, there were consistencies in the vaccine production, and the validation of the tests used

in this study was confirmed.

Considering the results of this study, the following concepts should be considered by vaccine program managers:

1. Since three viruses in the MMR vaccine are highly thermolabile, the recommended temperature as a cold chain is necessary for the storage and transport of this vaccine.

2. The best temperature for the maintenance and transportation of MMR is 2-8°C.

3. The time of this vaccine exposure to higher temperatures is very important (the exact times are discussed in the discussion); therefore, to decide whether to use or not to use the vaccine should be considered.

4. The light did not affect the efficacy of these vaccines under the conditions of this study; however, if the time and frequency of light exposure increases, they may be affected.

5. Based on the sensitivity of the vaccine to environmental conditions, the development of plans for storage and transportation of vaccines in different situations and vaccine injection sites, as well as training are necessary for vaccination teams by vaccine manufacturers.

## Authors' Contribution

Study concept and design: S. S.
Acquisition of data: S. R.
Analysis and interpretation of data: S. S.
Drafting of the manuscript: S. R.
Critical revision of the manuscript for important intellectual content: S. S.
Statistical analysis: S. S.
Administrative, technical, and material support: S. S.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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