Original Article

IJCIH
Converging Healthcare & Technology

**International Journal of Convergence in Healthcare**

Published by
IJCIH & Pratyaksh Medicare LLP

www.ijcih.com

# Validation Protocol of Vaccine Vials for Quality Storage Temperature under Laboratory Conditions

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## Abstract

Quality of liquid vaccine product in vaccine glass vials is a critical issue and an also important to deliver it with safe and potent conditions to the recipient for preventing disease burden in public. In case of multi-dose vaccine vials, safety and potency delivery to the recipient is more critical as compare to single dose vaccine vial. Vaccine vial of liquid multi-dose oral polio vaccine is under taken for validation study during its storage conditions. Therefore, current study was designed and performed with objective for validating to prevent breakage of liquid bivalent oral polio vaccine (LbOPV) vials with acceptance criteria of their quality attributed during the period of two years from manufacturing to expiry date of the vaccine for quality storage at -200C ±20C temperature in deep freezer under laboratory conditions.

***Keywords:*** *Validation, Deep freezer, Quality storage, Vaccine, laboratory conditions, Quality attributes.*

## Introduction

According to WHO, Good Manufacturing Practices (GMP) covers complete aspects of manufacturing of quality product with ensured quality for product consistence and controlled to meet quality standards for indented use as described in table 1. Quality assurance of the product is not just produced and tests the product to meet its final specification, as well as every batch of the product was produced by the same procedures, conditions and trained personnel to maintain its consistency with controlled the quality standard [1].

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As per GMP guidelines, consistency and controlled with quality stands are maintained by various ways. Validation is a systematic, crucial and effective way and also plays an important role for producing quality product consistently. Validation ensures and control of facility systems, equipments, processes, and tests procedures. According to the WHO "Validation is defined as the establishing by documented evidence which provides a high degree of assurance that will consistently perform according to the intended specified outcomes". Validation has great impact and an effective tool that is performed for analytical tests, equipments, facility systems like air, water, stream and for processes cleaning, sterilization, ascetic processes (Blending, filling etc.), and other manufacturing & process quality control tests. The system and process have been validated; it is expected that it remains control without any changes. If any change or modification in system, process, and equipment replaced or relocate or new are made, revalidation is performed. Critical equipment and process are routinely revalidated at annual interval to maintain the control on process and equipment [1,2].

Bharat Immunologicals & Biologicals Corporation Limited (BIBCOL) is a biotechnology industry situated in Bulandshahr city of Uttar Pradesh, India and actively engaged in production of Oral polio vaccine (OPV) in GMP approved facility. The facility has defined & approved process and quality testing for the vaccine manufacturing. In-house validation of equipments is performed on annual basis like Autoclave, Dry heat sterilizers (DHS) etc. [3,4]. Deep freezer is one of the critical equipment due to storage of finish vaccine product. Therefore, there is need to prevent vaccine vial breakage of the stored product during this life cycle as shown in figure 1. The life cycle comprises from date of manufacturing to expiry is two years and retainer vaccine vials samples is stored another one years for future quality testing purpose [5].

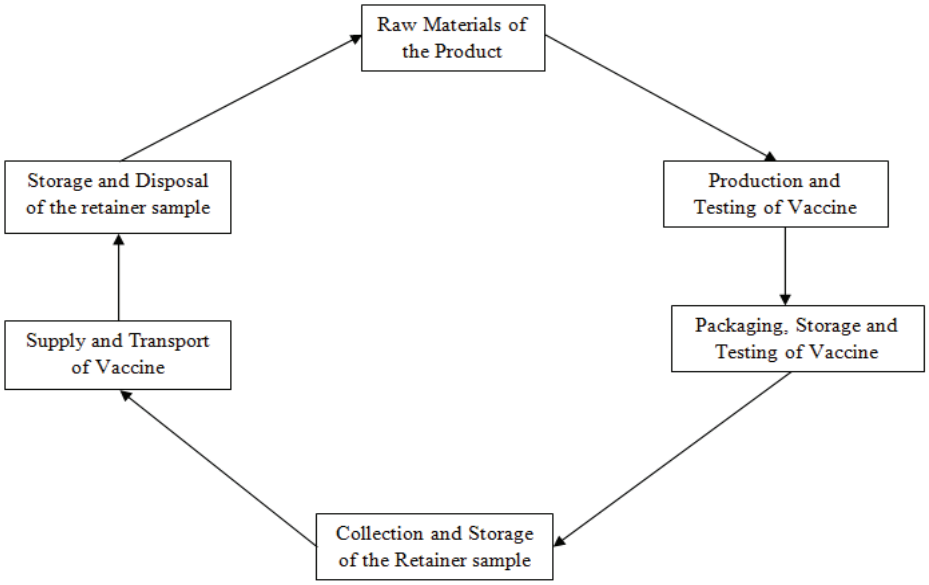


*Figure 1: Life cycle of the Vaccine*

Validation provides a high degree of assurance that a specific process, method, or system will consistently produce a result meeting pre-determined acceptance criterion of the product. There is need a written pre-defined procedure with planed manner in form of approved document and it is called as a validation protocol. The protocol will be conducted and defining acceptance criteria as per the approved plan from start to end. For example, the protocol for a manufacturing process identifies processing equipment, critical process parameters/operating ranges, product characteristics, sampling, test data to be collected, number of validations runs, and acceptable test results. In this study, validation of the equipment is established on the basis entire life cycle of the product and the data was collected and compiled. Therefore, current study was designed and performed with objective for validating to prevent breakage of liquid bivalent oral polio vaccine (LbOPV) vials with maintain acceptance criteria of their quality attributed during the period of two years from manufacturing to expiry date of the vaccine for quality storage at -20$^0$C ±2$^0$C temperature in deep freezer under laboratory conditions.

## Materials and Methods

**Qualification of deep freezer:** Deep freezer is a crucial equipment to perform validation of vaccine vials for preventing the breakage during the storage of the vaccine vials at -20$^0$C ±2$^0$C temperature during the two years. Following qualifications of the equipment was needed and confirm through documentation before performing the study [1]:

- Use requirement specification (URS) of the equipment was critically assessed for its specifications for requirement of the study. The freezer should always maintain consistency with the temperature range from -10$^0$C to -30$^0$C.
- Documents of the equipment were carefully examined and verified for its design qualification (DQ), operational qualification (OQ), performance qualification (PQ) and installation qualification (IQ) at the time of factory acceptance test (FAT), site acceptance test (SAT), and equipment installation report.
- Temperature mapping and validation of the equipment were completed and examined the comprehensive

report as per cGMP guidelines. The report was compiled with complete information of temperature mapping protocol, methodology, results, and conclusion and also including any corrective actions that were taken at the time of temperature mapping and validation of the equipment.

**Trained and authorization of person:** Current study was conducted and performed by only trained and authorized person. The people have key role with the responsibilities for the planning, execution, data interpretation and conclude the outcome of the study and also capable to prepare & submit the report [6].

**Collection and storage of samples:** Bharat Immunologicals & Biologicals Corporation Limited (BIBCOL) is well known biopharmaceutical industry and involved in production and testing of viral vaccine, and other biopharmaceutical products in Bulandshahr city of Uttar Pradesh, India. A total 25 nos. of liquid bivalent oral polio vaccine (LbOPV) vials from three subsequent produced batches (LbOPV-01 to 03) were collected as sample and used for validation study for quality storage of the vaccine vials in corrugated boxes at -20$^0$C ±2$^0$C in deep freezer [7]. All three batches were qualified following inclusion criteria:

- Expiry date of these batches has not been passed.
- 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) and kanamycin antibiotics (15µg per dose) according to Indian Pharmacopeia 2014 [4].
- Storage conditions of these batches were satisfied as per GMP guidelines and the selected batches with location code are summarized in table 2.
- Locations of the collected samples are stored at -20$^0$C ±2$^0$C temperature in deep Freezer as showing in figure 2.

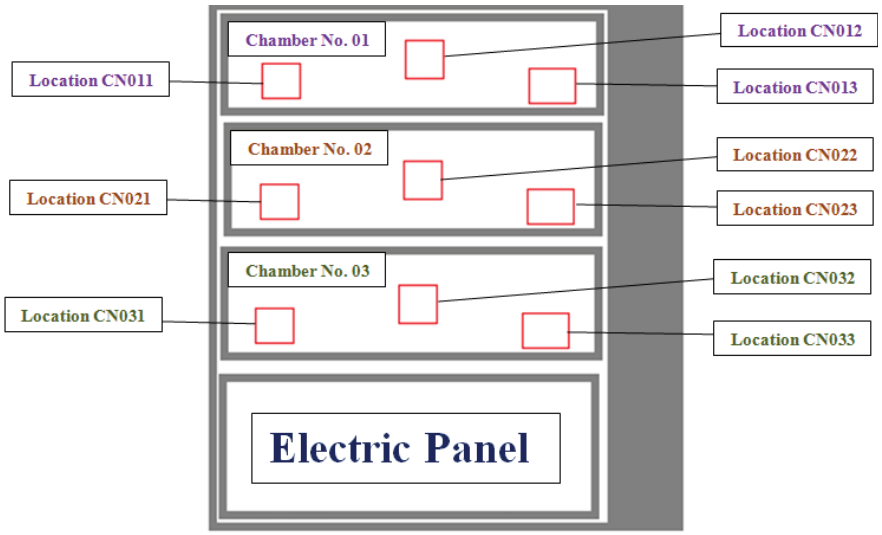


*Figure 2: Chamber-wise sample locations with the codes during the study of vaccine vial validation at -200C ±20C in Deep Freezer*

**Validation protocol for the vaccine vials:** Current study was exclusively designed and conducted to validate the breakage of the vaccine vials with acceptance criteria of the quality attributed as per GMP guidelines in WHO manual [1]. Collected vaccine samples of the three batches were stored at three temperatures viz. -10$^0$C, -20$^0$C, and -30$^0$C for the period of two years. Locations of the samples are stored at respective temperature in deep freezer as showing in figure 2. According to the validation protocol, all samples were checked after the subsequent monthly scheduled interval i.e. 3$^{rd,}$ 6$^{th}$, 9$^{th}$, 12$^{th}$, 15$^{th}$, 18$^{th}$, 21$^{st}$, and 24$^{th}$. Each vial of three batches was thoroughly checked individually by trained and authorized persons and also cross checked by another trained and authorized person. Results of the validation were recorded and compiled in respective table for analysis purpose.

**Quality attributes of the vaccine vials:** Collected samples from -10$^0$C, -20$^0$C, and -30$^0$C temperatures for the period of two years were tested parallel for the quality attributes. A total of nine quality attributes of the samples were studied and performed during the entire study period i.e. potency, identity, sterility, stability, pH, drop test, volume, kanamycin activity and vaccine vial monitor (VVM) [7, 8]. These tests were performed on a monthly interval up to two years and the results of all the quality

attributes were recorded and compiled in respective table for analysis purpose. The quality attributes and statistical analysis of the data were performed as described by Kumar et. al., (2020)[8].

## Results and Discussion

The study was designed and performed by the trained and authorized persons with the objective for validating breakage of the vaccine vials with maintaining acceptance criteria of all the quality attributed as per Good Manufacturing Practice (GMP) guidelines. Good manufacturing practice is an essential part of production of human drugs, veterinary drugs, biological and biotechnology products, and pharmaceutical ingredients[1,6,8]. These commercial processes are subject to regulatory oversight and must ensure that every aspect of the production process is carefully scrutinized. The purpose of any process validation is to collect data and scientifically analyze the process from conception to large scale production. An updated process validation protocol is essential to ensuring product quality and consistency. Many laws have been established to mandate process validation in order to protect consumers, especially in the case of pharmaceutical products

The validation protocol is pre-prepared & approved and comprises in the different stages. There are three main stages to any process validation protocol for any biologicals and pharmaceutical products in the biopharmaceutical industry as described in table 3. These stages of the process validation protocol should be completed for a high-quality product with reliability after complains the GMP guidelines.

**Table 1: Aspects covered by GMP for quality product**

| S.No. | Aspects covered by GMP |
|---|---|
| 1 | Suitable premises for manufacturing and quality control testing |
| 2 | Adequate laboratory facilities with grade A, B, C, & D |
| 3 | Qualified and trained personnel for manufacturing, quality control and other activities |
| 4 | Validation of critical steps involved in manufacturing, testing etc. |
| 5 | Defined and approved manufacturing process and testing procedures |
| 6 | Approved written procedures and instructions |
| 7 | Qualified and suitable storage for product |
| 8 | Approved suitable transport with appropriate conditions |
| 9 | Records of steps-wise defined and approved procedures for every activity |
| 10 | Full traceability of product through batch process records and distribution records |
| 11 | Approved system for product recall and investigation of market complains |

**Table 2: Detail of studies LbOPV batches with batch & location code**

| Batch Number | Batch Code | Chamber Number | Location Code |
|---|---|---|---|
| LbOPV - 01 | BN 01 | 01 | CN011, CN012, and CN013 |
| LbOPV - 02 | BN 02 | 02 | CN021, CN022, and CN023 |
| LbOPV - 03 | BN 03 | 03 | CN031, CN032, and CN033 |

**Table 3: Main stages of process validation protocol**

| Stage No. | Stage title | Description of process validation stages |
|---|---|---|
| Stage – 01 | Process Design | Process design is the research and development stage of the process. Methods of process design for creating the product are established and tested. Crucial parts of this work are performed at only small scale for identifying & correcting these problems with authentic documentation for record in prompt manner. These records shall be used as basic knowledge about the production strategy, and can use later for identifying optimal methods and troubleshooting problems. |
| Stage – 02 | Process Qualification | Process qualification is an important step, where the process design is carefully studied to capable for producing a consistently reliable & authentic product. Standard operating procedures (SOP's) are written, approved & tested at this stage for production of the product. The product must be tested for all quality attributes, analyzed & acceptable for commercial production and also every aspect of the established process is scrutinized including to ensure the production facility, sampling points with unique code. The transportation and storage of raw materials storage must be appropriate and consistent to ensure integrity and safety of the final product. All persons must be trained to perform and document each step of the process with accuracy and precision. It is also helpful at this stage to consider any opportunities for things to go wrong and to develop contingency plans. Any documented mistakes or failures during the process design stage will be useful at this stage. |

| Stage No. | Stage title | Description of process validation stages |
|---|---|---|
| Stage - 03 | Continued Process Verification | Production process is qualified with its reliability and consistency and then successfully completed. Even then continued process verification is important to continue product sampling, analysis, and verification. Trained and approved person is required for ensuring quality control and also encouraged to report any anomalies with document proof. The facility and equipments maintenance schedules must be followed as per approved standard operating procedures. |

Even in the research lab, it can be helpful to follow process validation protocols because it can improve consistency between experiments and reproducibility of the results. Consistency between batches and an improvement in working time and efficiency are all benefits of using process validation throughout the process; it can even help to avoid reproducibility issues related to any process. In case of commercial production of vaccines in industry, validation is most important and crucial part for any involved process, equipments and other critical required materials like vaccine vials. Ultimate goal in vaccine industry is still to fill a vial with final vaccine product under aseptic conditions in closed-vial technology with following three major advantages [(5)].

- Increased safety for the patient: the vial remains closed during the process, significantly reducing the risk of a contaminant entering the vial compared with other containers remaining fully open, resulting in a two-log reduction of contamination risk due to exposure.
- Simplification of the filling process: the vial is delivered clean and sterile, allowing the vaccine manufacturer to eliminate the preparation of the container components i.e. washing, siliconization, and de-pyrogenization for classical glass vials.
- Easier handling for healthcare professionals: the handling, the opening, the piercing of the stopper and collection of the product are facilitated compared with glass vials, because of the special shape of the stopper without a recess area.

On the basis of available literature, there are huge literature on quality vaccine vials, manufacturing, quality testing, storage, transportation and validation of equipments, methods & processes except validation of vaccine vial breakage. In the vaccine industry, breakage of vaccine vials is very common due to its glass material. So current study was undertaken about breakage of vaccine vials as a critical aspect during storage and transport of vaccine and no study was conducted and published on the breakage of polio vaccine vials during the period of storage up to two years. As per GMP guidelines, there is a need of validation study on the breakage of the vaccine vials during the storage. Therefore, current study was designed and performed to examine the breakage of the vaccine vials during its storage period from date of manufacturing to expiry of the polio vaccine under the laboratory condition in our vaccine industry i.e. two years.

A total 03 batches of LbOPV were included in the study and storage condition of these batches was maintained as per GMP guidelines [4]. Locations of the collected samples from these batches were stored at -20$^0$C ±2$^0$C temperature in deep Freezer as showing in the above figure 2. In current study, validation of breakage of the vaccine vials was conducted at different range of storage temperatures i.e. -10$^0$C, -20$^0$C, and -30$^0$C for the period of two years and all the vials were studied for their quality attributes according the monthly scheduled interval at 3$^{rd}$, 6$^{th}$, 9$^{th}$, 12$^{th}$, 15$^{th}$, 18$^{th}$, 21$^{st}$, and 24$^{th}$. Each vial of three batches was thoroughly checked individually by trained person and also cross-checked by another authorized person as earlier described in the material and methods section. All observations of the validation were recorded and compiled the results for data analysis in table 4.

**Table 4: Batch-wise validation record of liquid bivalent oral polio vaccine (LbOPV) vials**

| Batch Code | Storage Temp. at | Location Code | Recorded of broken vial on monthly basis | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 3rd | 6th | 9th | 12th | 15th | 18th | 21st | 24th |
| BN 01 | -10$^0$C | CN011 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN012 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN013 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN021 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN022 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN023 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN031 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN032 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN033 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| BN 02 | -20$^0$C | CN011 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN012 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN013 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN021 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN022 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN023 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN031 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN032 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN033 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| BN 03 | -30$^0$C | CN011 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN012 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN013 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN021 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN022 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN023 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN031 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |
| | | CN032 | NBV | NBV | NBV | NBV | NBV | NBV | BV* | NBV |
| | | CN033 | NBV | NBV | NBV | NBV | NBV | NBV | NBV | NBV |

**Note: 1.** BV means broken vial and NBV means no broken vial.

**2.** * In the Chamber no. 03, vaccine vial was found broken only 01 no. at single location and breakage in percentage-wise is 4% location-wise (location code CN032), while 0.44% chamber-wise at -30$^0$C temperature on 21st month of the study.

Collected samples from -10$^0$C, -20$^0$C, and -30$^0$C temperatures for the period of two years were tested parallel for the quality attributes. A total of nine quality attributes of the samples were studied and performed during the entire study period i.e. potency, identity, sterility, stability, pH, drop test, volume, kanamycin activity and vaccine vial monitor (VVM) [7,8]. These tests were performed on a monthly interval up to 02 years and the results of all the quality attributes were recorded, compiled and summarized in table 5 for analysis purpose. The quality attributes and statistical analysis of the data were performed as described by Kumar et. al., (2020) [8].

**Table 5: Results of quality attributed during the study period**

| Quality attributes | Test performed and result recorded on monthly interval | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3rd | 6th | 9th | 12th | 15th | 18th | 21st | 24th |
| Potency | Q | Q | Q | Q | Q | Q | Q | Q |
| Identity | Q | Q | Q | Q | Q | Q | Q | Q |
| Sterility | Q | Q | Q | Q | Q | Q | Q | Q |
| pH | Q | Q | Q | Q | Q | Q | Q | Q |
| Stability | Q | Q | Q | Q | Q | Q | Q | Q |
| Volume | Q | Q | Q | Q | Q | Q | Q | Q |
| VVM status | Q | Q | Q | Q | Q | Q | Q | Q |
| Drop test | Q | Q | Q | Q | Q | Q | Q | Q |
| Kanamycin activity | Q | Q | Q | Q | Q | Q | Q | Q |

**Note: 1.** Q means qualified as described in the section of materials and methods of this article.
**2.** NQ means not qualified the acceptance criteria as described in the section of materials and methods of this article.
**3.** Quality attributes testing of the broken vaccine vial did not perform on the 21st and 24th month during the study period.

Previous study presents a novel approach for continuous temperature monitoring in real time for various zones on ultra-low freezers, which provides a tool for maintaining the temperature chain of custody in real time and enabling remedial action shelf-by-shelf in real time [9] with current GMP guidelines [10]. The overall health of ultra-low freezers is continuously monitored by recording compressor performance, energy consumption, door position, room temperature, freezer temperature, and multi-zone temperature in real time. In the present study, author has considered all these points and found more reliable and consistency finding during these complete experiments.

## Conclusion

Validation of the equipment is established on the basis storage period of the product and the data was collected and compiled. Therefore, current study was designed and performed with objective for validating to prevent breakage of liquid bivalent oral polio vaccine (LbOPV) vials with acceptance criteria of their quality attributed during the period of two years from manufacturing to expiry date of the vaccine for quality storage at -20$^0$C ±2$^0$C temperature in deep freezer under laboratory conditions. The study will further extent for the validation of LbOPV vials through different modes of the transportation at different location.

Based on the study, author suggested that every training program should be investigated for its effectiveness towards each participant for further improvement in the course delivery.

**Conflict of interest:** Author declares that there is no conflict of interest.

**Source of Funding:** This work was carried out at Bharat Immunologicals and Biologicals Corporation Limited (BIBCOL), Chola, Bulandshahr, Uttar Pradesh, India and also supported by the organization.

**Ethical Clearance:** Nil

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