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Research Article

Stability of lyophilized measles vaccine: Stabilizers and Temperature parameters

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Abstract: The effectiveness of live measles vaccine is based on numerous factors like complete attenuation of the vaccine virus, appropriate stabilizer, pH, exposure to light, and also on the maintenance of cold chain (temperature) till the time vaccine is injected to the end-users which are normally infants in case of Measles. The purpose of the present study was the estimation of the thermal stability of the vaccine using three different types of stabilizers for lyophilization. All batches were prepared using different types (ST, SH & SG) of stabilizer and tested in triplicate against reference according to WHO's method of Spearman-Karber for calculation of vaccine titer. Potency and accelerated stability were carried out by incubating each of the three vaccines at 2-4°C and 37°C for seven days. For calculation of potency drop at increasing temperatures, titer was calculated for consecutive fourteen days at 24 hours interval, three vials from each vaccine type were incubated at 4°C, 25°C, 37°C, and 41°C. The potency observed was between log 103.64 to 104.89. Thus, making them fall within the WHO laid down criteria. These potency results also demonstrated that ST based stabilizer was comparatively more stable among the three. It also verified that vaccine remains stable for 14 days at 37°C but rapidly loses potency at 41°C even after 4 days. This study verifies that regardless of external hard weather conditions (e.g., Pakistan), maintenance of manufacturer recommended storage temperature and effective use of proper stabilizer is the only way to ensure the stability of the measles vaccine.

Keywords: Measles virus, Potency, Stabilizers, Quality control, Heat-stable, Storage

1. INTRODUCTION

The measles vaccine is a delicate and complex biological preparation that provides sufficient protection against this deadly disease worldwide. Personnel must involve in vaccine production, testing, transportation, storage, and administrating the vaccine and diluent must know the required recommended temperature and they must also be aware of the fact that reconstituted measles vaccine swiftly loses the potency at room temperature [1].

Those unvaccinated against the disease are at risk of severe health complications such as pneumonia, diarrhea, and encephalitis (a dangerous infection of the brain causing inflammation) and blindness [2, 3]. Even in a developed country like America from January 1st, 2019 to April 26th, 2019 total of 704 positive measles cases were reported which is highest since 1994 [4]. In 2017 total of 172939 positive measles cases were reported to WHO. It was highest in recent times. Four out of six WHO regions have shown an increase in the prevalence of measles. In 2017 it caused about 110000 deaths worldwide. 63% (626289) of total reported cases to WHO during the period of 2013-17 were categorized as programmatically preventable causes [5].

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Pakistan, one of the developing countries and situated in a tropical region with varied temperatures has lost hundreds of lives to measles. Various outbreaks and reported deaths took place in many different regions of Pakistan in recent years. In 2017 Pakistan reported 6780 cases of measles and in 2018 total of 32921 cases of measles were reported to WHO and measles became a problem for health care agencies, authorities, and common people. As a result, Pakistan started emergency measles immunization campaign nationwide for every child below 02 years of age and till June 2019 Pakistan reported 637 number of measles positive cases to WHO [6].

WHO has taken several important and mandatory regulations to reinforce national regulatory authorities and vaccine manufacturers to uphold the quality of vaccines to be used in immunization programs. Several international standards are developed by the WHO, to be fulfilled at every step until the injection of the vaccine to the end customer [7,8]. Steps like changes in vaccine formulation, freeze-drying of final product, improvement in storage, and transportation conditions are few to mention [9]. The heat stability of the freeze-dried measles vaccine has made a substantial effect on the quality of measles vaccines in the market [10].

The increased heat-stability under normal working conditions is particularly vital in the developing world. The use of appropriate stabilizers preserve the effectiveness of the vaccine by ensuring the stability of antigen and it also prevents the components of vaccine from clinging to the inner side of the vial [11, 12].

WHO has established the following obligatory criteria for the calculation of virus titre [13].

i. The freeze-dried vaccine must retain at least 1000 live virus particles in each human dose at the end of incubation at 37°C for seven days; and
ii. If during incubation, the virus titre has been decreased, then it shall have done so by not more than 1 log 10.

In Pakistan, NIH Islamabad is the only Public Sector Organization that is producing Basic Measles Vaccine. This study was carried out to know the thermal stability of the measles vaccine formulated with different types of stabilizers.

2. MATERIALS AND METHODS

Measles vaccine batches (using three types of stabilizers) were prepared and obtained from Measles Vaccine Production Laboratory (MVPL) of BPD, NIH using Edmonston-Zagreb strain. The measles vaccine virus was propagated in Vero cells which were maintained in Dulbecco's Modified Eagle Medium (DMEM) Gibco, to achieve high titer. All excipients were from Sigma-Aldrich, Trehalose, and D-sucrose from Ferro Pfanstiehl Laboratories, Mannitol, calcium chloride, phosphate, glutamate, sodium citrate from Fisher Chemicals, gelatin from GELITA. Human Albumin from CSL Behring GmbH, myoinositol (Sigma–Aldrich), fetal bovine serum and other tissue culture reagents, plastic and glassware of M/s Sigma, M/s Gibco, M/s Nunc were used. Vero cell line (CCL-81) used in the study was acquired from ATCC, USA. All these batches were tested for stability at elevated temperatures.

2.1 Formulation

Each batch was formulated using the 150µl of measles virus cell culture, which was mixed with 150µl of stabilizer (each type namely, Trehalose, human serum albumin, and sucrose- gelatin) to obtain the final concentration using the WHO method of measles vaccine production [13]. Each batch was designated after its stabilizers like ST-Trehalose stabilizer, SH for Human serum albumin, and SG for the gelatin-based stabilizer.

All solutions including cells, virus pools, and fetal bovine, human albumin were tested for the detection of adventitious agents, All Quality Control tested and released solutions were used for the formulation of final bulk. After release from quality control, all three bulks were filled and lyophilized as per the routine set procedure of NIH.

2.2 Residual moisture and pH

Five vials from each formulation were selected randomly for moisture testing using the Karl-fisher method [13]. The procedure was performed on the final product after lyophilization. pH was

determined at two stages one at the final bulk stage before filling and one at the final product stage i.e., after lyophilization.

2.3 Potency and Thermostability of Vaccines

At least three vials of each freeze-dried batch were reconstituted with diluents (Water for Injection) supplied with the vaccine and were incubated at -20°C & 37°C for seven days. Real-time potency and accelerated stability were calculated using the $CCID_{50}$ method of WHO [13] against the reference vaccine.

2.4 Stability of Vaccines

Fifteen vials of each batch were incubated at -00oC, at 4°C, at 25°C, at 37°C, and 41°C earlier for 7 days. All these samples were run parallel against the reference standard vaccine. To ensure the protection of laboratory persons, products, and the surrounding environment the procedure was carried out in Class IIA2 biological safety cabinet using proper PPE (personal protective equipment).

The Spearman-Karber formula was used for calculation of titer and was expressed in form of Cell culture infectious dose 50% ($CCID_{50}$) per human dose (virus dilution required to cause infection in 50% inoculated in cells) [13].

One vial from each incubation temperature i.e., 0°C, 4°C, 25°C, 37°C and at 41°C was taken out at 24h bases starting from 00 days to 14 days and was stored at -70°C till the test stage[14].

3. RESULTS AND DISCUSSION

In the present study measles vaccine batches constituting three different stabilizers were tested for stability and thermostability. The test was performed using the WHO method for potency testing of the Measles vaccine [13].

3.1 Residual Moisture and pH of Measles Vaccine

The highest and lowest values of residual moisture were seen in ST and SG vaccines. During these studies, it was noted that there was a trivial reduction in pH after lyophilization in all three vaccines. WHO has recommended the residual moisture must be lower than 3% in the measles vaccine.

3.2 Potency and Thermostability of Vaccines

The highest loss in titer was observed in the SH vaccine while the ST vaccine showed only 0.41% after exposure at elevated temperature of 37°C for one week as depicted in Fig 1.

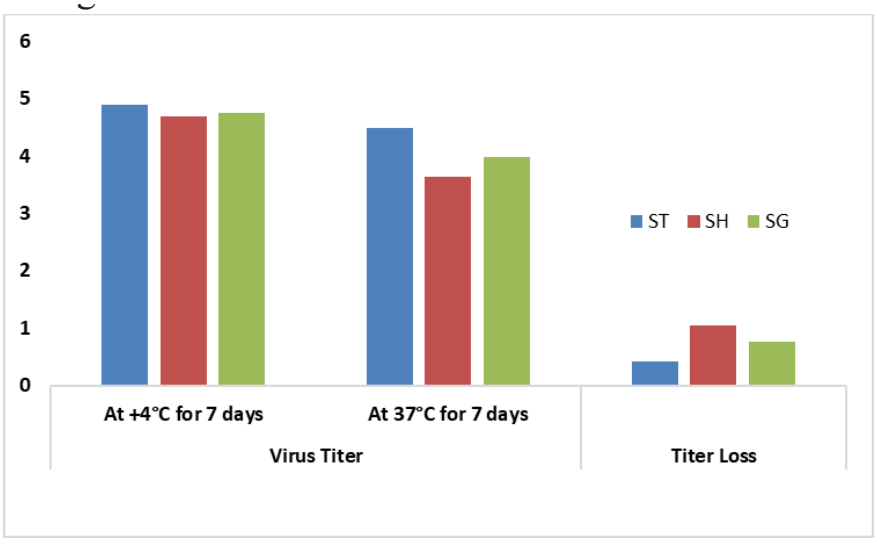

Fig 1. Potency and thermostability of lyophilized vaccines

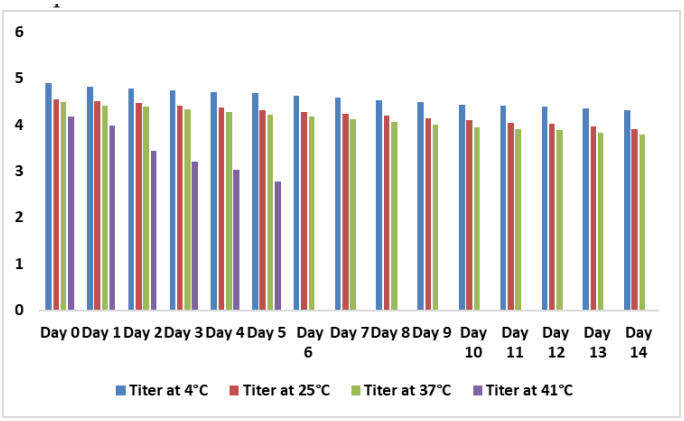

Fig 2. Virus titer in ST vaccine for 14 days incubation

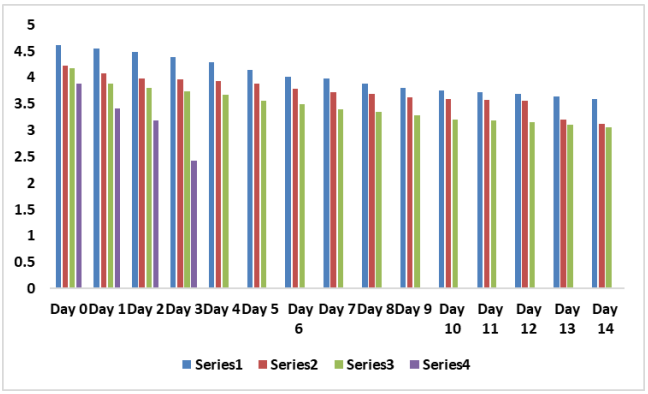

Fig 3. Virus titer in SH vaccine for 14 days incubation

3.3 Stability of measles vaccine at different temperatures in 14 days

Figures 2-4 show that vaccine virus titer was reduced at 24 hourly bases in 14 days with an increase in temperature. ST showed comparatively better results as compared to the other two vaccines. Results of all these three vaccines showed that at 41°C there is a sharp decrease in potency titer and titer reached below log 3 in ST on day 6 while in SG and SH the potency titer below log 3 reached on 4th day of incubation. Figure 5 reveals that vaccine virus titer and temperature are inversely proportional to each other, higher the temperature lower is the titer of the vaccine From these results we saw that rate of decrease in titer is slow in the ST vaccine at 4°C as compared to the other two vaccines. At 25°C the rate of titer drop is in ST and SG is better than in SH and 37°C the temperature drop in ST was only 0.70 as compared to the other two vaccines.

To maintain the efficacy and potency of vaccine heat stability at high temperatures is very important because in conditions where the cold chain cannot be maintained for some unforeseen situation potent vaccine can still be administered and immunization can be provided to the end-user [15, 16].

The effect of temperature on measles was studied by several scientists in the world [17, 18] these studies showed that effective immunization can only be achieved when the potency, stability, and safety of vaccines are properly maintained. Exposure of vaccines at elevated temperatures causes a significant reduction in potency of vaccines. Lyophilization is an effective method for stabilizing the vaccine and is being used the world over for vaccines, serums, and cultures to avert the inactivation of the virus [18, 19]. The freeze-dried measles vaccine is extremely effective and stable and maintains its potency for two years when kept at subzero temperatures [20, 21]

In the present study, three different stabilizers namely Trehalose based, human albumin-based, and gelatin-based are used for vaccine lyophilization. Residual moisture is an important test to determine the stability of the lyophilized product because it has an impact on the infectivity titer of the freeze-dried vaccine. As per WHO recommendations residual moisture must be lower than 3% in the measles vaccine [12, 13]. All these three stabilizers produced vaccines with residual moisture lower than 3% thus meeting the WHO requirement.

The stability test estimates the effectiveness of vaccines at different temperatures and storage conditions. Accelerated stability measures the efficacy of vaccines at elevated temperatures. The vaccine is incubated at 37°C for 7 days before testing [19]. In our study ST stabilizer was most effective as a reduction in potency was only 0.41 after exposure at 37°C for one week. A vaccine made with Trehalose stabilizer showed a minimum reduction in potency at all four temperatures as compared to the other two stabilizers. Trehalose dehydrates also preserve the infectivity of live attenuated Peste des Petits [22] It was also proved in regression analysis of stability data of mumps, measles vaccine showed that the vaccine formulated with Trehalose stabilizer gave better results [23].

When the vaccine is kept in refrigeration in the lyophilized form it retains its shelf life for two years but when it is exposed at 25°C it maintain its shelf life for one month, at 37°C freeze-dried measles vaccine keep its full immunogenic activity for 7 to 14 days [24, 25] but when the vaccine is exposed at an extremely hot temperature like 41°C it retains its effectiveness only from 3-7 days [17]. After reconstitution, the measles vaccine rapidly loses 50% of its potency within one hour at 20-25°C. At a temperature of 26°C, the vaccine reduces in titre to the minimum level in about 16 hours and at 37°C, it loses almost all its potency in one hour. Reconstituted measles vaccine should be kept cold during immunization procedures, must be discarded at the end of each immunization session, and must never be kept for use in subsequent sessions. Scientists have also shown that with improved techniques freeze-dried measles vaccine retains its potency up to 8 weeks after exposure to 37°C [22].

In the present study vaccine made with ST stabilizer revealed comparatively better results. When titer was calculated at different temperatures it showed less potency drop, so relatively it is more suitable for measles vaccine production [18]. MMR is recommended world over by WHO and CDC for the prevention of measles, mumps, and rubella [23]. Understandably, the measles vaccine used in Pakistan is of standard quality, but the more heat-stable vaccine can be manufactured as reported [25] claimed that the vaccine retained its claimed potency for 8 weeks when kept at 37°C.

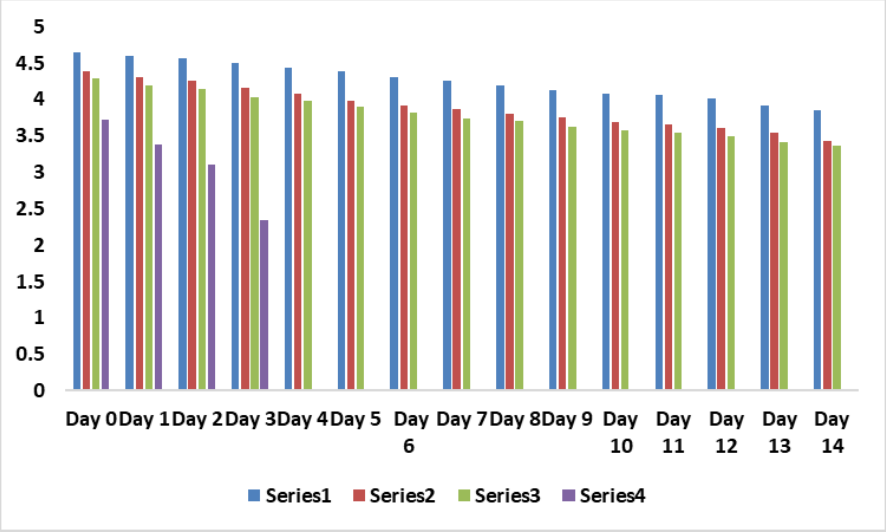

Fig 4. Virus titer in SG vaccine for 14 days incubation

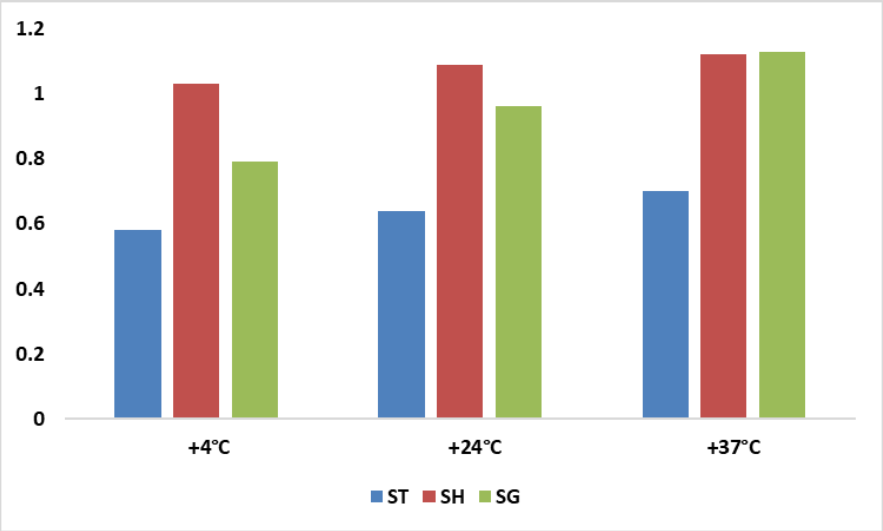

Fig 5. The average drop in virus titer at all four temperatures

4. CONCLUSIONS

The measles vaccine in its lyophilized form is highly stable. The use of appropriate stabilizers and retention of manufacturer-recommended temperature can result in more heat-stable measles vaccine to be used for the immunization programs of Pakistan where even today Government is facing major challenges due to the sprouting of measles cases repeatedly.

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