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Laboratory Investigations for safe and efficacious use of Opened Multi-Dose Vials of Bivalent Oral Polio Vaccine

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ABSTRACT

Aim: The aim of the current study was to laboratory investigation for safe and efficacious use of Opened Multi-Dose Vials of bivalent Oral Polio Vaccine containing type I and III.

Method: The proposed study was designed and conducted based on the safe and efficacious use of opened multi-dose bivalent Oral Polio Vaccine vials containing type I and III in subsequent immunization sessions on weekly intervals from 01st week to 09th week. Laboratory investigations of the vaccine quality attributes such as potency, identity, sterility, pH, kanamycin activity and Vaccine Vial Monitor status were performed after dose withdrawal from the multi-dose vaccine vials at +4°C temperature under laboratory conditions and obtained results were compared with the same batch samples at -20°C temperature.

Results: All quality attributes were qualified as per prescribed the acceptance criteria in Indian Pharmacopeia-2018. The opened multi-dose bivalent Oral Polio Vaccine vials can safe and efficacious use up to 56th day or 08 weeks after opening and stored at +4^{0}C temperature.

Conclusion: This study may helpful to provide well define information about the impact of duration and temperature on efficacy and safety of the bivalent oral polio vaccine after withdrawal immunization sessions on different weekly intervals.

Keywords: bivalent oral polio vaccine, Opened Multi-Dose Vial, Dose withdrawal, Subsequent sessions, efficacy, safety

INTRODUCTION

Oral Polio Vaccine (OPV) has been in use since the early 1960s and for most countries, remains the polio vaccine of choice in routine immunization schedules and supplementary immunization activities to finish the Polio Eradication Endgame. The objective 2 of the WHO Polio Eradication and Endgame Strategic Plan 2013 to 2018 requires the cessation of all OPVs in the long term, beginning with a switch from trivalent Oral Polio Vaccine (tOPV) to bivalent Oral Polio Vaccine (bOPV) containing type 1 & 3 in April 2016, removing the type 2 component (OPV2) from current immunization programmes [1]. Now-a-days, only the bOPV is using in pulse-polio and National immunization programmes.

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As we know that the bOPV is a multi-dose vial and is containing 20 doses in one vial. During the immunization, the single vaccine vial may be used in single and subsequent sessions. Use of one vaccine vial is completely depending on availability of vaccine receiver's numbers, if the receivers are at least in twenty numbers and the vaccine vial is used in single session after opening one vial. This is ideal condition and is considered as good attempt. Therefore, there was need standard guideline to use of opened multi-dose vials of the vaccine in subsequent immunization sessions. WHO policy statement was first published the guidelines in 1995 [2] and further replaced with the same title by World Health Organization (WHO), Geneva in 2000 [3]. WHO was revised the policy statement again in 2014 [4]. According to the current World Health Organization policy statement, the multi-dose vaccine vial after open should be kept between +2°C and +8°C. Multi-dose vials of OPV from which one or more doses of vaccine have been removed during an immunization session may be used in subsequent immunization sessions for up to a maximum of 28 days (04 weeks). Therefore, present study was designed and conducted with special emphasis on use of opened multi-dose bivalent Oral Polio Vaccine (bOPV) containing type 1 & 3 in subsequent immunization sessions and it may also beneficial to reduce the wastage of remaining vaccine doses. The study was performed to find out impact of storages temperature at $+4^0$C on quality attributes of bOPV under laboratory conditions and it is directly concern to potent and safe of the vaccine in vials as well as the same storage condition are using during subsequent immunization sessions in field.

MATERIALS AND METHODS

Sample's collection from bOPV batches with exclusion criteria

Sample of multi-dose vials from five bOPV batches were collected with following exclusion criteria; continuous produced batches, Expiry date of these batches has not been passed. Storage conditions of these batches were satisfied as per GMP guidelines and the selected batches are summarized in table 1. These batches were qualified the acceptance criteria of the quality attributes such as potency of type I (not $< 10^{6.00}$), potency of type III (not $<10^{5.80}$), Sterility (Sterile product), pH (6.50 to 6.80), Vaccine Vial monitor ($\geq 0.25 \pm 0.02$) and kanmycin antibiotics (15µg per dose) according to Indian Pharmacopeia - 2018 [5].

Sample Preparation, Handling and Storage of the bOPV vials

A total five batches of bOPV with codes are arranged in a serial order to easy understand as described the labeling code in Table 1. Samples of multi-dose bOPV vaccine vials were collected individually from each batch in separate small corrugated boxes. The vaccine vials were prepared after removal of aluminum cap and rubber stopper aseptically under laminar air flow (LAF) and the vials were sealed with pre-sterile dropper individually under sterile conditions in LAF. All prepared samples from five bOPV were divided in to two types on the basis of stored temperature after opening these vaccine vials; one type of the samples were stored at +4°C temperature and second type of the samples from the same batches were also stored at -20°C temperature and were kept individually in small corrugated boxes for further laboratory investigations. The vaccine vials samples were labeled as described in table 2. During the study, storage conditions of both types of samples from five bOPV batches were maintained and samples stored at +4°C temperature was used in the as such form, but the samples were stored at -20°C temperature was prepared in liquid form by thawing process at +4°C temperature for 10 to 15 minutes before the laboratory investigations. Finally results of the samples were compared with each other.

S. No.	Batch Code	Opened vials of vaccine samples stored at	
		$+4^0$C temperature (One type)	-20^0C temperature (Second type)
1	MDOBV 001	MDOBV 011	MDOBV 021
2	MDOBV 002	MDOBV 012	MDOBV 022
3	MDOBV 003	MDOBV 013	MDOBV 023
4	MDOBV 004	MDOBV 014	MDOBV 024
5	MDOBV 005	MDOBV 015	MDOBV 025

Table 1: Labeling description of multi-dose sample vials of bOPV batches.

Laboratory Investigations of the Opened vaccine Vials

In present study, laboratory investigations were performed with two types of the vaccine samples, which were collected individually from five bOPV batches. First type of the samples vials was stored at $+4^0$C temperature and the second type samples were stored at -20˚C temperature. Laboratory investigations like potency, identity, sterility, pH, kanamycin activity and Vaccine Vial Monitor (VVM) were performed to determine the vaccine quality on weekly intervals basis [01st week (07th day) to 09th week (63rd day)] under laboratory conditions.

Potency by cell culture technique

Both types of sample from five bOPV batches were determined for its potency in single dose containing type I and type III individually by cell culture technique [6]. The reference antisera standard of both types and HEp-2 (Cincinnati) cells were kindly provided by Central Drug Laboratory, Kasauli, Himanchal Pradesh, India. Confluent monolayer of HEp-2 cell was grown in MEM with 10% FBS in 25cm^2 tissue culture flask using standard cell culture techniques [7] and the viable cell count was performed using Neubauer's haemocytometer and trypan blue [8]. HEp2 cell concentration per well of microtitre plate was adjusted up to 10,000 cells/0.1ml by adding MEM with 5% FBS.

Each batch of both type's samples and reference antisera standard (Type I & III) were dilution serially in MEM with 2% FBS ranging from $10^{-3.0}$, $10^{-3.5}$, $10^{-4.0}$, $10^{-4.5}$, $10^{-5.0}$, $10^{-5.5}$, $10^{-6.0}$, $10^{-6.5}10^{-7.0}$ and $10^{-7.5}$. 0.05ml volume of each the prepared dilution was dispensed into each of 8 wells of flat-bottomed microtitre plate with lid, starting from higher dilution to lower dilution. The plates were incubated at 35.5˚C (± 0.5˚C) for 3 hours adding and vortex mixing of antisera. The incubation was required type-specific antiserum to neutralize of the other types of viral antigens. 0.1 ml of HEp-2 cell suspension (10,000 cells/0.1ml concentration) in MEM with 5% FBS was added to all the wells. The plate was sealed and incubated at 35.5˚C ($\pm 0.5^0$C) in carbon dioxide incubator. The plates were read microscopically on daily basis after 3rd , 5th and 7th day using an inverted microscope for cytopathic effect (CPE), wherein infected cells rounded up, showed shrinkage and marked nuclear pyknosis became refractile, degenerated and fell off the surface **[**9]. Kaerber's formula was used to obtain titre per dose (0.1 ml) of type I and type III of both vaccine samples [10]. Positive and negative controls for potency test were performed individually and result of the test was valid when positive and negative control of each experiment performed normal. Therefore the experiment with each sample of both types of the vaccine batches was performed in triplicate.

Identity by neutralization method

The identity of type I and III in opened bOPV vials was performed as previously described by Kumar and Tomar (2019) [11]. In brief, the vaccine is containing the two types of poliovirus; titration of the individual serotypes is undertaken

separately, using mixtures of appropriate type-specific antiserum to neutralize each of the other types present. Therefore, the tests was performed separately as confirmatory test to find out impact of storage condition at +4°C and at -20°C temperature of bOPV and the results of the both types of the samples were also compared with each other.

Sterility by direct inoculation method

Sterility test was carried out with both types of samples of bOPV on Nutrient Agar Medium (NAM) by direct inoculation method [12]. Positive and negative controls for bacterial and fungal were performed individually and the experiment with each sample of the vaccine was performed in triplicate.

For direct inoculation, one dose (100µl) from both types of bOPV samples was transferred through streaking on pre-prepared NAM petri-plates. Finally all inoculated plates were incubated at 22°C ± 2°C for fungal growth and 350C ± 20C for bacterial growth for 14 days. After incubation period, all plates were examined and results were recorded for bacterial and fungal growth.

Determination of pH by digital pH meter

The pH of both types of bOPV samples was determined using digital pH meter by standard method. The bOPV was used in the as such form. But 20 doses of lyophilized sbOPV was prepared and reconstituted in 0.2ml pre-sterile distilled water under aseptic conditions and kept at room temperature for 10 to 15 seconds to dissolve the vaccine completely. Standard pH buffer solutions were used to calibrate the pH meter before check the vaccines pH. Finally pH of the both vaccines was measured and recorded as per standard procedure.

Kanamycin activity by disc-diffusion method

The disc-diffusion method is a standard method, which was already used for quantitative evaluation of kanamycin antibiotic activity in single dose of oral polio vaccine [13]. Discs (size 05mm diameter) were prepared individually by soaking of single dose of both type of the vaccine samples and were dried for 30 minutes at room temperature under aseptic conditions in LAF. The each disc was containing antibiotic quantity equal to one dose of bOPV i.e. 100 µl/disc. Finally kanamycin activity in both types of bOPV samples was determined and compared the obtained results.

In disc-diffusion method, pre-sterile petri plates with equally distributed 20ml of nutrient agar medium were used and 1.0×106 cells/ml concentrations was adjusted & spread for uniform growth on the medium surface of Bacillus Subtilis (Gram-positive); and E. coli (Gram-negative) bacterial strains. Pre-prepared each disc containing 100µl/disc quantity of bOPV was aseptically transfer on the petri plates containing nutrient agar medium with bacteria and incubated at 37°C ± 1 for 24 hours. After incubation, kanamycin activity in both types of the samples was observed and measured the diameter of inhibition zone in millimeters.

Optical density of Vaccine Vial Monitor (VVM) by densitometer

In the study, both types of samples of bOPV batches were included and the same vial was studied to measure optical density (OD) of VVM, which was used on weekly interval basis for laboratory investigation purpose. The OD of each vial VVM was taken three times of reference circle and indicator square by densitometer on weekly interval after used for quality testing. OD Mean of indicator square was substrate from OD Mean of reference circle and final OD of VVM was recorded

[14]. Table 2 provided by the manufacturer to decide the discard point of the VVM in days at standard and constant temperature and this is for only reference purpose.

Constant temperature in day and night	Time for VVM to reach 'discard point'
At room temperature: +25˚C	8 days
At room temperature: +20˚C	20 days
In a refrigerator: +4˚C	180 days
In a freezer: -20˚C	Over 2 years

Table 2: Times recorded for a VVM attached to a vial of bOPV.

Statistical analysis of data

Each test was designed and conducted in triplicate and test-wise mean for each test was calculated. Finally mean value was recorded and summarized in respective table of test results. The results of each test were checked and observed by two experts.

RESULTS

A total five batches of bOPV were studied from batch code number MDOBV 001 to MDOBV 005. Collected sample vials from these batches were divided in to two types on the basis of storage temperature and given different sample codes; MDOBV 011 to MDOBV 015 for stored at +4˚C and MDOBV 021 MDOBV 025 stored at -20˚C. Finally, both types of the sample vials were opened in aseptic conditions under Laminar Air Flow (LAF) and covered with pre-sterile droppers to withdrawal samples on subsequent sessions during weekly intervals (01st to 09th week) for laboratory investigations such as potency, identity, sterility, pH, kanamycin activity and VVM status.

Laboratory Investigations of first type of opened bOPV Vials

In India, bOPV containing type 1 & 3 is using for routine and national immunization program for eradication of polio. Therefore current study performed with prime objective to investigate safe and efficacious uses of the opened multi-dose bOPV vials. Before start the study, these opened multi-dose bOPV vials properly sealed with pre-sterile droppers were stored at +4ºC storage temperature in laboratory conditions and the opened vials were studied after dose withdrawal from the multi-dose bOPV vials in subsequent session manner on weekly intervals from 01st week (7th day) to 09th week (63rd day) to determine quality of the vaccine and performed laboratory investigations based on various quality tests i.e. pH, sterility, potency, identity, vaccine vial monitor status and kanamycin antibiotic efficacy. The quality tests were performed to determine the safety and efficacy of opened multi-dose vial of bOPV at +4˚C storage temperature after weekly interval.

Potency test

In potency test, cell culture technique was used to measure the potency, in form of titre of each sampled vial of bOPV on weekly intervals and the results for the potency test are compiled and tabulated in Table 3. We have seen from the data of the potency that up to 49th day there is negligible variation among the bOPV samples but at the end of 08th week there is minor declination observed but it was found within acceptable range. Due to gradual declination in titre of type I & III of bOPV vaccine vials, potency test did not performed on 63rd day in the opened bOPV samples.

Batch			Titre of the vaccine vial at Day									
Code	**Vial No.**	**Poliomyelitis Virus**	**Sampling**	**07th**	**14th**	**21st**	**28th**	**35th**	**42nd**	**49th**	**56th**	**63rd**
	11	Type I	6.13	6.13	6.13	6.13	6.13	6.13	6.13	6.13	6.1	NP
MDOBV 01		Type III	5.93	5.93	5.93	5.93	5.93	5.93	5.93	5.93	5.9	NP
	12	Type I	6.13	6.13	6.13	6.13	6.13	6.13	6.13	6.13	6.11	NP
		Type III	5.93	5.93	5.93	5.93	5.93	5.93	5.93	5.93	5.9	NP
	21	Type I	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.03	NP
MDOBV 02		Type III	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	NP
	22	Type I	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	NP
		Type III	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	NP
	31	Type I	6.07	6.07	6.07	6.07	6.07	6.07	6.07	6.07	6.07	NP
MDOBV 03		Type III	5.88	5.88	5.88	5.88	5.88	5.88	5.88	5.88	5.88	NP
	32	Type I	6.07	6.07	6.07	6.07	6.07	6.07	6.07	6.07	6.07	NP
		Type III	5.88	5.88	5.88	5.88	5.88	5.88	5.88	5.88	5.88	NP
	41	Type I	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	NP
MDOBV 04		Type III	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	NP
	42	Type I	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	NP
		Type III	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.88	NP
	51	Type I	6.11	6.11	6.11	6.11	6.11	6.11	6.11	6.11	6.09	NP
MDOBV 05		Type III	5.93	5.93	5.93	5.93	5.93	5.93	5.93	5.93	5.89	NP
	52	Type I	6.11	6.11	6.11	6.11	6.11	6.11	6.11	6.11	6.1	NP
		Type III	5.93	5.93	5.93	5.93	5.93	5.93	5.93	5.93	5.9	NP
	61	Type I	6.14	6.14	6.14	6.14	6.14	6.14	6.14	6.14	6.12	NP
MDOBV 06		Type III	5.95	5.95	5.95	5.95	5.95	5.95	5.95	5.95	5.94	NP
	62	Type I	6.14	6.14	6.14	6.14	6.14	6.14	6.14	6.14	6.1	NP
		Type III	5.95	5.95	5.95	5.95	5.95	5.95	5.95	5.95	5.92	NP

Table 3: Potency test data of Opened Multi-Dose bOPV Vials stored at $+4^0$C by Cell Culture Technique.

Note: Type 1 Poliomyelitis virus Sabin strain is not less than $10^{6.0}$ $CCID_{50,}$ Type 3 Poliomyelitis virus Sabin strain is not less than $10^{5.8}$ $CCID_{50}$ and potency was not performed as indicated in table as NP at 63rd day.

Identity test

Neutralization assay for identification of type I & III in bOPV samples was performed to find out the impact of subsequent withdrawal of doses on weekly interval from 01st week to 09th week. The results of the test indicated that there is no adverse impact on presence of type I & III in all first type samples of bOPV batches.

Sterility test

Sterility test of the samples was performed on nutrient agar medium plates by direct inoculation method and the results were compiled to analyze the sterility data in table 4. In this experiment we observe that, the opened bOPV samples stored at +4˚C shows no contamination up to the end of 08th week but at the end of 09th week (i.e. 63rd day), contamination was found in few samples. Hence the results prove that all sample of bOPV was found sterile up to 08th week after opening and storage at +4˚C under the laboratory conditions.

Determination of the pH

The pH results of opened multi-dose bOPV vials at $+4^0$C are summarized in table 5, which was analysed by pH meter. The obtained results showed that there is negligible variation in pH of the sample vials up to 08 weeks (56th day) after the opening of bOPV vials, when the samples were stored at $+4^0$C and it was found within acceptable limit ranging from 6.50 to 6.60. On the end of 09th week (63rd day), few sample did not performed the test due to presence of contamination; DPC as

indicated in table 5. This data shows that the experiment was successfully analysed up to 08 weeks after the opening of vial in aseptic condition and storage at the temperature of $+4^0$C.

Batch		Sterility Status at Day									
Code	**Vial No.**	**Sampling**	**07th**	**14th**	**21st**	**28th**	**35th**	**42nd**	**49th**	**56th**	
MDOBV 01	11	S	S	S	S	S	S	S	S	S	S
	12	S	S	S	S	S	S	S	S	S	S
MDOBV 02	21	S	S	S	S	S	S	S	S	S	C
	22	S	S	S	S	S	S	S	S	S	S
MDOBV 03	31	S	S	S	S	S	S	S	S	S	S
	32	S	S	S	S	S	S	S	S	S	C
MDOBV 04	41	S	S	S	S	S	S	S	S	S	C
	42	S	S	S	S	S	S	S	S	S	S
MDOBV 05	51	S	S	S	S	S	S	S	S	S	S
	52	S	S	S	S	S	S	S	S	S	S
MDOBV 06	61	S	S	S	S	S	S	S	S	S	C
	62	S	S	S	S	S	S	S	S	S	C

Table 4: Sterility data of Opened Multi-Dose bOPV Vials stored at $+4^0$C.
Abbreviations: S stands for Sterility; C stands for Contamination.

Batch		pH value at Day									
Code	**Vial No.**	**Sampling**	**07th**	**14th**	**21st**	**28th**	**35th**	**42nd**	**49th**	**56th**	**63rd**
MDOBV 01	11	6.63	6.52	6.52	6.52	6.54	6.55	6.56	6.57	6.6	6.63
	12	6.62	6.51	6.51	6.51	6.53	6.54	6.55	6.57	6.59	6.61
MDOBV 02	21	6.61	6.53	6.53	6.53	6.53	6.53	6.55	6.58	6.6	DPC
	22	6.61	6.51	6.51	6.51	6.52	6.53	6.55	6.58	6.61	6.62
MDOBV 03	31	6.62	6.52	6.52	6.52	6.53	6.55	6.56	6.57	6.58	6.61
	32	6.63	6.52	6.52	6.52	6.53	6.54	6.56	6.58	6.61	DPC
MDOBV 04	41	6.65	6.51	6.51	6.51	6.52	6.53	6.55	6.58	6.6	DPC
	42	6.66	6.53	6.53	6.53	6.54	6.55	6.56	6.57	6.6	6.62
MDOBV 05	51	6.61	6.5	6.5	6.5	6.51	6.53	6.54	6.55	6.6	6.61

Table 5: pH data of Opened Multi-Dose bOPV Vials stored at $+4^0$C by pH meter.

Antibiotic efficacy of Kanamycin in bOPV

Antibacterial activity of kanamycin antibiotic containing in bOPV vial was performed against *Bacillus subtilis* (Gram positive bacteria) and *Escherichia coli* (Gram negative bacteria) by disc diffusion. Preliminary activity of kanamycin was conducted with different quantity i.e. 6, 9, 12, 15 and 18µg/disc. Control disc containing sterile distilled water was performed simultaneously with all experiments to check the activity of the sterile distilled water as a solvent. In present study, *Bacillus subtilis* and *E. coli* were used to check antibacterial efficacy of kanamycin and the bacteria were maintained on the nutrient agar medium.

In present investigation, all quantities of kanamycin showed significant inhibitory activity against *Bacillus subtilis* with zone of inhibition ranging from 19.00 mm to 26.00 mm in diameter and against *E. coli* with mean of zone of inhibition ranging from 20.00 mm to 27.00 mm in diameter. Antibacterial activity of standard kanamycin against bacteria at 15µg/disc is 24.00 mm for *Bacillus Subtilis* and 25.00 mm for *E.Coli.*

Kanamycin efficacy in opened multi-dose bOPV vials stored at $+4^{\circ}$C was performed by Disc-Diffusion method. The inhibition zone size was observed up to 49th day shows no variation, at the end of 08th week some of the samples shows

lesser inhibition zone against the bacterial strains, at the end of 09th week, there is no zone formed in each samples. The results for Kanamycin antibiotics efficacy test are shown in Table 6.

Batch			Inhibition zone size at Day									
Code	**Vial No.**	**Strain Code**	**Sampling**	**07th**	**14th**	**21st**	**28th**	**35th**	**42nd**	**49th**	**56th**	**63rd**
MDOBV 01	11	BSGP	24	24	24	24	24	24	24	24	20	0
		BSGN	25	25	25	25	25	25	25	25	22	0
	12	BSGP	24	24	24	24	24	24	24	24	21	0
		BSGN	25	25	25	25	25	25	25	25	25	0
MDOBV 02	21	BSGP	24	24	24	24	24	24	24	24	20	C
		BSGN	25	25	25	25	25	25	25	25	23	C
	22	BSGP	24	24	24	24	24	24	24	24	22	0
		BSGN	25	25	25	25	25	25	25	25	24	0
MDOBV 03	31	BSGP	24	24	24	24	24	24	24	24	20	0
		BSGN	25	25	25	25	25	25	25	25	23	0
	32	BSGP	24	24	24	24	24	24	24	24	21	C
		BSGN	25	25	25	25	25	25	25	25	24	C
MDOBV 04	41	BSGP	24	24	24	24	24	24	24	24	20	C
		BSGN	25	25	25	25	25	25	25	25	24	C
	42	BSGP	24	24	24	24	24	24	24	24	23	0
		BSGN	25	25	25	25	25	25	25	25	25	0
MDOBV 05	51	BSGP	24	24	24	24	24	24	24	24	20	0
		BSGN	25	25	25	25	25	25	25	25	25	0
	52	BSGP	24	24	24	24	24	24	24	24	23	0
		BSGN	25	25	25	25	25	25	25	25	24	0
MDOBV 06	61	BSGP	24	24	24	24	24	24	24	24	23	C
		BSGN	25	25	25	25	25	25	25	25	25	C
	62	BSGP	24	24	24	24	24	24	24	24	21	C
		BSGN	25	25	25	25	25	25	25	25	24	C

Table 6: Kanamycin Sensitivity in Opened multi-dose bOPV Vials at $+4^0$C by Disc-Diffusion Method.

Vaccine Vial Monitor (VVM) status

The Vaccine vial monitor is a new type of monitor device applied directly to each vaccine vials by the manufacturer. The VVM progressively changes color with heat exposure and gives a visual indication when exposure has occurred. The vaccine itself of course exhibits no visible change with heat exposure.

A total five bOPV batches were involved in the study and optical density (OD) of vaccine vial monitor (VVM) of each bOPV were recorded by densitometer on weekly interval after use and then stored at +4˚C. The study was carried out up to 63 day or 09 weeks. There is no change found in OD of the VVM up to fourth week at +4˚C. After this, OD's of the VVM were recorded continuously decreased from fifth to eight week without any fixed pattern. On the basis of present study, we observed that open multi-dose of bOPV vials can be used for 56 day or 08th week because OD of bOPV VVM was recorded within acceptable criteria. On 63rd day, the same had found below the acceptable OD Value criteria ($\geq 0.25 \pm 0.02$). Recorded OD value of each vial VVM of sample batches were recorded and summarized in Table 7.

Batch		OD Value at Day									
Code	Vial No.	Sampling	07th	14th	21st	28th	35th	42nd	49th	56th	63rd
MDOBV 01	11	0.34	0.34	0.34	0.34	0.33	0.3	0.29	0.26	0.25	0.22
	12	0.34	0.34	0.34	0.34	0.32	0.29	0.28	0.26	0.24	0.21
MDOBV 02	21	0.35	0.35	0.35	0.35	0.34	0.33	0.31	0.29	0.26	0.18
	22	0.34	0.34	0.34	0.34	0.33	0.31	0.3	0.28	0.26	0.2
MDOBV 03	31	0.34	0.34	0.34	0.34	0.33	0.3	0.29	0.26	0.25	0.22
	32	0.34	0.34	0.34	0.34	0.32	0.29	0.28	0.26	0.24	0.21
MDOBV 04	41	0.35	0.35	0.35	0.35	0.34	0.33	0.31	0.29	0.26	0.18
	42	0.34	0.34	0.34	0.34	0.33	0.31	0.3	0.28	0.26	0.2
MDOBV 05	51	0.34	0.34	0.34	0.34	0.33	0.3	0.29	0.26	0.25	0.22
	52	0.34	0.34	0.34	0.34	0.32	0.29	0.28	0.26	0.24	0.21
MDOBV 06	61	0.35	0.35	0.35	0.35	0.34	0.33	0.31	0.29	0.26	0.18
	62	0.34	0.34	0.34	0.34	0.33	0.31	0.3	0.28	0.26	0.2

Table 7: VVM data of Opened Multi-Dose bOPV Vials stored at $+4^0$C.

Laboratory Investigations of second type of opened bOPV Vials

Simultaneously, the same laboratory investigations were also performed on weekly intervals basis [01st week (07th day) to 09th week (63rd day) to check the safety and efficacy of second type of each batch sample vials that were stored at -20˚C temperature. The sample vials were used for laboratory investigations like potency, identity, sterility, pH, kanamycin activity and VVM status only after thawing at +4˚C for 10 to 15 minutes. The results were found consistency (within acceptable limits) of each quality test throughout the study (data not shown). The obtained results of quality attributes were used as reference standard for first type of stored samples at +4˚C temperature and were compared with each other.

DISUSSION

Currently, there have already been published literature and available on polio viruses, poliomyelitis, history, taxonomy, properties, clinical manifestation, & pathogenesis as well as conventional and hypothetical future vaccines including currently using vaccines Oral Polio Vaccine (OPV) and Inactivated Polio Vaccine (IPV)), vaccination schedules & their administration routes and it is also available on public domain [15 -17]. According to World Health Organization (WHO), poliomyelitis is well known viral contagious disease among five year age of children worldwide. It has already been eradicated from many developed countries through vaccination with Sabin strains formulated live attenuated trivalent and bivalent Oral Polio Vaccine such as USA [18], but not in developing countries such as Pakistan, Afghanistan etc. Many scientific and technical factors are responsible and playing tremendous role to success and/or failure of OPV and its vaccination program especially Vaccine-Associated Paralytic Polio (VAPP), Vaccine-Derived Paralytic Polio (VDPP), cold chain system, short shelf life vaccine and trained personnel [19 -22]. In addition, storage of OPV as finished product is well established to maintain its quality attributes and it is also safe and potent for pediatric use. As per WHO recommendation, the vaccine is potent if stored at -20˚C or below -20˚C until the expiry date indicated on the vial i.e. for a period of two years from the date of manufacture. It can be stored for up to six months between +2˚C and +8˚C. It means that OPV is easily damaged by heat but is not harmed by freezing.

Now-a-days, very few literatures have searched through various search engines on internet. One of them was published policy in September month of 2014 by WHO. The Policy for Multi-Dose Vail applies to all vaccine vials, including those

that have been transported in the cold chain for outreach immunization sessions, provided that standard handling procedures are followed for example bivalent Oral Polio Vaccine (bOPV). This means that opened vials can be used in subsequent immunization sessions, in different sites, over several days, provided that they have been stored in vaccine carriers or cold boxes with a suitable number of frozen icepacks [4]. According to the policy, safe and efficacious use of bOPV is only 28th day (04 weeks) after opening, if it is stored at +4°C and the current investigation is also suggested to safe and efficacious uses with accepted limit of all quality attributes up to 56th day (08 weeks) under laboratory conditions.

CONCLUSION

World Health Organization allow opened vaccine vials, including bivalent oral polio vaccine, to be used in subsequent immunization sessions up to 28th day after opening with assurance of vaccine safety and efficacy. Once opened, multi-dose vials should be kept between +2°C and +8°C (Ideally at +4°C). It can be stored for up to six months at +4^{0}C (when the vials are not opened). Vaccine is potent if stored at not higher than -20°C until the expiry date indicated on the vial. The vaccine should be used only when the Vaccine Vial Monitor attached on the vial has not reached the discard point. Therefore care should be taken to avoid the situations, especially during transport. Since, the bOPV delivered to the child at the temperature of +4°C, it would necessary to examine the stability of the vaccine at the exposure. Current investigation suggested that opened multi-dose vials of bOPV are qualified for their safe and efficacy use up to 08 week or 56th day under the laboratory conditions, if these vials are stored at +4°C.

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CONFLICT OF INTEREST

The authors have disclosed no potential conflict of interest.

REFERENCES

1. World Health Organization (WHO) (2015) Meeting of the Strategic Advisory Group of Experts on Immunization, conclusion and recommendations. Weekly Epidemiological Record 90: 261-278.
2. WHO Policy Statement (1995) The use of opened vials of vaccine in subsequent immunization sessions. World Health Organization (WHO), Geneva.
3. WHO Policy Statement (2000) The use of opened multi-dose vials of vaccine in subsequent immunization sessions. World Health Organization (WHO), Geneva.
4. WHO Policy Statement (2014) Multi-dose Vial Policy (MDVP) “Handling of multi-dose vaccine vials after opening. World Health Organization (WHO), Geneva.
5. Indian Pharmacopeia (2018) The Indian Pharmacopeia Commission 1st (Vol.), Government of India, Ministry of Health and Family Welfare, India Pharmacopeia Laboratory, Ghaziabad.
6. WHO expert committee on biological standardization requirements for poliomyelitis vaccine (oral) technical report series, No. 800, 1990.

7. Determination of total virus content of OPV (1996) Laboratory manual on potency determination of oral polio vaccine. Regional polio reference laboratory National institute of communicable diseases. New Delhi: 13-17.
8. Cell counting by using haemocytometer (1996) Laboratory manual on potency determination of oral polio vaccines Regional polio reference laboratory NICD, New Delhi: 25-26.
9. Melnick JL, Wenner HA, Phillips CA (1979) Enteroviruses, In: Lennette EH, Schmidt NJ (Eds.), Diagnostic procedures for viral, rickettsial and chlamydial infections. Washington American Public Health Association; 5th (Edn.): 487-491.
10. Spearman C, Kaerber G (1974) In: Bibrack B, Whittmann G (Eds.), Virologische Arbeitsmethoden. Stuttgart: Fischer Verlag: 37-39.
11. Kumar A, Tomar V (2019) Development of potent and stable lyophilized Sabin formulated live attenuated bivalent oral polio vaccine. European Journal of Biomedical and Pharmaceutical Sciences 6(11): 218-226.
12. World Health Organization (WHO) (2012) Forty-six WHO Experts Committee on specification for pharmaceutical preparation. International Pharmacopeia in 4th (Edn.), Document QAS/11.413FINAL.
13. Kumar A, Singh SP, Verma S, et al. (2014) Quantitative evaluation of kanamycin in single dose of oral polio vaccine in vitro ISST. Journal of Applied Chemistry 5(1): 9-12.
14. Umit H, Kartoglu (2019) The Book of VVM: Yesterday-today-and-tomorrow, Extensio et. Proggressio.
15. Singh AK, Kumar A (2011) Poliomyelitis: viruses and vaccines. ASMJ 6(7).
16. Kumar A, Tomar V (2019) Review on current polio vaccines: Vaccination schedules and administration routes. Journal of Pharma Research 8(4): 194-204.
17. Chandrakant L (2014) A brief history of vaccine and vaccination in India. Indian Journal of Medical Research 139(4): 491-511.
18. Aylward B, Tangermann R (2011) The global polio eradication initiative: lessons learned and prospects for success. Vaccine 29(4): D80-D85.
19. Platt LR, Estívariz CF, Sutter RW (2014) Vaccine associated paralytic poliomyelitis: A review of the epidemiology and estimation of the global burden. The Journal of Infectious Diseases 210(1): S380-S389.
20. Polio Global Eradication Initiative (2014) Background and technical rationale for introduction of one dose of inactivated polio vaccine (IPV) in routine immunization schedule.
21. Polio Eradication and Endgame Strategic Plan 2013-2018
22. Brandau DT, Jones LS, Wiethoff CM, et al. (2003) Thermal stability of vaccines. Journal of Pharmaceutical Sciences 92(2): 218-231.