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Full Article

Stability Study of Razi Trivalent and Monovalent Oral Poliomyelitis Vaccine

Soleimani*, S., Bordbar, N.

Department of Viral Vaccine Quality Control, Razi Vaccine & Serum Research Institute, Karaj,Iran

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ABSTRACT

Stability studies play a critical role in assuring product quality at all points in the vaccine life cycle. These studies used to determine vaccine expiry date and vaccine efficacy. Accelerated stability and long term stability study performed for three batches of trivalent oral poliomyelitis vaccine (OPV) and three batches of monovalent OPV (type1) manufactured by Razi institute. After sampling, the samples tested intervals in months for long term stability and after 48^{h} incubation in 37 ^{0}C for accelerated stability. All of quantitative and qualitative control tests including Potency, Sterility, Mycoplasma detection and Physicochemical testes performed in each period. Potency test for trivalent OPV (tOPV) in three batches of vaccines until 26 months and in two batches, until 27 months after production, met the specification. Potency test for monovalent OPV (mOPV) in all batches of vaccines until 18 month after production met the specification. Sterility, mycoplasma and physicochemical testes on these samples for mOPV and tOPV until expiry date and after that, passed. All batches of vaccines in accelerated stability met the specification after incubation and the reduction titer of vaccines was less than 0.5(-LogCCID50/dose). Results of this research indicated razi polio vaccines are stable for 24 months if the cold chain considered properly.

Keywords: OPV, Stability study, Expiry date, Potency test, Quality control test

INTRODUCTION

Poliomyelitis is a disease caused by poliovirus. This virus has three types 1, 2 and 3. Type 1 is most epidemic. Poliovirus inactivated in 55 ^{0}C for 30 minutes, but 2 mol/liter Mg2+ prevented from virus inactivation (plotkin & orenstein, 2004). The virus is stable against low pH, proteolytic enzymes, alcohol 70%, lyzol 50%, quaternary ammonium compound, ether and bile but is sensitive to formaldehyde 0.3%, 0.3–0.5 ^{ppm}, free chlorine without organic components and 50 ^{0}C for one hour (Knip & Howley 2007). There are two types poliomyelitis vaccine: Killed polio vaccine (salk vaccine) which is stable for 1 – 4 years in 2-8^{0}C (Galazka *et al* 1998, ICH 2001 & Chand *et al* 2008) and live polio vaccine (sabin vaccine) (OPV) that is very sensitive to temperature (Highly thermo-labile) and need cold chain for maintenance and transportation. This vaccine has most stability, if it has been kept in -20 ^{0}C (WHO/IVB/06010). Efficacy and safety of vaccines is related to the components of

*Author for correspondence.Email: sina.soleimani@gmail.com

vaccine contains, proteins, carbohydrates, lipids, inactive or attenuated microorganisms with stabilizers, adjuvants and preservatives. Vaccines especially live vaccines are sensitive to environmental factors (Plotkin & orenstein 2004). So, Stability of vaccines has major impact on the success of immunization programs world wide.

Stability study is one of the most quality control tests of vaccine (Chung 2008, biologicals 37 2009 & WHO/BS/06.2049 2006), and includes 4 groups:

1. Accelerated stability study: Studies 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.
2. Long term stability study (real time/ real condition): Studies 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.
3. Ongoing stability study: The purpose of this study is to monitor the vaccine and to determine that the vaccine remains and can be expected to remain, within the specification under the storage conditions indicated on the label, within the re– test period in all future batches.
4. Stress tests: Studies performed to determine the impact of extreme environmental factors such as light and extreme temperature. These studies not usually performed as part of a stability program.

Totally, the purpose of the stability study is preparation of document for:

1. The quality of vaccine ingredients
2. Vaccine changes by different effective factors such as temperature, humidity, light.
3. Determination of vaccine shelf life or vaccine expiry date.
4. Estimation of vaccine re- test time.
5. Recommendation of suitable maintenance condition.

So, in this study, accelerated and long term stability study performed for Razi oral poliomyelitis vaccine.

MATERIALS AND METHODS

Sampling. 300 samples from three consecutive batches of trivalent oral poliomyelitis vaccine were sampled randomly (20 samples for each test period). For monovalent oral poliomyelitis vaccine, 200 samples from three consecutive of monovalent vaccine, sampled randomly. Then, the samples stored in the frozen state at a temperature below -20 ^{0}C similar to packing and condition that recommended in the vaccine leaflet by manufacturer. The accuracy of freezer temperature checked by temperature observation, two times in a day and rechecked by cool vision system that recorded and controlled the temperature every one hour.

Long term Stability study. Trivalent vaccine were tested 13 times over 3 years after production in 0,3,6,9,12,15,18,21,24,26,27,30 and 36 months and monovalent vaccines were tested 10 times in 0,3,6,9,12,15,18,21,24 and 27 month after production.

Potency test. In each period of the study, according to the standard WHO protocol (WHO, BLG, 1995) after preparation of HeLa cell (ATCC CCL-2) (Freshny R. Ian, 2005) titration was performed by two methods: Microplate and macro tube culture. In micro method the samples were diluted and dispensed into flat –bottomed Nunc microtitre plate. Then HeLa cell suspension contains 2×10^{5} cells $_{/ml}$ was added. In macro method, the samples were tested in tube contain monolayer of HeLa cell that prepared previously. After 4-7 days, the cells in microplate and tubes were observed by invert microscope for cytopathic effects. The CCID50/dose of vaccines calculated by Spearman-Karber method with estimation of the 50% end –point (Muhammad T. et al 2010). Then the geometric mean titre (GMT) calculated by macro and micro method duplicate data. According to WHO requirements, titre of trivalent vaccine should not be less than $10^{6.15}$ CCID50/dose and for monovalent vaccine type 1

should not be less than 10^6 CCID50/dose as minimal protective titre (WHO, TRS, No. 904, 2002).

Sterility and mycoplasma test. The samples 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 test, the vaccines cultured in PPLO broth and sub cultured in PPLO agar (European Pharmacopoeia, 2004). These tests performed for all of the vaccine in each period in long term study.

Physicochemical tests. In each period Physicochemical tests including appearance, airtightness, labeling, stabilizer content (MgCl2), pH and extractable volume were performed for all of the samples. Color, consistency, transparency and any visible particle were considered for appearance.

In airtightness and labeling inspection, tube airtightness and stability of label were inspected. For stabilizer evaluation, the MgCl2 content tested by complexometric titration. By hydrogen ion content, pH of samples was tested and the volume of vaccine was evaluated by drops count (European Pharmacopoeia, 2004).

Accelerated Stability Study. After sampling, 5 tubes of each trivalent and monovalent vaccine incubated at 37 ^{0}C for 48 hours. Then the exposed and unexposed vaccines tested for potency in macro & micro titration method concurrently. Titre of the trivalent vaccines should not be less than $10^{6.15}$ CCID50/dose and for monovalent vaccine should not be less than 10^6 CCID50/dose. The difference between exposed and unexposed monovalent and trivalent samples should not be more than $10^{0.5}$ CCID50/dose.

Table 1. Potency (CCID50/dose) of trivalent polio vaccine in long term stability study

Lot number	0	3th month		6th month		9th month		12th month		15th month		18th month	
	Titre [a]	Titre	Reduction [b]	Titre	Reduction	Titre	Reduction	Titre	Reduction	Titre	Reduction	Titre	Reduction
861017	6.42	6.42	0	6.42	0	6.42	0	6.42	0	6.42	0	6.30	0.12
861018	6.42	6.30	0.12	6.30	0.12	6.30	0.12	6.30	0.12	6.30	0.12	6.17	0.25
861019	6.30	6.30	0	6.30	0	6.17	0.13	6.17	0.13	6.17	0.13	6.17	0.13

Table 1. (Cont'd): Potency (CCID50/dose) of trivalent polio vaccine in long term stability study

Lot number	21th month		24th month		26th month		27th month		30th month		36th month	
	Titre [a]	Reduction [b]	Titre	Reduction	Titre	Reduction	Titre	Reduction	Titre	Reduction	Titre	Reduction
861017	6.17	0.25	6.17	0.25	6.17	0.25	6.17	0.25	5.92	0.50	5.80	0.62
861018	6.17	0.25	6.17	0.25	6.17	0.25	6.17	0.25	5.80	0.62	5.67	0.75
861019	6.17	0.13	6.17	0.13	6.17	0.13	6.05	0.25	5.67	0.63	5.55	0.75

a- Titer (Log 10) b- Reduction in Titre (Log 10)

b- Reduction in Titre (Log 10)

For test validation, system suitability was checked and potency of a standard preparation determinated in parallel of test samples. The difference between this titre and the standard titre was $10^{0.38}$ CCID50/dose (less than $10^{0.5}$). So the tests were valid (ICH, Q2 R1, 2005).

RESULTS

Vaccine potency in long term stability study. All of the three batches of trivalent vaccines met the specification (WHO, TRS, No. 904, 2002) during the 24 even till 26 month after production in potency test. In 27th month, only one batch didn't pass the specification. In 30th month all of the vaccines had titre out of specification. For monovalent vaccine, all of the three batches met the specification until 18 month in potency test. But in 21th month all of the vaccines had titre out of specification. The potency results of trivalent and monovalent vaccines in long term stability study, has been shown in table 1 and 2 respectively.

Sterility and mycoplasma tests in long term stability study. All of the samples (trivalent and monovalent) were free from bacterial (aerobic and anaerobic), fungal and mycoplasma agents during the study.

Physicochemical tests in long term stability study. All of the trivalent and monovalent polio vaccines met the specification (Red color, transparent, free of any visible particle, airtight and readable in appearance, airtightness and labeling test) in each period of long term study**.** In Mgcl2 content, pH and extractable volume tests, met the specification too (table 3).

Accelerated stability study. All of the trivalent and monovalent vaccine samples**,** passed the thermo stability test. As shown in table 4 the reduction titre after incubation at 37 ^{o}C for all of the samples was not more than 0.5(-LogCCID50/dose).

DISCUSSION

Vaccines are combination 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 occurs. So, stability study of biological products plays an important role for determination of product changes in maintenance period and ensuring of safety and efficacy of vaccines. Stability is the ability of a vaccine to retain its chemical, physical, microbiological and biological properties within specified limits throughout its shelf – life. Several factors effect stability of vaccines such as, stabilizer such as MgCl2, heavy water (D2O) (Der yuan *et al* 2000), lyophilization process, vials or tubes of vaccines, freeze thawing cycles (0.07 and 0.67 CCID50/dose reduction was recorded respectively at the end of 10 and 50 cycles of freeze thawing (Chand *et al* 2008)) process and equipments used in production and the cold chain used for maintenance and transportation of vaccines.Oral polio vaccine being highly thermo – labile needs a stringently monitored cold chain (Yuan Wang *et al* 2000). This vaccine is known to retain its potency over a long period if stored at -20 ^{0}C (Galazka *et al* 1998 and Chand *et al* 2008) but is stable less than 6 months in 3-5 ^{0}C (Sokhey *et al* 1988). There was little loss in virus titre when samples were kept at -20 and 4-8^{o}C for 21 days, whereas the samples exposed to 36^{0}C for 21 days showed almost complete loss in virus titre (Sokhey *et al* 1988). The average loss in virus titre in a year (Log $CCID_{50}$) was 0.47 at -20^{o}C and 0.65 at 4-8^{0}C (Sokhey *et al* 1988). So, cold chain is necessary for this vaccine (Yuan Wang *et al* 2000 & Der yuan *et al* 2000). There are some documents about polio vaccine thermostability (Muhammad *et al* 2010, Chand *et al* 2008, Sokhey *et al* 1988 & Yuan wang *et al* 2000) but there is no any documented for polio vaccine long term stability. So, in this study, stability study performed for polio vaccine. Long term stability is used to recommend storage conditions and to establish the shelf life and the release specifications. Stability indicating parameters are used to assess product suitability throughout the shelf life. Determination of stability parameters should result in quantitative values with the detectable rate of change.

Table 2. Potency (CCID50/dose) of monovalent polio vaccine in long term stability study

Lot number	0	3th month		6th month		9th month		12th month	
	Titre [a]	Titre	Reduction[b]	Titre	Reduction	Titre	Reduction	Titre	Reduction
876001	6.30	6.30	0	6.17	0.13	6.17	0.13	6.05	0.25
876002	6.17	6.17	0	6.17	0	6.17	0	6.17	0
876003	6.17	6.17	0	6.17	0	6.17	0	6.17	0

Table 2(Cont'd). Potency (CCID50/dose) of monovalent polio vaccine in long term stability study

Lot number	15th month		18th month		21th month		24th month		27th month	
	Titre [a]	Reduction [b]	Titre	Reduction	Titre	Reduction	Titre	Reduction	Titre	Reduction
876001	6.05	0.25	6.05	0.25	5.92	0.38	5.92	0.38	5.80	0.50
876002	6.17	0	6.17	0	5.92	0.25	5.80	0.37	5.67	0.50
876003	6.05	0.12	6.05	0.12	5.80	0.37	5.67	0.50	5.55	0.62

a- Titer (Log 10) b- Reduction in Titre (Log 10)

Table 3. Physicochemical tests of polio vaccines in long term stability study

Test	Specification	Result [a]	
		tOPV	mOPV
MgCl2 Content	1.00 M ± 0.1	0.95	0.92
pH	6.50 - 7.50	7.02	7.13
Extractable Volume	At least 30 drops/tube	32	31

a- Mean of 13 times test for tOPV and 10 times for mOPV

Table 4. Accelerated stability study in polio vaccines

Vaccine	Lot umber	Potency	Accelerated Stability titre	Reduction titre	Specification
tOPV	**861017**	6.42	6.25	0.17	Titre not less than 6.15 Reduction titre not more than 0.5 (LogCCID50/dose)
	861018	6.42	6.25	0.17	
	861019	6.30	6.17	0.13	
mOPV	**876001**	6.30	6.05	0.25	Titre not less than 6.00 Reduction titre not more than 0.5 (LogCCID50/dose)
	876002	6.17	6.10	0.07	
	876003	6.17	6.10	0.07	

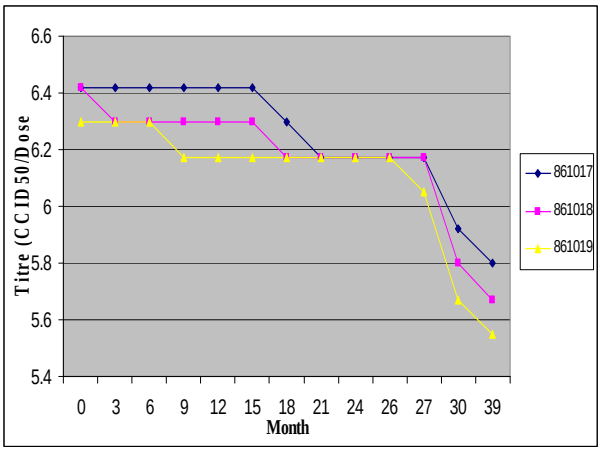

Figure 1. Potency of trivalent polio vaccines in long term stability study.

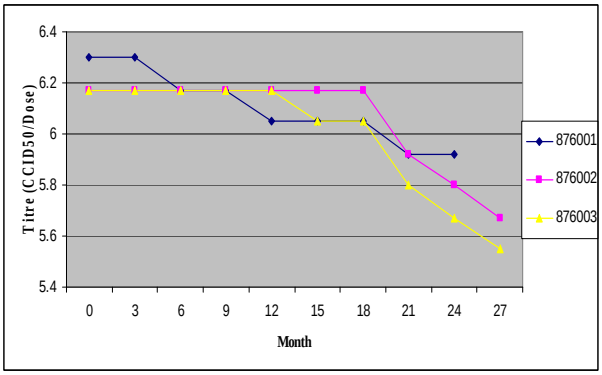

Figure 2. Potency of monovalent polio vaccines in long term stability.

Qualitative parameters such as sterility, mycoplasma and physicochemical tests could also be considered.

The result of potency test of the trivalent polio vaccine in duration of the long term stability indicated, all of the vaccines had titre more than $10^{6.15}$ CCID50/dose (WHO trivalent vaccine specification) until the end of 26 month after production. In 27th month, two batch met the specifications but titre of another one was reduced to $10^{6.05}$ CCID50/dose. In 30th and 36th month (the end of 3th year) all of three batches of vaccine, had titre less than vaccine specification. Potency test of monovalent polio vaccine in duration of the study showed, all of the vaccines had titre more than 10^{6} CCID50/dose (WHO monovalent vaccine specification) until the end of 18 month after production. In 21st month all of the vaccines had titre less than vaccine specification. The reduction titre of these vaccines increased in 24th and 27th month. Appropriate statistical evaluation of vaccine stability data promotes 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 should be employed to model potency decay (Stability guidelines, biological 37). The use of such tools provides incentive to properly design vaccine stability studies. An understanding of the principles of degradation, as well as the statistical tools for measuring product stability, is essential to management of product quality. Vaccine shelf life is best managed through determination of a minimum potency release requirement, which helps assure adequate potency throughout expiry. The case of measuring the degradation rate, testing at the beginning and the end of the study improves the precision of this estimate. As shown in Fig. 1 and 2, there is no any significant difference between all of the three batches of mOPV and three batches of tOPV and all of the samples had similar regression. This result indicated, there is consistency in production of the polio vaccines.

The result of the other quality control tests for three batch of trivalent and monovalent polio vaccine indicated all of the samples in sterility, mycoplsma and physicochemical tests met the WHO specifications until expiry date and even after that. The result of accelerated stability indicated all of trivalent and monovalent vaccine had less than 0.5 (-Log CCID50/dose) reduction titre after incubation and met the WHO accelerated stability specifications (table 5). 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 transport (WHO/GPV/98.07).

This study showed the trivalent polio vaccine is stable until at least 24th month and the monovalent polio vaccine is stable until at least 18th month after production with consideration of cold chain. All of the quality control tests met the specifications in this time because of good condition in production of the vaccines. Totally oral polio vaccines as manufactured by Razi institute met the WHO requirements for biological for at least two years in -20 ^{0}C and 48 hours at 37 ^{0}C. For good production and maintenance of vaccine, the following concepts are recommended:

1. Polio vaccine is highly thermo–labile vaccine, so the cold chain is necessary for maintenance and transportation of the vaccine (MOH guideline, 2005).
2. Planning of training about maintenance, transportation and use of vaccine for vaccinators by manufacturer.
3. Virus harvesting with optimum titre: For this purpose the manufacturer needs cell bank and seed bank according to GMP regulations.
4. Formulation of vaccine: The amount of virus and stabilizer is very important in stability of vaccine.

For further study, stress tests are very useful for evaluation of vaccine stability and the effect of environmental factors on potency of vaccine. These tests perform in different condition in maintenance of vaccine.

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