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RESEARCH ARTICLE

Real-time Study for Uses of Opened Multidose Vials of Live Attenuated Bivalent Oral Polio Vaccine

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ABSTRACT

Introduction: This study was aimed to find out real-time for the use of opened multidose vials of live attenuated bivalent oral polio vaccine (bOPV) containing type I and III. **Materials and Methods:** The study was performed in continuation of a preliminary study with opened multidose bOPV vials of only five batches and established use of the safe and efficacious form of the vaccine up to 56th day (8 weeks) after opening and then stored at +4°C temperature. In the current study, collected samples from ten bOPV batches were divided into two types; one type samples were stored at +4°C temperature and second type samples were stored at −20°C temperature and both types samples were studied in the real-time study for safe and efficacious use of the opened multidose vials in subsequent immunization sessions on weekly intervals from 1st week to 8th week. A total of six critical and quality attributes of the vaccine were studied, that is, potency, identity, sterility, pH, kanamycin activity, and vaccine vial monitor (VVM) status. After completion of the 8th week, the study was performed to find out the real-time and temperature for the uses of the vaccine, and all quality attributes were performed on a daily basis to the withdrawal of the subsequent sessions. **Results and Discussion:** Results of all the quality attributes (potency, identity, sterility, pH, kanamycin activity, and VVM status) were qualified the acceptance criteria as mentioned in Indian Pharmacopeia-2018 and obtained results of one type samples (stored at +4°C temperature) were compared with the same batches second type samples; stored at −20°C temperature use as a reference standard. The real-time study suggested that opened multidose live attenuated bOPV vials can use in safe and efficacious conditions up to 59th day after opening and stored at +4°C temperature. **Conclusion:** This study provides scientific strength to decide the real-time uses of efficacious and safe conditions of opened multidose live attenuated bOPV vials after withdrawal subsequent sessions only up to 59th day after opening and stored at +4°C temperature.

Keywords: Dose withdrawal, duration, efficacy, and safety, opened multidose bivalent oral polio vaccine vial, subsequent sessions, temperature, validation

INTRODUCTION

At present, live attenuated bivalent oral polio vaccine (bOPV) is using in pulse-polio and national immunization programs after switching from trivalent oral polio vaccine (tOPV) containing monovalent type I, II, and III to bOPV containing monovalent Type I and III after April 2016.[1] The vaccine vial contains 20 doses and it is considered as a multidose vaccine vial. The multidose vaccine vial after open should be stored in between +2°C and +8°C, including monovalent, bivalent, and tOPV as per guidelines of the World Health Organization (WHO) policy statement (2014).[2] Multidose vials of bOPV from which one or more doses of vaccine

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have been removed during an immunization session may be used in subsequent immunization sessions for up to a maximum of 28 days after opening the vaccine vial stored in between +2°C and +8°C temperature. Our laboratory has already been conducted a preliminary study to determine the safe and efficacious use of opened multidose bOPV vial up to 8th weeks or 56th day if the vials are stored at +4°C temperature.[3] The current WHO guidelines have addressed two concerns for the use of vaccine in opened multidose vials in subsequent sessions; the first concern is the potency of vaccine and the second concern is the safety of vaccine administration. It has well established the impact of time and other factors on potency and safety of opened multidose vials and the risk of contamination is higher in a multidose vial than in a single-dose vial because the vaccine is repeatedly exposed every time for dose withdrawal purpose. Therefore, there is a need to determine the potency in opened multidose vaccine after overtime for the stability of the particular vaccine and the safety of the vaccine in a multidose vial is primarily dependent on the risk of contamination with a pathogenic organism and bacteriostatic activity of preservatives in the vial. In the account of vaccine potency and safety issues, our laboratory has decided to extend the previous study for safe and potent consumption of multidose bOPV vial on a real-time basis up to the last twentieth dose. This study was further started previously up to 8th-week completion to conclude the real-time at +4°C temperature for the uses of the vaccine and after that, all quality attributes were performed on a daily basis to the withdrawal of the subsequent sessions.

MATERIALS AND METHODS

This study turned into performed in Bharat Immunological and Biologicals Corporation Limited (BIBCOL), Village Chola, Bulandshahar, Uttar Pradesh, India. In 1989, BIBCOL has well-certified Good Manufacturing Practices and Good Laboratory Practices accredited laboratory for production and quality control testing of the oral polio vaccine and also have well-qualified, dedicated, and experienced manpower explosive at each level for production, quality control, and quality assurance. Validated all of the kinds of the involved types of equipment and processes have been done in-house and third party by qualified vendors on an annual basis. In-house validation of all production and quality attributes equipment and crucial processes were performed by the experienced officer (not below the rank of Manager, Quality Assurance) on an annual basis and the documents were prepared and verified by the senior officers at least Assistant General Manager rank officer.

Collection, preparation, and storage of the vaccine samples

A sample of multidose vials from ten bOPV batches with batch codes was collected with previously described exclusion criteria. The batches were qualified the acceptance criteria of the quality attributes as mentioned in Indian Pharmacopeia-2018; the potency of type I (not $<10^{6.00}$), the potency of type III (not $<10^{5.80}$), sterility (Sterile product), pH (6.50–6.80), vaccine vial monitor (VVM) ($\geq 0.25 \pm 0.02$), and kanamycin antibiotics (15 μg per dose).[4] The procedure for the sample handling, preparation, and storage were adopted as previously described by Kumar *et al*.[3] Finally, the prepared samples from ten bOPV batches were divided into two types based on stored temperature after opening and also sealed aseptically with pre-sterile dropper all the vaccine vials; one type of the samples was stored at +4°C temperature and the second type of the samples from the same batches was also stored at −20°C temperature. Detail of the studied samples is described in Tables 1 and 2 summarized detail of required bOPV sample vials from every batch to carry out six quality attributes, namely, potency, identity, sterility, pH, kanamycin activity, and VVM. Detail of studied bOPV batches was given with batch code from MDOBV 001 to MDOBV 010 and the collected bOPV samples were divided into two types on the basis of stored temperature; one type was stored at +4°C temperature and second type was stored −20°C temperature.

Quantity of bOPV sample vials from each batch was summarized to perform all quality attributes,

Table 1: Labeling description of multidose sample vials of bOPV batches

Batch code	Opened vials of vaccine samples stored at	
	+4°C temperature (one-type)	−20°C temperature (second-type)
MDOBV 001	MDOBV 011	MDOBV 021
MDOBV 002	MDOBV 012	MDOBV 022
MDOBV 003	MDOBV 013	MDOBV 023
MDOBV 004	MDOBV 014	MDOBV 024
MDOBV 005	MDOBV 015	MDOBV 025
MDOBV 006	MDOBV 016	MDOBV 026
MDOBV 007	MDOBV 017	MDOBV 027
MDOBV 008	MDOBV 018	MDOBV 028
MDOBV 009	MDOBV 019	MDOBV 029
MDOBV 010	MDOBV 020	MDOBV 030

Table 2: Detail of required bOPV sample vials from each batch

Quality attributes	Test-wise required bOPV sample vial (in no.) for both types	
	One-type (+4°C temperature)	Second-type (−20°C temperature)
Potency	2	2
Identity	2	2
Sterility	1	1
pH	1	1
Kanamycin activity	1	1
VVM status	1	1
Total	8	8

namely, potency, identity, sterility, pH, kanamycin activity, and VVM.

Quality attributes of the opened vaccine vials

In the present study, a total of six quality attributes are studied in both types of samples of opened bOPV vials from the ten batches such as potency, identity, sterility, pH, kanamycin activity, and VVM. These tests were performed on a weekly interval basis up to 56th day (8th week) and the study also continued after completion of 8th week on daily interval till 63rd day after opening the vaccine vials.

Potency test

Cell culture technique was used to determine the potency of both types of samples opened vial from each bOPV batch in single-dose containing type I and type III individually.[5] The standard antisera of bOPV (type I and III for reference purpose) and cell line of human epithelial (HEp)-2 (Cincinnati) were given to BIBCOL by Central Drug Laboratory, Kasauli, Himachal Pradesh, India. In our laboratory, standard cell culture techniques were used to prepare a confluent monolayer of HEp-2 cell in minimum essential medium (MEM) with 10% fetal bovine serum (FBS) in 25 cm^2 tissue culture flask[6] and the viable cell count was done with the help of trypan blue dye using Neubauer hemocytometer.[7] A 10,000 cells/0.1ml concentration of the cell per well was adjusted by adding MEM with 5% FBS in 96-flat bottom microtiter plate for the experiment. Serially dilution of each batch samples and standard antisera of type I and III was performed 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 $10^{-7.5}$. 0.05 ml volume of each the prepared dilution was dispensed into each of 8 wells of a flat-bottomed microtiter plate with lid, starting from higher dilution to lower dilution. The plates were incubated at 35.5°C (± 0.5°C) for 3 h 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/0.1 ml concentration) in MEM with 5% FBS was added to all the wells. The plate was sealed and incubated at 35.5°C (+0.5°C) in a carbon dioxide incubator. The plates were read microscopically on a daily basis after 3rd, 5th, and 7th day using an inverted microscope for cytopathic effect[8] and Kaerber's formula was used to obtain titer per dose (0.1 ml) of type I and type III in both types of vaccine samples.[9] Positive and negative controls for the potency test were performed individually and the result of the test was valid when positive and negative control of each experiment performed independently.

Identity test

The neutralization method was used to identity type I and III in the opened bOPV sample vials as the previously described method of Kumar and Tomar (2019).[10] 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 were performed separately as a confirmatory test to find out the impact of storage condition at +4°C and −20°C temperature of bOPV vials, and the results of both types of the samples were also compared with each other.

Sterility test

The direct inoculation method for sterility test was used in both types of the opened bOPV samples on Nutrient agar medium (NAM).[11] In brief, one dose (100 µl) of each opened bOPV samples was taken with the help of an inoculation loop for streaking on pre-prepared NAM Petri-plates after passes the growth-promoting test and all the plates were transferred in incubated for 14 days to check the bacterial growth at 35 ± 2°C and also check fungal growth at 22 ± 2°C and the experiment with each sample of the vaccine was performed in triplicate. Positive and negative controls were also performed for bacterial and fungal growth individually. After completion of the incubation period, these plates were examined by two experts and the results were recorded.

Determination of pH by digital pH meter

Digital pH meter was used to determine the pH of both types; opened vials of bOPV samples were determined using digital pH meter as described by Kumar *et al*.[3] In brief, type one samples of bOPV were used in the as such form because it was stored at +4°C and the second type of the bOPV samples was stored at −20°C, it was thawing first before checking the pH. Finally, the pH of the vaccine samples was measured and recorded after the calibration of the equipment using the standard buffers as per the standard procedure.

Kanamycin activity test

The disk diffusion method was used to determine kanamycin activity in a single dose (100 µl/disc) of the opened bOPV as described by Kumar *et al*.[12] In brief, disks (size 5 mm diameter) were prepared individually by soaking and drying of a single dose of both types of samples of the vaccine at room temperature under aseptic conditions in laminar airflow. In this method, pre-sterile Petri plates containing 20 ml of NAM and 1.0×10^6 cells/ml concentrations of Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacterial strains were used and the single dose of the vaccine containing pre-prepared disk was aseptically transferred on the NAM with bacterial strains, individually. All the plates were incubated at 37 ± 1°C for 24 h. After completion of the incubation, kanamycin activity in both types of the vaccine samples was examined after measuring the diameter of the inhibition zone in millimeters. Finally, kanamycin activity by disk diffusion method was recorded and compared to the obtained results in both types of bOPV samples.

Optical density (OD) of VVM test

Densitometer was used to measure OD of VVM of both types of vaccine samples in bOPV batches.[13] The OD of each vial VVM in the vaccine sample vials was measured at least 3 times of reference circle and indicator square on a weekly interval after used for quality testing purpose and the mean was calculated for reference circle and indicator square, individually. The calculated mean of indicator square was substrate from reference circle mean and the OD value was recorded for further laboratory investigation purposes.

Statistical analysis

Statistical analysis of the quality attributes was done as the previous method of Kumar *et al*.[3] In this study, experiments for the quality attributes in both types of vaccine samples were initially designed and performed in triplicate. At the end of the experiment, the test-wise mean was calculated and the value was recorded as a result and summarized in the respective table for each quality attributes. The results of each test were checked and observed by two experts.

RESULTS AND DISCUSSION

In this study, only ten bOPV batches with the code were studied, namely, MDOBV 001 to MDOBV 010 and the samples were divided into two types based on storage temperature and labeled with the codes; MDOBV 011 to MDOBV 020 for stored at +4°C and MDOBV 021 MDOBV 030 stored at −20°C temperature. Both types of the sample vials were used to check the status of quality attributes (potency, identity, sterility, pH, kanamycin activity, and VVM status) after dose withdrawal in subsequent sessions on weekly interval up to 8th week (56th day) and the results of each quality test were found consistency within acceptable limits as above-mentioned. Finally, the obtained results of both types of vaccine samples were compared with each other. The current study was further proved that the bOPV batches are usable in terms of safety and potency up to 56th day after opening the multidose vials and stored at +4°C temperature. After the completion of the 56th day of both types of sample vial, further study was continued with type one samples on daily intervals from 57th to 63rd day and the results are compiled in some qualified samples out of the total studied batches [Table 3]. All quality attributes were found in acceptable limits up to the 59th day of the study in the studied batches. However, there was a gradual decrease in the number of qualified batches based on their quality attributes. On the 63rd day, only four batches were qualified in potency, identity, sterility, and pH test, while three batches were qualified in the Kanamycin activity test and six batches were qualified in VVM status.

There was the main difference in the withdrawal of subsequent sessions on the daily interval in the study, while it was on weekly intervals in the previous study. Finally, Table 4 has compiled to compare the result of previous and current studies in the percentage of the qualified batches on quality attributes wise on 56th and 63rd day in both types of bOPV samples.

All studied bOPV batches were qualified in both type 100% on 56th day in previous and current studies, while overall studied bOPV batches were qualified 100% on 63rd day except 10% not qualified sterility by second type sample in the study. In one type samples of the previous study on 63rd day, the quality attributes were found qualified 100% by potency, kanamycin activity, and VVM status, while 60% qualified by identity, sterility, and pH

Table 3: Result of one-type samples of bOPV batches

Quality attributes	Results recorded on the day							
	57th	58th	59th	60th	61st	62nd	63rd	57th
Potency	10/10	10/10	10/10	10/10	9/10	7/10	6/10	4/10
Identity	10/10	10/10	10/10	10/10	9/10	8/10	7/10	4/10
Sterility	10/10	10/10	10/10	10/10	9/10	7/10	6/10	4/10
pH	10/10	10/10	10/10	10/10	8/10	7/10	6/10	4/10
Kanamycin activity	10/10	10/10	10/10	10/10	9/10	7/10	5/10	3/10
VVM status	10/10	10/10	10/10	10/10	9/10	8/10	7/10	6/10

Table 4: Comparison of both types of samples results at 56th and 63rd day in previous and current study

Quality attributes	Results recorded on the day							
	Previous studied samples				Current studied samples			
	One type		Second type		One type		Second type	
	56th	63rd	56th	63rd	56th	63rd	56th	63rd
Potency	100	100	100	100	100	40	100	100
Identity	100	100	100	100	100	40	100	100
Sterility	100	60	100	100	100	40	100	90
pH	100	60	100	100	100	40	100	100
Kanamycin activity	100	60	100	100	100	30	100	100
VVM status	100	100	100	100	100	60	100	100

tests only. While, the quality attributes were found one type sample qualified in the current study; 40% by potency, identity, sterility, and pH, 60% by VVM status, and 30% by kanamycin activity test only.

One-type samples of the bOPV batches were used to perform six quality attributes such as potency, identity, sterility, pH, kanamycin activity, and VVM status on a daily basis from 56th to 63rd day after opening the vaccine vials. The mean value was recorded as a result.

The quality attributes, namely, potency, identity, sterility, pH, kanamycin activity, and VVM status were studied at 56th and 63rd day of one-type and second-type samples of all bOPV batches in the current study and obtained results were compared with the previous study. The mean value was recorded as a result.

Simultaneously, all quality attributes were also performed of second type bOPV samples and obtained results were compared with each other in previous and current studies, as shown in Table 4. The second type of sample was used as a reference standard for first type samples of bOPV. The results of second type samples were found consistency (within acceptable limits) of each quality attribute throughout the previous study, but 10% of second type samples were found out of the acceptable limit of sterility test. Based on the current study, this was happened due to the change in sample withdrawal on a daily interval.

Overall results show that opened multidose bOPV vials may consume up to 59th day with safe and potent quality of the vaccine instead of 56th day as per the previous finding. The current study indicates the frequency of interval is also a crucial parameter to ensure the vaccine quality in multidose vials. Table 5 is showing various conditions to use safe and potent multidose bOPV vials.

Based on the available literature, live attenuated oral polio vaccine is a well-established and used in many countries for the prevention of polio through pulse and national immunizations programs. According to the WHO, poliomyelitis is well known viral contagious disease among 5-year age of children worldwide. It has already been eradicated from many developed countries through vaccination with Sabin strains formulated live attenuated trivalent and bOPV such as the USA,[14] but not in developing countries such as Pakistan and Afghanistan. Many scientific and technical factors are responsible and playing a tremendous role in the success and/or failure of OPV.[15-17] Out of all these, the vaccine storage is considered as a key factor to maintain its quality attributes to deliver a safe and potent vaccine 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, that is, for 2 years from the date of manufacture. It can be stored for up to 6 months between +2°C and +8°C.

In addition to the above, WHO has issued guidelines for all multidose vaccine vials in 2014, including OPV.[4] The policy for multidose vial 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, 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. Despite this, limited studies were conducted and published in

Table 5: Conditions for safe and potent use of opened multidose bOPV vials

Condition	Temperature	Day	Duration (in interval)	Reference
Ideal	+4°C	1st	within 1–4 h	Ref. no. 2
WHO recommended	+4°C	28th	Different subsequence sessions	Ref. no. 2
Optimized	+4°C	56th	8 weeks	Ref. no. 3
Optimized	−20°C	63rd	9 weeks	Ref. no. 3
Optimized	+4°C	59th	8 weeks and followed by another 3 days	Current study
Optimized	−20°C	63rd	9 weeks	Current study®

Legend 4: Summary for safe and potent use of opened multidose bOPV vials was prepared in ideal, optimized conditions and as recommended by WHO and correlate with sessions interval in duration and days after storage at +4°C and −20°C temperatures. ®Except 10% of the samples not qualified in the sterility test

this regard. In recent, Kumar *et al*. investigated the use of safe and efficacious bOPV doses with an acceptable limit of all quality attributes up to 8 weeks (56th day) under laboratory conditions if the vaccine stored at +4°C temperature and the dose withdrawal subsequent on the weekly interval.[3] In continuation of this, the current study is conducted for further evaluation of the use of safe and efficacious vaccine on real-time and duration, thereafter obtained results were compared previous study with found slightly differed. The current results revealed that bOPV can be used safe and efficacious form with all quality attributes up to 59th day after opening the vial and stored at +4°C temperature instead of 56th day. Another finding suggested that the handing of the vaccine vials is very crucial during immunization and also trained the personnel for immunization purposes because 10% of the second type of the vaccine (stored at −20°C temperature) were not meet the acceptance criteria of sterility test.

CONCLUSION

WHO allows opened multidose vaccine vials, including the live attenuated bOPV, to be used in subsequent immunization sessions up to the 28th day after opening with assurance of vaccine safety and efficacy. It was decided after considering various points such as handing of vaccine vials, storage conditions during its immunization, administration route, dose withdrawal, and subsequent sessions. The current study was conducted under laboratory condition to find out the real-time for safe and efficacious use of opened multidose live attenuated bOPV at +4°C temperature (stored condition of one type samples) and −20°C temperature (stored condition of second type samples) and the sufficient data of all quality attributes are compiled in an easy and scientific manner after taking in account of all the observations. Finally, the current investigation provides scientific strength to decide the real-time uses of efficacious and safe conditions of opened multidose live attenuated bOPV vials after withdrawal subsequent sessions only up to 59th day after opening, if these vials are stored properly at +4°C.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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