Determination of Factors Affecting Bivalent (Type 1 and 3) Stability of Oral Poliomyelitis Vaccine

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Received: 04 Mar 2019
Revised: 01 Jun 2019
Accepted: 19 Jun 2019

ABSTRACT

Background and Objectives: In recent years, due to the eradication of type 2 poliovirus, a bivalent vaccine containing types 1 and 3 is used in Iran. Since it is a highly thermolabile vaccine, it should be stored at a recommended temperature. Since cold chain may not be applied in Iran's tropical weather conditions, potency of the vaccine may be subjected to change. Therefore, we evaluated the effective factors on the potency of this vaccine.

Methods: We evaluated stability of a bivalent oral poliovirus vaccine produced by Razi Institute of Iran to ensure consistency of virus at different temperatures (-20, 2-8, 22-25 and 35-37 ºC), time intervals and freeze/thaw cycles. Three consecutive batches produced during full-scale production were randomly sampled.

Results: At -20 ºC, there was no change in vaccine vial monitor (VVM). The potency of the samples exposed to 2-8 ºC for 60 days and to 22-25 ºC for five days met the specification. The mean potency of the samples was 6.17, 6.00, 5.83, 5.75 and 5.54 (log CCID50/dose) after 10, 20, 30, 40 and 50 freeze/thaw cycles, respectively. In addition, the mean degradation of VVM was 0, 23.33, 60 and 100% for samples exposed to -20, 2-8, 22-25 and 35-37 ºC, respectively.

Conclusion: The results indicate the effects of environmental factors on the potency of the vaccines and the correlation between the VVM grade, color change and the vaccine potency for programming vaccine distribution networks at different transit levels. Based on the results of our study, the best temperature for maintenance and transportation of bivalent oral poliovirus vaccine is -20 ºC. Furthermore, freeze/thaw cycles lower the potency of the vaccine and change the VVM grade significantly.

Keywords: Poliovirus vaccine, Refrigeration, Temperature, Freezing, Vaccine potency, Iran, Immunization programs, Vaccination, Protein stability

This paper should be cited as: Soleimani S [Determination of Factors Affecting Bivalent (Type 1 and 3) Stability of Oral Poliomyelitis Vaccine]. mljgoums. 2020; 14(1): 36-43

INTRODUCTION

Due to risk of rapid potency loss, vaccines should be kept under recommended temperatures (1). Because of lack of ice packs, freezer and efficient transport subtractions, the cold chain under field situations is often challenging (2). Therefore, vaccine vial monitor (VVM), a heat and time-sensitive sticker, is glued to WHO-prequalified vaccines based on polymerization technology (3). When exposed to a high temperature, the potency of vaccines will drop and the VVM inner square changes to a dark color. When the color of the inner square merges with the outer circle, the VVM endpoint is reached and the vaccine should be discarded. This indicates whether the vaccine remains sufficiently potent for use, even when the cold chain is not trusted. Studies have determined a correlation between the VVM grade and the vaccine potency (4).

Currently, the oral poliovirus vaccine (OPV) is the best choice to prevent polio outbreaks and transmission of wild viruses (5). After eradication of wild-type 2 poliovirus, some countries including Iran introduced the bivalent OPV (bOPV) such as type 1 and type 3 polioviruses (6). This vaccine is highly thermolabile (inactivated at 55 ^{o}C for 30 minutes) and needs VVM2, with an endpoint at 37 ^{o}C for two days or more (7) and is stable for two years at -20 ^{o}C (8). Because kinetics is used for vaccine degradation rate estimation following inappropriate storage and transportation conditions (9), the samples in this study were stored at 2-8, 22-25 and 35-37 °C. The overall objective of this study is to measure and monitor the potency of vaccines in different storage conditions and to evaluate the relationship between OPV potency, VVM and heat exposure.

MATERIALS AND METHODS

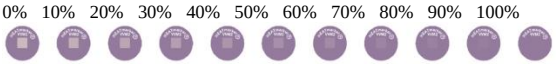
From three consecutive batches of the bOPV, 300 tubes were randomly sampled. Then, the samples were stored at -20, 2-8, 22-25 and 35-37 ºC (temperature of tropical regions and most parts of Iran). Second series of samples were exposed to 10, 20, 30, 40 and 50 freeze/thaw cycles. The validity of these temperatures was checked by observation two times a day and rechecked by recording the temperature every hour by a cool vision system.

After each vaccine challenge, for detection any contamination, each sample was cultured in brain heart infusion agar, tryptic soy broth, thioglycolate broth and blood agar. For mycoplasma detection, the samples were cultured and subcultured in pleuropneumonia-like organism broth and agar (selective medium for mycoplasma cultivation and maintenance), respectively (10).

Physicochemical tests including stabilizer content ($MgCl_2$), airtightness, appearance, labeling, pH and extractable volume were done at the beginning and after each stress situation. When testing the appearance, consistency, color, transparency and any visible particle were considered. Stability of label and tube airtightness were inspected. The $MgCl_2$ content was tested by complexometric titration assay, and pH of the samples was determined by assessing the hydrogen ion content. Finally, volume of each vial was estimated by drop count (8).

All samples were tested for potency after exposure to freeze/thaw cycles and temperatures of -20, 2-8, 22-25 and 35-37 ºC for 2, 4, 7, 10, 14, 21, 30 and 60 days (11): After preparation of the HeLa cells (ATCC CCL-2) (12), the vaccine was diluted and added into a microtiter plate (Nunc). Then, a cell suspension (2×10^5 cells/ml) was added to the plate. After 4-7 days, the cells were observed for cytopathic effects. The vaccines' CCID50 was determined by estimating the 50% endpoint by the Spearman-Karber method per dose (13). Later, the geometric mean titer was determined in triplicate. The titer of the bivalent vaccine must be more than 10^6 CCID50/dose as the minimal protective titer according to the WHO requirements (14).

The VVM was classified as follows:



In parallel to each potency test, the VVM of the vials in different conditions was evaluated and classified at baseline and after each vaccine challenge based on the above VVM grading.

For validation of the tests, system suitability was controlled and potency of a vaccine standard was determined in parallel to test vaccines. The difference was $10^{0.36}$ CCID50/dose (less than $10^{0.5}$ on the base of requirements) verifying the assay's validity (15).

The least squares regression analysis and a

linear regression model were used for analysis of vaccine stress test data.

RESULTS

All polio vaccine samples were free from fungal, bacterial (aerobic and anaerobic) and mycoplasma contamination during the study process. The vaccine samples met the specification after each physicochemical test and vaccine challenge condition (Table 1).

Table 2 shows the results of the potency test in different conditions. At -20 ^{o}C, there was no reduction in the potency in the test periods and there was no change in VVM (VVM grade was zero). The potency of the samples exposed to 2-8 ^{o}C for 60 days met the specification. At 22-25 ^{o}C, the samples met the specification until five days after which the potency of the vaccines decreased to 5.05, 5.05 and 4.93 (log CCID50/dose) for each batch, respectively. The VVM minimum grade of the samples, 60 days after exposure to 2-8 ^{o}C and 22-25 ^{o}C was 40% and 70%, respectively. Exposure to 35-37 ^{o}C for two days reduced the potency and the potency of three batches reached the lowest level (<4.00) after 21 days. As shown in table 2, the VVM grade was 100%, 21 days after exposure to 35-37 ^{o}C. Figures 1 and 2 show the linear regression fit of data obtained from table 2.

The mean potency of the samples was 6.17, 6.00, 5.83, 5.75 and 5.54 (log CCID50/dose) after 10, 20, 30, 40 and 50 freeze/thaw cycles, respectively. The linear regression fit of data from table 3 is shown in figure 3. The mean degradation of VVM was 0, 23.33, 60 and 100% for samples exposed to -20, 2-8, 22-25 and 35-37 ^{o}C, respectively (Table 2). The results of vaccines VVM gradation in different freeze/thaw cycles are shown in table 3.

Table 1– Physicochemical tests of polio vaccines in long term stability evaluation

Sample	Test	Specification	Results*									
			-20 °C		2-8 °C		22-25 °C		35-37 °C		Freeze/thaw cycles	
			Beginning	End	Beginning	End	Beginning	End	Beginning	End	Beginning	End
A	$MgCl_2$ Content	1.00 M ± 0.1	0.93	0.93	0.92	0.93	0.92	0.91	0.93	0.90	0.92	0.90
	pH	6.50 - 7.50	7.13	7.15	7.13	7.15	7.14	7.18	7.14	7.21	7.13	7.18
	Extractable volume	At least 30 drops/tube	30	30	31	31	30	30	30	29	30	30
	Sterility and Mycoplasma	To be free from fungal and bacterial agents and mycoplasma	Approved	Approved	Approved	Approved	Approved	Approved	Approved	Approved	Approved	Approved
B	$MgCl_2$ content	1.00 M ± 0.1	0.93	0.92	0.92	0.92	0.92	0.92	0.92	0.93	0.92	0.92
	pH	6.50 - 7.50	7.12	7.15	7.13	7.15	7.16	7.18	7.14	7.14	7.15	7.12
	Extractable volume	At least 30 drops/tube	30	30	30	30	30	30	30	29	30	30
	Sterility and Mycoplasma	To be free from fungal and bacterial agents and mycoplasma	Approved	Approved	Approved	Approved	Approved	Approved	Approved	Approved	Approved	Approved
C	$MgCl_2$ content	1.00 M ± 0.1	0.92	0.92	0.92	0.93	0.92	0.92	0.91	0.92	0.90	0.90
	pH	6.50 - 7.50	7.13	7.15	7.13	7.15	7.14	7.18	7.14	7.21	7.13	7.18
	Extractable volume	At least 30 drops/tube	30	30	30	30	30	30	30	30	30	30
	Sterility and Mycoplasma	To be free from fungal and bacterial agents and mycoplasma	Approved	Approved	Approved	Approved	Approved	Approved	Approved	Approved	Approved	Approved

Table 2- Potency (log CCID50/dose) and VVM grade of polio vaccine exposed to heat

Sample	Initial titer [a]	Temperature	2 Days			5 Days			7 Days			10 Days		
			Titer	VVM Form	VVM Grade	Titer	VVM Form	VVM Grade	Titer	VVM Form	VVM Grade	Titer	VVM Form	VVM Grade
A	6.42	-20 °C	6.42		0%	6.42		0%	6.42		0%	6.42		0%
		2-8 °C	6.42		0%	6.42		0%	6.42		0%	6.30		0%
		22-25 °C	6.30		10%	6.30		10%	6.17		10%	5.80		30%
		35-37 °C	6.17		10%	6.05		20%	5.67		30%	5.17		50%
B	6.42	-20 °C	6.42		0%	6.42		0%	6.42		0%	6.42		0%
		2-8 °C	6.42		0%	6.42		0%	6.42		0%	6.42		0%
		22-25 °C	6.30		10%	6.30		20%	6.05		30%	5.80		40%
		35-37 °C	6.17		10%	6.05		20%	5.17		40%	5.17		50%
C	6.30	-20 °C	6.30		0%	6.30		0%	6.30		0%	6.30		0%
		2-8 °C	6.30		10%	6.30		10%	6.17		20%	6.17		20%
		22-25 °C	6.30		10%	6.17		20%	6.17		20%	5.92		40%
		35-37 °C	5.92		30%	5.55		50%	5.42		60%	5.30		60%

Table 2– Potency (log CCID50/dose) and VVM grade of polio vaccine exposed to heat (Continued)

Sample	Initial titer [a]	Temperature	14 Days			21 Days			30 Days			60 Days		
			Titer	VVM Form	VVM Grade	Titer	VVM Form	VVM Grade	Titer	VVM Form	VVM Grade	Titer	VVM Form	VVM Grade
A	6.42	-20 °C	6.42		0%	6.42		0%	6.42		0%	6.42		0%
		2-8 °C	6.30		10%	6.30		10%	6.17		10%	6.17		10%
		22-25 °C	5.80		30%	5.67		30%	5.17		40%	5.05		50%
		35-37 °C	4.67		80%	4.12		100%	<4.00		100%	<4.00		100%
B	6.42	-20 °C	6.42		0%	6.42		0%	6.42		0%	6.42		0%
		2-8 °C	6.17		10%	6.17		10%	6.17		20%	6.05		20%
		22-25 °C	5.80		40%	5.67		40%	5.05		60%	5.05		60%
		35-37 °C	4.12		90%	<4.00		100%	<4.00		100%	<4.00		100%
C	6.30	-20 °C	6.30		0%	6.30		0%	6.30		0%	6.30		0%
		2-8 °C	6.05		30%	5.80		30%	5.67		40%	5.55		40%
		22-25 °C	5.80		40%	5.55		50%	5.05		60%	4.93		70%
		35-37 °C	5.05		70%	<4.00		100%	<4.00		100%	<4.00		100%

Table 3- Effect of freeze/thaw cycles on potency (log CCID50/dose) and VVM grade of polio vaccine

Sample	Initial titer [a]	10 Cycles			20 Cycles			30 Cycles			40 Cycles			50 Cycles		
		Titer	VVM Form	VVM Grade	Titer	VVM Form	VVM Grade	Titer	VVM Form	VVM Grade	Titer	VVM Form	VVM Grade	Titer	VVM Form	VVM Grade
A	6.42	6.17		0%	5.92		20%	5.92		30%	5.80		40%	5.55		40%
B	6.42	6.42		0%	6.17		10%	5.92		20%	5.92		20%	5.67		30%
C	6.30	6.05		10%	5.92		20%	5.67		40%	5.55		50%	5.42		50%

DISCUSSION

Vaccine stability is critical for successfully delivery of global immunization programs (9).

Several factors affect vaccines stability such as preservatives, lyophilization process, vials or tubes, monitoring process, vaccine production, maintenance equipment, transportation and cold chain (16).

Nowadays, a majority of vaccination failures results from thermal instability (17). The most important step to vaccine stability monitoring is an analytical method adoption for determination of specific vaccine ability as the "potency test", which is calculated by appropriate laboratory tests. Long-term maintenance, transit to health centers and the period immediately prior to injection might reduce vaccine potency (18). Preserving the vaccine potency by cold chain and maintenance during transportation seem to be more critical for developing countries, especially the tropical countries such as Iran where the cold chain is usually not available. A successful immunization program requires vaccines to be stored and transported properly while being under continuous temperature monitoring that helps detection of cold chain failure (19, 20).

Figure 1- Linear regression fit of data for mean virus titer (log CCID50/dose) of bOPV samples at different temperatures and time intervals.

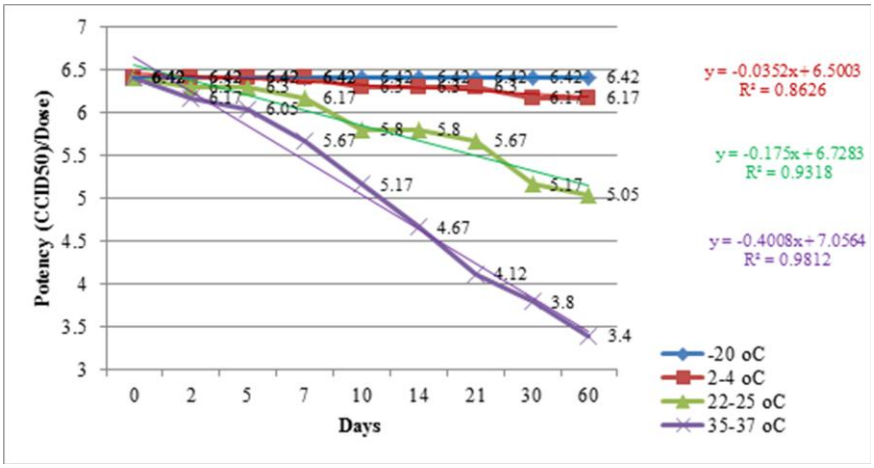


Figure 2 -Linear regression fit of data for bOPV VVM status at different temperatures and time

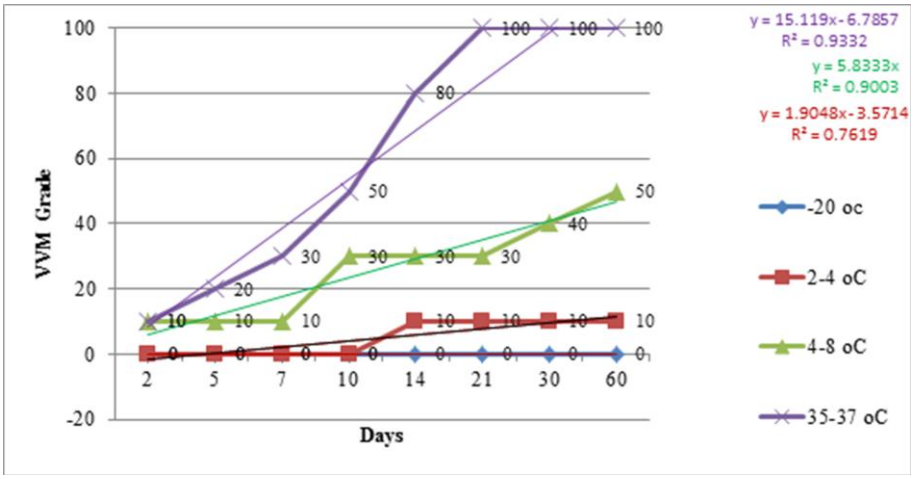
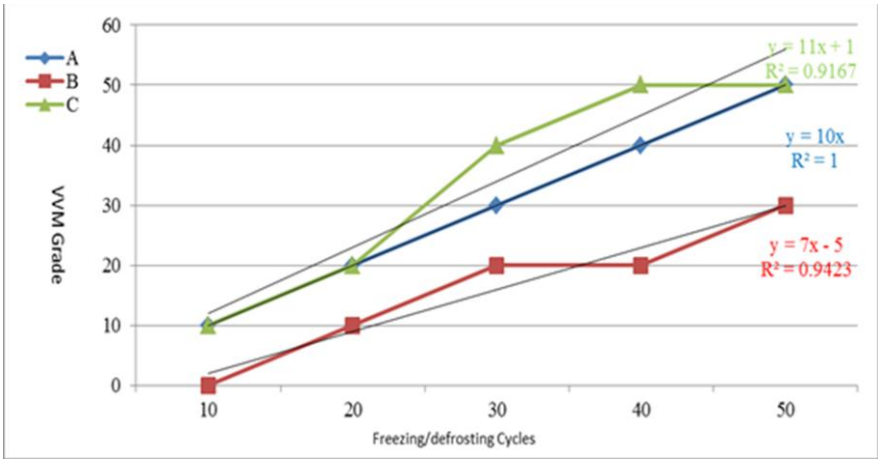


Figure 3- Linear regression fit of data for correlation of freeze/thaw cycles and VVM grade



Polioviruses are resistant to some common inactivating factors including ethanol, ether, chloroform and non-ionic detergents but sensitive to ultraviolet light, heat (55 ^{o}C), chlorine and formaldehyde (21).

From 17 April until 1 May 2016, the first stage of OPV cessation was successfully implemented in more than 150 countries and territories: a globally synchronized "switch" from the trivalent OPV to bOPV, which contains poliovirus type 1 and 3 (22).

Thermostability of polio vaccines has been the subject of many studies over the past few decades, particularly in polio-endemic countries (8, 23-26). According to biophysical studies, polioviruses can be inactivated by moderate heat exposure (42-45 ^{o}C) (13, 27). At -20 ^{o}C, the potency of this vaccine retains over a long period (8, 28), but at 3-5 ^{o}C, the stability of the vaccine is less than six months (29). Magnesium chloride is used as a stabilizer in OPV (18) with no significant loss of potency for as long as nine freeze/thaw cycles (30). Some researches employed VVM to specify the effects of OPV heat exposure. In a study by Samant et al., heat exposure in the last stage of the vaccine cold chain damaged 9% of the vaccines in health centers of India. The authors reported ice packs as well as storage and transportation temperature as the most important factors in temperature-induced OPV damage (24). In a study in a remote area of Mali, storing OPV without the cold chain during an OPV vaccination campaign was proven acceptable as long as the vaccine does not reach the endpoint (based on the VVM reading) (25). A study in Chad reported similar results regarding OPV storage (26) .

We designed this study based on VVM grade scoring for vaccine vials and testing of the vaccine for total OPV potency according to the standard WHO protocol. The results of the bOPV potency test in recommended temperature showed that all samples had a titer of more than $10^{6.15}$ CCID50/dose (WHO bivalent vaccine specification, as the total titer is fixed in bOPV, composite titer estimation is sufficient to assess the thermal stability) and no change was made to the VVM for 60 days. The same result was reported in previous studies until 24 months (8). Storage in refrigerator (2-8 ^{o}C) decreased the potency but the VVM grade was still somewhat favorable. One week storage at room temperature (22-25 ^{o}C) decreased the potency of the samples to below 10^{6} (CCID50/dose) and changed the VVM grade. After 60 days, the potency reached 10^{5} and the VVM grade was changed to 70%. In incubator (35-37 ^{o}C), the specification of the vaccine was not acceptable after five days. According to our findings, this vaccine is stable for 60 days, <10 days and <5 days at 2-8, 22-25 and 33-37 ^{o}C, respectively.

When evaluating the effect of freeze/thaw cycles on the vaccine, we found that 20 cycles decreased the vaccine's potency below the specification and altered the VVM to 20%. In addition, 40 freeze/thaw cycles decreased the potency to a very low level and the VVM grade reached 50% so that the vaccine was no longer usable. The results showed that all tested bOPV vaccines met the WHO specifications at the beginning and at the end of the study period, which indicates the favorable production conditions at the Razi Institute.

CONCLUSION

We revealed the stability of three consecutive batches of bOPV vaccines at different test conditions. The results indicated the effects of environmental factors on the potency of the vaccines and the correlation between the VVM grade, color change and vaccine potency for programming at vaccine injection sites. Based on the results of our study, the followings should be considered by vaccine program managers:

1. The bOPV is a highly thermolabile vaccine that requires cold chain for storage and transport.
2. The best temperature for maintenance and transportation of bOPV is -20 ^{o}C.
3. Duration of exposure to high temperatures is very important.
4. Freeze/thaw cycles lower the potency of the vaccine and change the VVM grade significantly.

ACKNOWLEDGMENTS

This study received financial support by the Razi Vaccine and Serum Research Institute (research projects No: 2-18-18-86034). The authors would like to thank the staff of human viral vaccine production, viral vaccine quality control and biobank departments and the Razi Vaccine and Serum Research Institute for their cooperation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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