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Evaluation of the thermal stability of live-attenuated Rubella vaccine (Takahashi strain) formulated and lyophilized in different stabilizers

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ABSTRACT

Live attenuated viral vaccines are difficult to handle and often sensitive to temperature. The viral titer may drop during the processing and storing stage, especially at high temperatures. Using live attenuated viral vaccines successfully depends on keeping the sufficient potency required for an immune response. Although freeze-drying makes the vaccine more stable, in the absence of appropriate stabilizer the process may affect the structure and viability of the viruses. Therefore, the formulation of vaccine by means of an appropriate stabilizer plays a crucial role in the stability of viral structure and potency of the vaccine. This study aimed to evaluate the effect of two new stabilizers, including a Trehalose-based stabilizer (T) and a stabilizer containing sucrose, human serum albumin and sorbitol (S) on the thermal stability of lyophilized live-attenuated Rubella virus (Takahashi strain). Two Rubella vaccines were formulated using different stabilizers and were lyophilized. The potency of produced vaccines was investigated using accelerated stability test. To determine the pattern of thermal stability of reconstituted vaccines in 24 h, incubating at three different temperatures and continuous sampling was also included in this study. The viral titer was calculated by $TCID_{50}$ method. The regression analysis revealed that T vaccine found the sufficient stability compared to commercial Rubella vaccine containing a gelatin-based (G) stabilizer.

1. Introduction

Rubella virus (family Togaviridae, genus Rubivirus) causes Rubella disease which is an acute infection. The virus spreads from person to another through droplets. The disease has a mild clinical course characterized by low-grade fever, a short-lived morbilliform rash, and lymphadenopathy (Zanga et al., 2017). The pregnant women are mostly at risk of rubella virus (Shokri et al., 2013). Rubella infection in the first trimester may cause congenital rubella syndrome (CRS) or even fetus death in the first trimester. CRS has serious consequences such as miscarriage, stillbirth and severe birth defects in infants (Herini et al., 2017; Mori et al., 2017).

Rubella infection could be prevented by active immunization using live attenuated vaccines. In 1969, a live attenuated rubella virus strain was licensed. In the early 1970s, a combined vaccine containing attenuated measles virus, attenuated mumps virus and attenuated rubella virus (MMR) was introduced (Plotkin, 2006). Takahashi strain is used in rubella and MMR vaccines in Razi Serum and Vaccine Institute (RVSRI).

The ability of live attenuated viruses for immune system stimulation could be influenced by several factors such as temperature, pH, vaccine formulation, exposure to light, freezing-thawing and inactivating agents (Hansen et al., 2015). Changes in vaccine formulation and preparing the finished product in Freeze-dried (lyophilized) form are among measures which improves thermal stability and preserves vaccine potency during production and transport, particularly when cold chain is difficult to meet (Cardoso et al., 2017; Colinet et al., 1982; Tang et al., 2005).

To produce more stable live viral vaccines, the vaccine manufacturing companies try to improve the thermal stability of vaccines by making changes in the vaccine formulations (Hill et al., 2016). Previous researches showed that heat stability in other live viruses depends on the components of applied stabilizers (Jamil et al., 2014; Riyesh et al., 2011; Shayestehpour et al., 2012). Main ingredients of those stabilizers were trehalose, hydrolyzed gelatin, sucrose, sorbitol, sodium phosphate, potassium phosphate, glycine, monosodium glutamate and

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bovine serum albumin (Jamil et al., 2014; Kamali Jamil et al., 2017). Suitable stabilizers not only help preserve a vaccine's effectiveness by keeping the antigen and other components of vaccine stable during the storing process, but also prevent the components of vaccine from adhering to the vaccine vial inner side (Uchimoto et al., 2011).

The aim of this study was to investigate the thermal stability of live attenuated rubella viruses (Takahashi strain) formulated in new stabilizers and lyophilized under a routine freeze-drying program.

2. Materials and methods

2.1. Virus culture

Rubella virus, Takahashi strain, was used for preparation of Rubella vaccines that obtained from the RVSRI (10^{5} $TCID50_{/ml}$ as per relevant certificate of analysis). The virus was propagated on human diploid (MRC-5) cell line. MRC-5 cells were cultivated using Dulbeco's Modified Eagle's Medium (DMEM, Gibco BRL) containing kanamycin–neomycin (Biosera, United Kingdom) as bactericidal agents in 175 cm^2 flasks. The medium was supplemented with 5% fetal bovine serum (FBS, Gibco BRL). Monolayer cells were washed with phosphate-buffered saline (PBS) and inoculated with Rubella virus at a 0.1 multiplicity of infection (MOI) at room temperature for one hour. Fresh medium was added to the inoculated flasks and incubated at 33 °C for 7 days. Two flasks were left un-inoculated for controls. All flasks were observed daily using inverted light microscope for development of any cytopathic effect (CPE). The cell supernatants were harvested on day 7, filtered through a 0.22 µm membrane filter and stored at −70 °C until the stage of formulation. Samples were taken from harvested viruses and tested for virus potency and sterility.

2.2. Preparation of stabilizers

Three different types of stabilizers were prepared as below:

Stabilizer (T): 111 g/l trehalose dihydrate, 111 g/l hydrolyzed gelatin, 1.2 g/l KH2 PO4, 88.87 g/l sodium glutamate and 5.59 g/l Na2HPO4 in water.

Stabilizer (S): 1.24 g/l human serum albumin, 60.0 g/l sucrose, 70.2 g/l sorbitol, 10.59 g/l Na2HPO4, 40.2 g/l hydrolyzed gelatin, 0.88 g/l sodium bicarbonate and 5.07 g/l NaCl in water.

The gelatin-based stabilizer (G) was provided by the RVSRI.

All stabilizers were filter-sterilized immediately after being prepared and dispensed in sterile bottles.

2.3. Formulation and freeze-drying

To make the final bulks, harvested viruses were mixed with the same volumes as the prepared stabilizers separately. Final bulks were dispensed into vaccine vials (1 ml per vial) and lyophilized through a routine procedure. At the end of lyophilization cycle, vaccine vials were sealed and stored at −70 °C.

2.4. Solubility test

Three randomly selected lyophilized vaccine vials from each formulation were reconstituted in 1 ml sterile distilled water and the quality of solubility was checked visually.

2.5. Determination of residual moisture

Residual moisture of five randomly selected lyophilized vaccine vials from each formulation were determined using Karl-Fisher method to determine very low amount of water in a samples (May et al., 1982). In previous studies, this method has been used to determine the residual moisture in other vaccines (Shahkarami et al., 2009).

2.6. Measurement of pH

At the time of formulation, a sample was taken and the pH was determined. Following lyophilization, three randomly selected lyophilized vaccine vials were reconstituted in 1 ml sterile distilled water, then immediately pH was determined. Comparison of these two pH values is a useful way to reveal the effect of lyophilization process on the pH of the product.

2.7. Virus titration

Tissue Culture Infectious Dose 50% ($TCID_{50}$) assay was used for virus titration and stability testing (Reed and Muench, 1938). RK-13 cells were prepared in cell culture tubes and used to determine TCID50 infectivity end points. 10-fold serial dilutions (10^{-1} to 10^{-8}) of reconstituted vaccine were prepared in DMEM medium containing 5% FBS. 0.1 ml of each dilution was inoculated to four tubes, after one hour incubation at room temperature media were added and the tubes were kept at 37 °C for 7 days. Multiplication of Rubella virus was evaluated by Interference assay using Vesicular Stomatitis Virus and viral titers were calculated using Spearman-Karber formula (Ramakrishnan, 2016).

2.8. Vaccine thermostability test

Thermostability of prepared vaccines were measured using accelerated stability test. Three randomly selected lyophilized vaccine vials from each formulation were incubated at 37 °C (also the same quantity at −70 °C for further comparison) for 7 days, then tested for viral titer using $TCID_{50}$ method. Based on WHO instructions and previous experiments (Galazka et al., 1998; Shahkarami et al., 2009), the stability of vaccines was determined through the extent of reduction in titer of viruses in vaccines that had been kept at 37 °C compared to those kept at −70 °C.

2.9. Stability of reconstituted vaccine

Seven randomly selected lyophilized vaccine vials from each formulation were reconstituted in 1 ml sterile distilled water and incubated at 4 °C, 25 °C and 37 °C. One vial was taken from each formulation/temperature at 0, 4, 8, 12, 16, 20 and 24 h from incubation and immediately were stored at −70 °C until the titration stage (Jamil et al., 2014).

2.10. Sterility test

Samples taken before and after lyophilization process were tested for bacterial and fungal contamination using fluid Thioglycolate and Soybean-Casein Digest broth, respectively. Inoculated tubes were observed for 14 days and checked for any contamination.

2.11. Statistical evaluation

The statistical significance of all stability test data was determined using Sigma Plot 11 and R 3.5.0 software and Wald Statistic, Regression and Z-test have been used.

3. Results

3.1. Residual moisture and pH of prepared Rubella vaccines

Residual moisture and pH of prepared vaccines are summarized in Table 1. The highest and lowest residue of moisture were seen in S and T vaccines, respectively. Slight decrease in pH (0.2–0.3) were seen in all three vaccines following lyophilization.

Table 1
Residual moisture and pH of the live-attenuated rubella vaccines before and after lyophilization.

Vaccine	Residual moisture (%)	pH	
		Before lyophilization	After lyophilization
S[a]	2.9 ± 0.2	7.1 ± 0.36	6.8 ± 0.26
T[b]	1.3 ± 0.35	7.2 ± 0.16	7.0 ± 0.31
G[c]	1.5 ± 0.19	7.0 ± 0.24	6.8 ± 0.18

[a] Vaccine which is formulated using stabilizer S.
[b] Vaccine which is formulated using stabilizer T.
[c] Vaccine which is formulated using stabilizer G.

Table 2
Rubella vaccine titers before and after lyophilization.

Vaccine	Virus titer ($log_{/ml}$)		Titer loss
	Before lyophilization (Final balks)	After lyophilization (vaccines)	
S[a]	6.0 ± 0.32	5.21 ± 35	0.79
T[b]	6.5 ± 28	6.0 ± 30	0.5
G[c]	6.5 ± 30	6.0 ± 25	0.5

[a] Vaccine which is formulated using stabilizer S.
[b] Vaccine which is formulated using stabilizer T.
[c] Vaccine which is formulated using stabilizer G.

3.2. Potency and thermostability of the lyophilized vaccines

The effect of lyophilization process on the viral titer and accelerated stability data are summarized in Table 2 and 3, respectively. The highest loss in titer due to lyophilization process was seen in S vaccine. The best result of stability test was seen in T vaccine, as only 0.1 Log decrease in titer was seen.

3.3. Stability of reconstituted Rubella vaccines at different temperatures in 24 h

Tables 4–6 shows how the viral titer in reconstituted vaccines (G, S and T respectively) was reduced at 4 h intervals during a 24 h period. Regression analysis was also applied accordingly (Table 7 and Fig. 1). Graphical representation revealed an inverse relationship between virus titer and temperature.

Viral titer was reduced at the slowest level, when the vaccines were kept at 4 °C. After 24 h the number of infectious particles in samples was acceptable so that is required for successful immunization. At 4 °C, the rate at which rubella virus titer dropped was also significantly slower in T vaccine compared to the S vaccine (p = 0.037), which were not significantly different between G and S vaccines (P = 68). At 25 °C the viral titer of S vaccine was significantly greater in the last sample (24 h following reconstitution) compared to T vaccine (p = 0.034), which was not significantly different between T and G vaccines (p = 0.08). At 37 °C, rapid decreases in rubella virus titer occurred in all three vaccines as evidenced by the significantly steeper slopes of the regression lines compared the same stabilizers incubated at 25 °C or 4 °C.

Data extrapolated from these regression analyses showed the relative rates of infectivity loss per hour and were used to estimate the half-life of each vaccine formulation at defined temperatures (Table 7). Regardless of the type of stabilizer, half-life of all three prepared vaccines were reduced in higher temperatures. However, as temperature went up, the T and G vaccines exhibited reserved levels of virus infectivity (8.6 h, 8.8 h respectively)

Table 3
Lyophilized vaccines titers at −70 °C and 37 °C after 1 week.

Stabilizer	Virus titer ($log_{/ml}$)		Titer loss
	In 37 °C after 1 week	In −70 °C after 1 week	
S[a]	4.9 ± 0.13	5.4 ± 0.18	0.5
T[b]	6.0 ± 0.14	6.1 ± 0.17	0.1
G[c]	5.6 ± 0.17	6.0 ± 0.14	0.4

[a] Vaccine which is formulated using stabilizer S.
[b] Vaccine which is formulated using stabilizer T.
[c] Vaccine which is formulated using stabilizer G.

Table 4
Viral titers in reconstituted G vaccines during a 24-hour period.

Hours	($log_{10}TCID_{50/ml}$)		
	4°C	°C 25	°C 37
0	0.11 ± 6.0	0.11 ± 6.0	0.11 ± 6.0
4	0.25 ± 5.8	0.14 ± 5.1	0.32 ± 4.1
8	0.17 ± 5.5	0.26 ± 4.2	0.19 ± 4.1
12	0.24 ± 5.5	0.20 ± 4.0	0.17 ± 2.5
16	0.18 ± 4.5	0.13 ± 3.1	–
20	0.37 ± 4.0	–	–
24	0.23 ± 3.7	–	–

Table 5
Viral titers in reconstituted S vaccines during a 24-hour period.

Hours	($log_{10}TCID_{50/ml}$)		
	4°C	°C 25	°C 37
0	0.13 ± 5.5	0.13 ± 5.5	0.13 ± 5.5
4	0.26 ± 5.3	0.14 ± 5.0	0.10 ± 4.2
8	0.17 ± 4.9	0.33 ± 4.1	0.25 ± 2.5
12	0.14 ± 4.8	0.25 ± 3.5	–
16	0.39 ± 3.7	0.22 ± 3.0	–
20	0.15 ± 3.5	–	–
24	0.18 ± 3.0	–	–

Table 6
Viral titers in reconstituted T vaccines during a 24-hour period.

Hours	($log_{10}TCID_{50/ml}$)		
	4°C	°C 25	°C 37
0	0.10 ± 6.0	0.11 ± 6.0	0.13 ± 6.0
4	0.14 ± 6.0	0.21 ± 5.6	0.22 ± 4.2
8	0.12 ± 5.9	0.32 ± 5.0	0.17 ± 2.3
12	0.18 ± 5.5	0.28 ± 3.6	0.12 ± 2.0
16	0.15 ± 5.1	0.15 ± 2.0	–
20	0.11 ± 4.5	–	–
24	0.21 ± 4.3	–	–

Table 7
Effect of temperature on the degradation rates and half-lives of three live-attenuated rubella vaccines with different stabilizers at 4 °C, 25 °C and 37 °C.

Temp (°C)	Vaccine	Regression equation ($y = ß_0 + ß_1X$)	Half Life (hour)	Degradation rate (hour)
4	S	5.704-0.110x	25	0.110
	T	6.282-0.079x	37.9	0.079
	G	6.232-0.103x	29.1	0.103
25	S	5.520-0.163x	16.8	0.163
	T	6.440-0.250x	12	0.250
	G	5.860-0.173x	17.3	0.173
37	S	5.567-0.375x	7.3	0.375
	T	5.710-0.348x	8.6	0.348
	G	5.700-0.338x	8.8	0.338

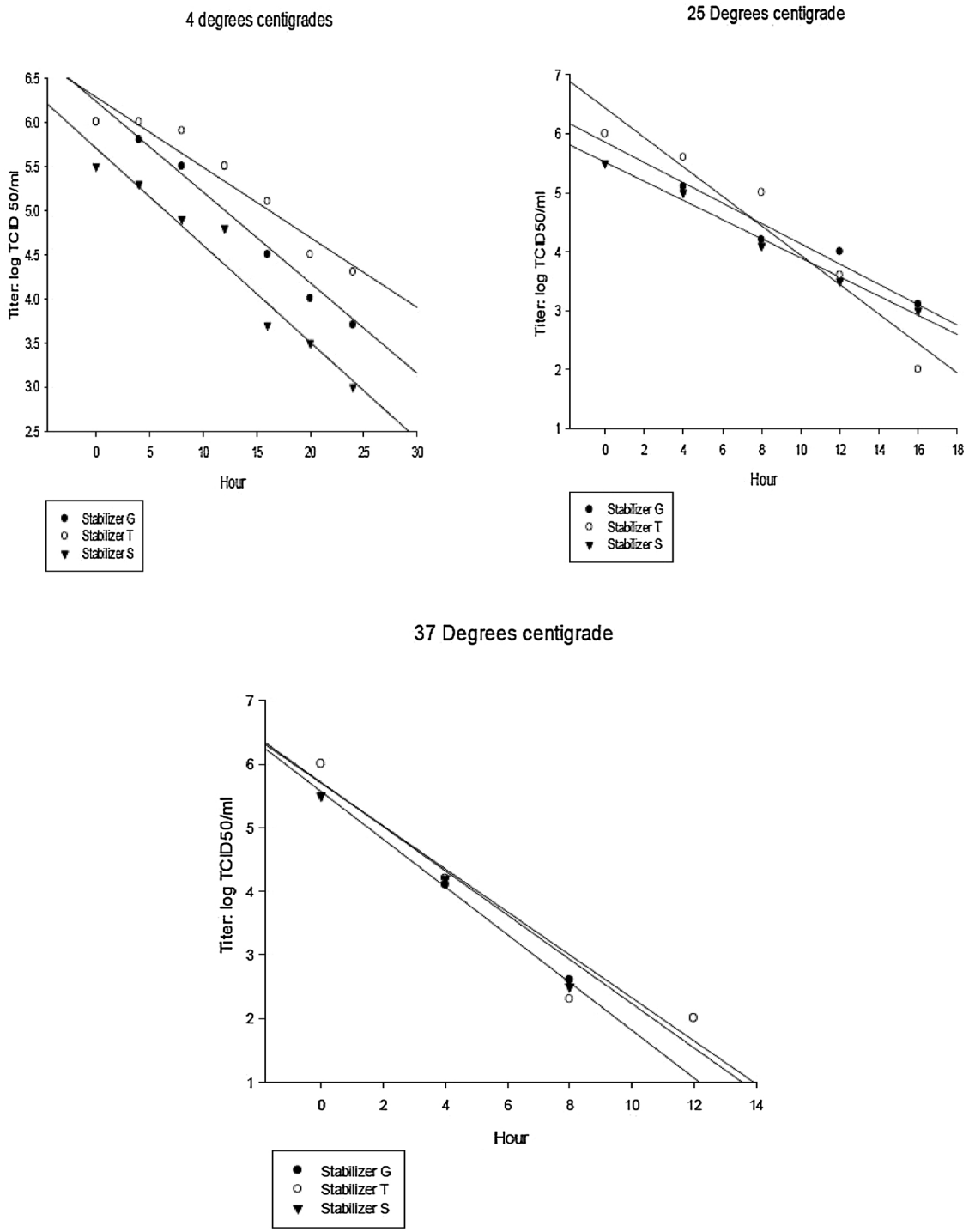

Fig. 1. Regression analyses of the three reconstituted rubella vaccines incubated at 4 °C (A), 25 °C (B) and 37 °C (C) for up to 24 h. The rubella vaccine potency titration results were used to calculate the regression lines for each vaccine.

4. Discussion

Over 100 000 infants are born with CRS every year worldwide (Lambert, 2007). Controlling and eliminating rubella infection is one of the goals of WHO. Vaccination, as the most effective preventive action has dramatically reduced the morbidity and mortality of the disease (Spika et al., 2003).

A successful immunization program primarily depends on the stability, efficacy, safety and potency of the vaccines (Naik et al., 2017). High temperatures can reduce the potency and stability of vaccines (Pastorino et al., 2015). To ensure the optimal potency, vaccines should be stored and handled carefully (Kartoglu and Milstien, 2014). Vaccines could be developed as either lyophilized or liquid formulations which depend on the nature of immunizing ingredients.

Freeze-drying (lyophilization) is a well-established technique for stabilizing live attenuated viral vaccines. This method has also been used successfully for the preservation of serum, vaccines, bacterial cultures and other biological materials (Hansen et al., 2015). It is known that lyophilization requires appropriate stabilizers to prevent inactivation of the virus (Pastorino et al., 2015).

In the present study, the effect of sucrose, trehalose and sorbitol on stabilizing rubella virus (TAKAHASHI strain) was compared with a

gelatin-based stabilizer.

The majority of live vaccines are unstable unless they are stored as a dry product with low residual moisture. Residual moisture affects the infectivity titer of freeze-dried vaccines (May et al., 1982). According to the World Health Organization documents and European pharmacopeia's guideline, the residual moisture in lyophilized vaccines should not be more than 3% (World Health Organization, 1995). It has been shown that MMR vaccines produced at RVSRI had met this requirements (Shahkarami et al., 2009). Following lyophilization of all three prepared formulations (S, T and G stabilizers), the residual moisture remained below 3%.

Accelerated stability test evaluates the virus titer when the lyophilized vaccine is kept at 37 °C for one week (Mariner et al., 2017). According to the World Health Organization instructions, the virus titer should not be decreased by over 1.0 log10 during incubation when it is compared to those one kept at 0 °C or lower temperatures (Kumru et al., 2014). In this study, the trehalose dihydrate-based stabilizer (T) was the most effective as only 0.1 log10 reduction in virus titer was seen.

Based on WHO recommendation, lyophilized viral vaccines may lose their potency quickly when they are reconstituted. Accordingly, they should be kept at 2–8 °C following reconstitution and should be used within 8 h particularly in hot weather. Stability pattern of reconstituted S, G and T vaccines was evaluated in whole day at 4, 25, 37 °C every four hours. All three formulations were able to retain virus titer in an acceptable range up to 24 h when the relevant reconstituted vaccines were kept at 4 °C. Despite the above fact, the virus titer of reconstituted vaccines decreased rapidly in higher temperatures.

Infectivity profile regression analyses of stability data revealed that vaccine formulated using stabilizer 'T' was significantly better than that one formulated using stabilizer 'S'. Kamali Jamil R et al.(Jamil et al., 2014) and Shayestehpour M et al. (Shayestehpour et al., 2012) had reported almost the same findings showing the higher protection of the mumps and measles viruses during lyophilization process.

It was also previously shown that a stabilizer based on trehalose dihydrate preserves the infectivity of live attenuated peste des petits (Mariner et al., 2017) and VLP-based vaccines (Wang et al., 2017). Trehalose dihydrate is a sugar (disaccharide) that preserves the protein structure. Interaction between a protein and trehalose allows the protein to remain in its native form and prevents denaturing during desiccation, heat shocks, cold storage and lyophilization process (Olsson et al., 2016).

Briefly, improving the thermal stability of live viral vaccines using advanced stabilizers, particularly in hot weather, ensures the viability of the viruses during lyophilization, transportation, storage and administration processes.

Based on the outcomes of the present study, the trehalose dehydrate-based stabilizer is recommended for the formulation of live-attenuated rubella vaccine (produced by TAKAHASHI strain). Previous studies had also shown that a trehalose-based stabilizer ensures the stability of attenuated mumps and measles viruses. Since CDC recommends protection against measles, mumps and rubella diseases by getting MMR vaccine (McLean et al., 2013), in parallel with routinely used gelatin-based stabilizer, a trehalose-based stabilizer seems to be another applicable candidate for mass production of MMR vaccine as well.

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References

Cardoso, F.M.C., Petrovajova, D., Hornakova, T., 2017. Viral vaccine stabilizers: status and trends. Acta Virol. 61, 231–239.
Colinet, G., Rossignol, J., Peetermans, J., 1982. A study of the stability of a bivalent measles–mumps vaccine. J. Biol. Stand. 10, 341–346.
Galazka, A., Milstien, J., Zaffran, M., 1998. Thermostability of Vaccines. World Health Organization, Geneva.
Hansen, L.J., Daoussi, R., Vervaet, C., Remon, J.P., De Beer, T.R., 2015. Freeze-drying of live virus vaccines: a review. Vaccine 33, 5507–5519.
Herini, E.S., Gunadi, Triono, A., Mulyadi, A.W., Mardin, N., Rusipah, Soenarto, Y., Reef, S.E., 2017. Hospital-based surveillance of congenital rubella syndrome in Indonesia. Eur. J. Pediatr. 176, 387–393.
Hill, A.B., Kilgore, C., McGlynn, M., Jones, C.H., 2016. Improving global vaccine accessibility. Curr. Opin. Biotechnol. 42, 67–73.
Jamil, R.K., Taqavian, M., Sadigh, Z.A., Shahkarami, M.K., Esna-Ashari, F., Hamkar, R., Hosseini, S.M., Hatami, A., 2014. Evaluation of the thermal stability of a novel strain of live-attenuated mumps vaccine (RS-12 strain) lyophilized in different stabilizers. J. Virol. Methods 199, 35–38.
Kamali Jamil, R., Shayestehour, M., Azita Sadigh, Z., Taqavian, M., Shahkarami, M.K., Esna-Ashari, F., Shahbazi, R., Mohammadi, A., 2017. The effect of various stabilizers on preserving immunogenicity of lyophilized mumps vaccines. J. Res. Health Sci. 17, e00393.
Kartoglu, U., Milstien, J., 2014. Tools and approaches to ensure quality of vaccines throughout the cold chain. Expert Rev. Vaccines 13, 843–854.
Kumru, O.S., Joshi, S.B., Smith, D.E., Middaugh, C.R., Prusik, T., Volkin, D.B., 2014. Vaccine instability in the cold chain: mechanisms, analysis and formulation strategies. Biologicals 42, 237–259.
Lambert, S.R., 2007. Congenital Rubella Syndrome: the End Is in Sight. BMJ Publishing Group Ltd.
Mariner, J.C., Gachanja, J., Tindih, S.H., Toye, P., 2017. A thermostable presentation of the live, attenuated peste des petits ruminants vaccine in use in Africa and Asia. Vaccine 35, 3773–3779.
May, J.C., Grim, E., Wheeler, R.M., West, J., 1982. Determination of residual moisture in freeze-dried viral vaccines: karl Fischer, gravimetric and thermogravimetric methodologies. J. Biol. Stand. 10, 249–259.
McLean, H.Q., Fiebelkorn, A.P., Temte, J.L., Wallace, G.S., 2013. Prevention of measles, rubella, congenital rubella syndrome, and mumps, 2013: summary recommendations of the Advisory Committee on Immunization Practices (ACIP). Morbidity and Mortality Weekly Report: Recommendations and Reports 62. pp. 1–34.
Mori, Y., Miyoshi, M., Kikuchi, M., Sekine, M., Umezawa, M., Saikusa, M., Matsushima, Y., Itamochi, M., Yasui, Y., Kanbayashi, D., Miyoshi, T., Akiyoshi, K., Tatsumi, C., Zaitsu, S., Kadoguchi, M., Otsuki, N., Okamoto, K., Sakata, M., Komase, K., Takeda, M., 2017. Molecular epidemiology of rubella virus strains detected around the time of the 2012-2013 epidemic in Japan. Front. Microbiol. 8, 1513.
Naik, S.P., Zade, J.K., Sabale, R.N., Pisal, S.S., Menon, R., Bankar, S.G., Gairola, S., Dhere, R.M., 2017. Stability of heat stable, live attenuated Rotavirus vaccine (ROTASIIL(R)). Vaccine 35, 2962–2969.
Olsson, C., Jansson, Hn, Swenson, J., 2016. The role of trehalose for the stabilization of proteins. J. Phys. Chem. B 120, 4723–4731.
Pastorino, B., Baronti, C., Gould, E.A., Charrel, R.N., De Lamballerie, X., 2015. Effect of chemical stabilizers on the thermostability and infectivity of a representative panel of freeze dried viruses. PloS One 10, e0118963.
Plotkin, S.A., 2006. The history of rubella and rubella vaccination leading to elimination. Clin. Infect. Dis. 43, S164–S168.
Ramakrishnan, M.A., 2016. Determination of 50% endpoint titer using a simple formula. World J. Virol. 5, 85.
Reed, L.J., Muench, H., 1938. A simple method of estimating fifty per cent endpoints. Am. J. Epidemiol. 27, 493–497.
Riyesh, T., Balamurugan, V., Sen, A., Bhanuprakash, V., Venkatesan, G., Yadav, V., Singh, R.K., 2011. Evaluation of efficacy of stabilizers on the thermostability of live attenuated thermo-adapted Peste des petits ruminants vaccines. Virol. Sin. 26, 324–337.
Shahkarami, M.K., Taqavian, M., Shafyi, A., Alirezaie, B., Esna-ashari, F., Soleimani, S., Shahbazi, R., 2009. Investigation of the relationship between the residual moisture and thermal stability of lyophilized MMR vaccine. Iran. J. Virol. 3, 25–28.
Shayestehpour, M., S.M, Shafyi, A., Taqavian, M., Kamali jamil, R., Esna-ashari, F., 2012. A study of the thermal stability of measles vaccine produced by AIK-C strain. Arak Univ. Med. Sci. J. 15, 26–33.
Shokri, S., Shahkarami, M., Shafyi, A., Mohammadi, A., Esna-ashari, F., Hamta, A., 2013. Evaluation of seroconversion in guinea pigs following inoculation of new formulations of rubella vaccine. Iran. J. Virol. 7, 12–16.
Spika, John S., Wassilak, S., Pebody, R., Lipskaya, G., Deshevoi, S., Güriş, D., Emiroglu, N., 2003. Measles and rubella in the world health organization european region: diversity creates challenges. J. Infect. Dis. 187, S191–S197.
Tang, X.C., Nail, S.L., Pikal, M.J., 2005. Freeze-drying process design by manometric temperature measurement: design of a smart freeze-dryer. Pharm. Res. 22, 685–700.
Uchimoto, T., Iwao, Y., Takahashi, K., Tanaka, S., Agata, Y., Iwamura, T., Miyagishima, A., Itai, S., 2011. A comparative study of glycerin fatty acid ester and magnesium stearate on the dissolution of acetaminophen tablets using the analysis of available surface area. Eur. J. Pharm. Biopharm. 78, 492–498.
Wang, N., Zhang, Y., Lei, X., Yu, W., Zhan, Y., Wang, D., Zhang, J., Wang, A., Xiao, L., Jiang, P., 2017. Optimized conditions for preserving stability and integrity of porcine circovirus type2 virus-like particles during long-term storage. J. Virol. Methods 243, 146–150.
World Health Organization, 1995. Manual of Laboratory Methods for Potency Testing of Vaccines Used in the WHO Expanded Programme on Immunization.
Zanga, J., Mbanzulu, M.K., Kabasele, A.F., Ngatu, N.R., Wumba, D.R., 2017. Rubella Seroprevalence and real-time PCR detection of RUBV among Congolese pregnant women. BMC Infect. Dis. 17, 250.