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A STUDY TO EVALUATE THE SAFETY AND CYTOTOXICITY OF 'ORAL POLIO VACCINE

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Abstract

Live attenuated oral polio vaccine (OPV) is used to eradicate the polio disease. Live, oral poliomyelitis vaccine is a clear apparent or light pink colored solution, attenuated poliomyelitis viruses (Sabin strains) which was propagated in primary monkey kidney cell culture (P.M.K.C.C). The main aim of the study was to generate bivalent oral polio vaccine for oral administration purpose. We have investigated various quality parameters like sterility, media preparation, microbial monitoring, GPT (growth promoting test), water drop, vaccine drop test, pH and kanamycin antibiotic activity. There are three serotypes of poliovirus have been identified as (PV1), (PV2), (PV3) each serotype with a slightly different in capsid protein. [3] The disease mostly transmits via fecal-oral route (INTESTINAL SOURCE) and the oral-oral route (OROPHARYNGAL SOURCE) Poliovirus come into oropharynx and proliferates in tonsils, lymph node of the neck after that in Peyer patches and small intestine. A medical rotation came after produced polio vaccine in 1950 by Salk and Sabin, presently two types of vaccine fit for the medical care of children purpose by several administration routes such as Oral Polio Vaccine (OPV) for oral route and Inactivated Polio Vaccine (IPV) for injectable administration. Our study potentially suggest that this bivalent vaccine is able to inactivate all virus strains spread among the country and passed all parameters and the study may help to maintain cold chain during transportation and storage of vaccine.

Keyword: Polio, Bivalent (OPV), Quality imputed, Sterility, GPT.

1.INTRODUCTION

Poliomyelitis word come from Greek word "polio" meaning Grey and "myelon" meaning marrow , as per according to the world health organization (WHO) this polio disease is most viral infectious disease up to 5 years age of children in worldwide. Since 1998 wild Poliovirus decreased by over 99% from an approximate 350,000 cases then to 33 covers cases in 2018. Poliomyelitis caused by member of genus Enterovirus belonging to family Picornaviridae called poliovirus (PV). Poliovirus is about 25-30nmin diameter the outer coat or capsid is frame of 60 protomers, each made of 4 virion proteins (VP1, VP2, VP3, VP4) set in icosahedral symmetry 8 strands of protein set in β beta group forming a β barrel. There are three serotypes of poliovirus have been identified as (PV1), (PV2), (PV3) each serotype with a slightly different in capsid protein. The disease mostly transmits via fecal-oral route (INTESTINAL SOURCE) and the oral-oral route (OROPHARYNGAL SOURCE) Poliovirus come into oropharynx and proliferates in tonsils, lymph node of the neck after that in peyer patches and small intestine. Michael underwood (English physician) concern to polio as "debility of the lower extremist ". A medical rotation came after produced polio vaccine in 1950 by Salk and Sabin, presently two types of vaccine fit for the medical care of children purpose by several administration routes such as Oral Polio Vaccine (OPV) for oral route and Inactivated Polio Vaccine (IPV) for injectable administration. As per national immunization program Sabin formulated bivalent oral polio vaccine (sbOPV) is using in India, changed from Sabin formulated trivalent oral polio vaccine (stOPV) from April 2016. A newly combine vaccine schedule of bOPV and IPV is using in India by WHO Guidelines, this has been confirmed that

Sabin (OPV) vaccine is safe, potent, effective and cost-effective. In previous decades so many assays done to originate more potent safe and efficient polio vaccine but such assay is continued with various quality parameters, from all necessary parameters this introduce about safety and sustenance as sustenance of Sabin formulated oral polio vaccine is must throughout its production process.

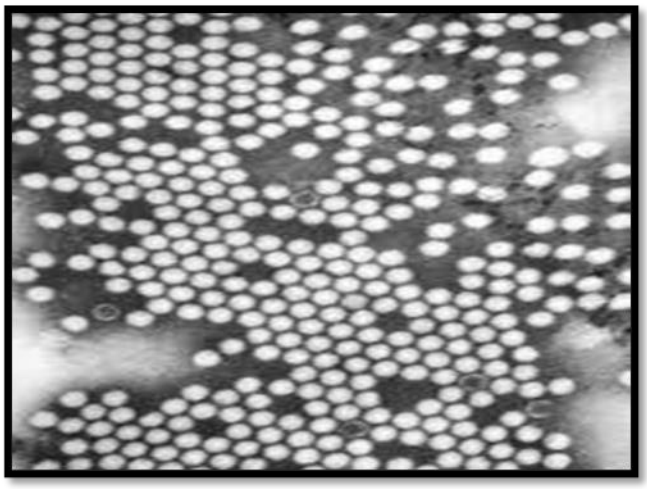

Figure 1 (electron micrograph of poliovirus)

2. REVIEW OF LITERATURE

OPV can cause (VAPP) vaccine–associated paralytic poliomyelitis in recipients and confirmation same symptoms to (WPV) wild type polio. Study on quality parameters of Sabin formulated oral polio vaccine with the aim of this study to maintain the (OPV) cold chain and prevention from (VAPP) further sustenance of storage and transportation. The bulk amount of OPV diluted with the solution of mgcl2 then the diluted solution filled in the vaccine vials approximately 2 ml in each vial (42-43) doses according to the given SOPs (Standard operating protocol) all performed assay mentioned in record book according to batch number. Further acquired samples from manufacturing department and attempted various quality attributes in laboratory such as media preparation (FTB, SCD, NA, TPVG, MEM) growth promoting test (GPT), determination of pH and microbial monitoring etc. Relevant media has prepared and store, microbial monitoring through distribution of selected NA plated to several palaces including manufacturing department, Media section 2, cell culture lab, filling area etc. then incubate them for 5 days subsequently check all plates and count colonies further discard them by decontamination autoclave.

GPT (Growth promoting test) which is used to check the prepared media. Towards sort out this need the strains of different micro- organism (ex andida albicans, Bacillus subtilis, Aspergillus niger) acquired loop of different strain and heat at 100°c then take sample from old SCD, FTB bottles (Growth presented) and poured in the new SCD, FTB bottles then keep Incubation for five days if the growth demonstration, then media is able to use. Identity of serotypes by neutralization, there are two types of polioviruses in OPV; titration conformed individually of both serotype by using appropriate type specific antisera to neutralize all of types present.

Determination vaccine pH by digital pH meter, filled vaccine vials acknowledged after manufacturing department and according to batch no. take out vials and open them by opener and empty vials in the glass beaker, further wash the probe with distilled water and checked pH, Oral Polio Vaccine pH was between the 6.4 to 6.8 as per according to the given SOPs. Record the data in documentary file overcome from all attributes recommend about oral polio vaccine (OPV) safety, storage and transportation and vaccination schedule.

3. AIMS/TARGET

1. To perform all quality attributes of bOPV safely
2. Distinguish the vaccine type, mechanism and route of administration
3. Maintain cold chain of OPV and stability of vaccine
4. Work under the guidelines provided by BIBCOL

4. MATERIALS & METHODOLOGY

4.1.1. Materials

- BIBCOL provided the items relevant to the media section which is including ftb powder, scd powder, NA powder, sodium hydrogen carbonate, gentamycin etc. which was collected and used for the experiment. Media used for the patterned quality parameters of oral polio vaccine (OPV)
- WFI (water for injection) by several pipelines

- (sbOPV) Sabin formulated bivalent oral polio vaccine were given by the manufacturing department for check the filled volume.
- Strains for GPT were made available.

INGREDIENTS MEM	FTB	SCD	NA	TPVG
Eagle's MEM with glutamine,	Casein enzymic hydrolase	Pancreatic digest of casein	Peptic digest of animal tissue	PBS powder (ca & mg free)
Sodium bicarbonate	Yeast extract	Papaic digest of soybean meal	Beef extract	Trypsin
Gentamycin sulphate solution	Dextrose and agar	Glucose	Sodium chloride	EDTA powder
Water for injection	Sodium chloride	Sodium chloride	Agar	Glucose powder
pH	L-cysteine	Dipotassium phosphate	-	Water for injection
-	Sodium thioglycollate	-	-	-
-	Resazurin sodium	-	-	-

Table:1. Detailed of ingredients used in the preparation of different media

4.2. Methods

4.2.1. Media Preparation

In order to check the all-quality attributes of bOPV (bivalent oral polio vaccine) media is require, different types of media require for different type of test such FTB, SCD media require for sterility test, NA media use for microbial monitoring, MEM (minimal essential media) is for cell culture of Hep 2 cell and for potency and TPVG (trypsin phosphate versene glucose) for trypsinization adhere cells.

1. MEM (MINIMAL ESSENTIAL MEDIA)

This media is use for cell culture of Hep2 cell and for the potency (titration) of bOPV

PROCEDURE

- Took 10 MEM powder packet and dissolve in 10-liter cold water
- Magnetic stirrer for 10 minutes
- Then weighed 22 gm. Sodium hydrogen carbonate and dissolve in the same bottle
- Add 12 ml gentamycin
- Adjust pH at 7+-2
- Filter the solution and transfer in another bottle
- Then fill into Mc cartney bottles
- Stored at 2 c

2. NA (NUTRIENT AGAR)

Nutrient agar media is used for growth of many types of microorganism to check the contamination and microbial monitoring.

PROCEDURE

- Weigh 248 gm. NA Powder
- Dissolved into 8-liter distilled water
- Then autoclave about 45 minutes
- Set the water bath at 90°c
- Put the bottle in water bath
- After then poured media onto plates
- Stored at 2°- 8°c

3. TPVG (Trypsin phosphate versene glucose)
TPVG use for the trypsinization of Hep 2 cell in cell culture technique

PROCEDURE

- Took 1-liter distilled water
- Weighed 9.6 gm. PBS (Phosphate buffer saline)
- Weighed 1 gm. glucose
- Then 1 gm. Trypsin

- 0.2 gm. EDTA
- Poured in the bottle
- After then magnetic stirrer 20- 30 minute
- Filter the solution by glass assembly
- Poured in Mc cartney bottle
- Store at 2°- 8°c

4. SCD (SOYBEAN CASEIN DIGEST)

SCD recommended as a general-purpose medium used for cultivation of a wide variety of microorganism and recommended for sterility test.

PROCEDURE

- Weighed 213 gm. Scd powder
- Dissolved in 7-liter distilled water
- Shake for 15 minutes
- Set water bath at 90 °c
- Put bottle in water bath for 10 minutes
- Poured in 55 bottles and 77 test tubes
- Covered bottles and test tube with cotton plug
- Autoclave for 25 min.
- Replaced cotton plug with rubber Cork
- Incubation 35°c for 3 days
- Stored at room temperature

5. FTB - FLUID THIOGLYCOLLATE BROTH

FTB media use for differentiate between anaerobic bacteria and aerobic bacteria. If the bacteria is aerobic then the resazurin ring appear on the top of the bottle and if the bacteria is anaerobic then the ring form in the bottom of bottle

PROCEDURE

- Weighed 210 gm. ftb powder
- Dissolved in 7 liter distilled water
- Shaking for 20 minute
- Autoclave 25 minute
- Poured in 55 bottles and 77 test tubes
- Covered bottle and test tube with cotton plug
- Autoclave 25 minute
- Replaced cotton plug with rubber Cork
- Incubation 35 °c for 3 days
- Stored at room temperature

6. MICROBIAL MONITORING

This method is used to check the contamination in different areas under the quality control lab and manufacturing department. In this took several NA plates and put on different places after that incubate the plates for few days on different temperature then count the numbers of colony, presented on the plates. On the basis of colony count concluded the clean room concept (Grade system). The clean room concept is based on the size of particle, grade increase according to the size number.

Requirement - Nutrient Agar plates, marker, thermal room, aluminum foil paper, thermometer, NA plate's carrier, Decontamination autoclave.

PROCEDURE

- Took the Nutrient Agar plates from cold room
- Mark them according to places(spot)
- Covered with the aluminum foil paper
- Put the plates into NA Plate carrier
- Put the plates according to marking which mentioned on plates
- Open the lid and keep in same for 2 hours
- After 2 hours collected all the plates
- Put them for Incubation at 23°c for 3 days
- After then incubate at 35°c for 2 days
- Count the colony numbers
- Discard the plates by decontamination autoclave.

6. VACCINE DROP TEST

This test is performed to count the drops of Sabin vaccine presented in a particular vial. The Oral Polio Vaccine come in bulk from Indonesia and then mix up the bivalent vaccine with the solution and filled in the vials. Vials filled in the manufacturing department from there vials received and replaced the seal (aluminum) with vaccine dropper. According to the SOPs 42 - 43 drops should be present in a vial the reason for this test is to calculate the number of doses can give to the recipients (infected).

Requirement - Vaccine vials, Dropper, Beaker, Opener

PROCEDURE

- Took the Vials from the manufacturing department
- Took the opener and removed the aluminum seal
- Put the opener on the surface of vials
- Then down drops one by one
- Collect in the beaker
- Count the numbers of drops presented in the vials
- Mentioned this in document
- Put empty vials in box and discard them

7. WATER DROP TEST

This test is used to detect the contamination in the water which is used in the QC Lab. To check the water contamination need water samples from different point, collect the water from washing, media 1 & 2 sections while in other hand carry nutrient agar plates. Keep all required items in the water testing lab, then marked the NA plates and took the syringe suck some water from each samples and pour on the plates then tilt the plates keep for Incubation for 5 days

Requirement - water from different samples, Nutrient Agar plates, syringe, gloves, LAF, thermal room, marker.

PROCEDURE

- First collect the samples from different water points
- Took Nutrient Agar plates and marked them
- Keep all items in the LAF
- Took syringe and suck some water from each sample
- Open the lid of the NA plates
- Poured 10-15 drops of water in each plates
- Tilt little bit and keep them for incubation for 5 days
- After 5 days see check the colony on the plates

8. GPT (GROWTH PROMOTING TEST)

GPT is one of the most important tests which are used to check the prepared media. Because if the media is wretched then conclusion will not be correct. To do this need the strains of different micro- organism (ex- *Candida albicans, Bacillus subtilis, Aspergillus niger)* took loop of different strain and heat at 100°c then take sample from old SCD, FTB bottles (Growth presented) and poured in the new SCD, FTB bottles then keep Incubation for five days if the growth show then media is able to use.

Requirement - LAF, loop sterilization, old and new SCD, FTB bottles, gloves, thermal room etc.

PROCEDURE

- Took old SCD, FTB bottles (Growth presented) of different micro-organism
- Took loop and heat at 100°c
- Pour the loop in old bottles took sample and poured in new bottles
- Keep Incubation of 5 days
- After the 5 days check out the growth
- Discard the old bottles
- Repeat the experiment when new media prepared

9. DETERMINATION OF PH BY DIGITAL PH METER

This test is used to check the pH of vaccine which is filled in the manufacturing department. The pH of oral polio vaccine should be 6.2-7.0

Requirement - pH meter, vaccine vials, tissue paper, beaker

PROCEDURE

- Took vaccine vials and discard in the beaker
- Wash the probe with distilled water
- Soak with tissue paper
- Put probe in beaker filled with vaccine sample
- Measure the pH of vaccine
- Make record of all readings

5. OBSERVATION AND RESULT.

MEDIA PREPARATION

- Several media powder has been taken from raw material lab (RML) and weighed appropriate amount and mix in water respectively. Solution was turbid after mixing further shaking for 20

minutes proceeding magnetic stirrer on the way to make solution clear. Due to different ingredients in different media need several types of sterilization certain through autoclaving such as ftb, scd, NA etc. and certain need filtration such as MEM, TPVG etc. and temperature, incubation period also vary

- Promote storage of media at 2-8° c and mentioned expiry date one month before manufacturing date

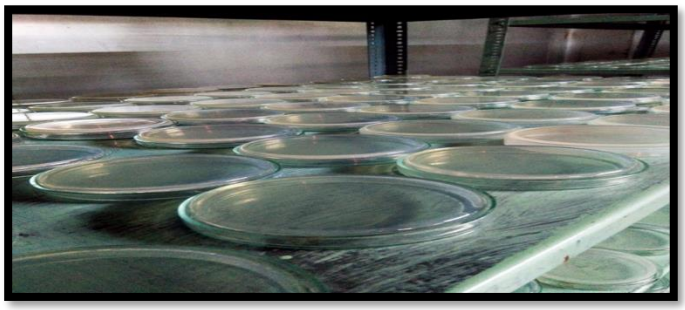

Figure 2. NA plates on incubation state to check any contamination occurred during preparation.

Figure 3. Positively primed media conveyed into mc cartney bottles.3.1. Section headings

5.1. Growth promoting test

● Perceived the prepared media before adding strains, the media was fresh and no significant growth appears, after adding the strains from old bottles to new ftb, scd bottles through heated loop for checking the media efficacy subsequently a definite incubation time (5 days) at 35◦ c the growth exist in new bottles.

● This provided the outcome that prepared media can use for the several quality attributes.Section headings ought to be centre supported, with the principal letter promoted and numbered successively, beginning with the Introduction. Sub-area headings ought to be in capital and lower-case italic letters, numbered 3.1, 3.2, and so on, and centre advocated, with second and resulting lines indented. You may need to embed a page break to keep a heading with its content.

IC_{50} (ug/ml)				
Sr.NO.	**Drug**	**Growth**	**Limit**	**Remarks**
1	Kanamycin	<10cfu/ml	<10cfu/ml	Absent
2	Without Kanamycin	350cfu/ml	<10cfu/ml	Present

Table 2 Activities with kanamycin or without kanamycin.

Figure 4. Media bottles-after transferred strains of Candida albicans, Aspergillus Niger.

5.2. Microbial monitoring

- Particular NA plates were disseminated to chosen areas under cover aluminum foil further open the lid for two hours and received the NA plates and incubate them for 5 days in which 3 days at 25° c and 2 days at 35° c further observed the colonies on NA plates and 10 colonies found on several plates
- Later NA plates discarded by decontamination autoclave, concluded based on NA lid marking clean room concept (Grade system)

Figure 5. Plates kept on different areas for microbial detection

5.3. VACCINE DROP TEST

- Vaccine vials established according to the batch number and open the vials safely by opener, and measured the total amount and drops (sbOPV) in a particular vial.
- Dignified amount by help of pipet and drops by the help of dropper, around 2ml of total solution and 42-43 drops presented in each vials.

5.4. WATER DROP TEST

- Identified the impurity of used water during testing different quality parameters, all collected water samples lead to the water testing lab while in other hand appropriated NA plates and marked respectively, later incubate after sample dispensed.
- Completion of incubation patterned plates and no colonies found on plates concluded supplied water in QC lab is free from impurity.

5.5. DETERMINATION OF PH

- Starting with the calibration of pH meter through standard solution of 4.0 and 7.0 further used for check the pH of each batch of sbOPV
- Found a batch-wise difference only slighter as compared to other batch.

6. DISCUSSION

Totally eradication of type 2 poliovirus almost from all countries, only type1 and type2 virus existing and these are also in several countries including Nigeria, Bangladesh etc. BIBCOL is producing the bivalent vaccine and for identification either type1 or type2 used antisera for type 1 is type3 and for type3 is type1, patterned through microscope after completion of incubation time (7 days) via CPE (cytopathic effect) on Hep 2 cells

Define to use only Hep 2 cell for cytopathic effect – because this is susceptibility to human poliovirus

Ftb media can be used for several testing including aerobic and anaerobic bacteria as well as isolation bacteria from blood culture after 14 days incubation if the colonies appear then subgroup the broth and inspect a toluidine blue stained smear for bacteria.

Possible reason for red ring appearance in ftb media is due to resazurin –ingredient present in ftb powder and effort as oxidation-reduction indicator that turns pink when increased oxidation, colorless when reduced.

GMP (Good manufacturing product) guidelines about the laboratory and company environment under schedule M.

7. CONCLUSION

To accomplish the well-developed sbOPV with quality aspects from as such form of liquid sbOPV with its active and excipients ingredients, further inquiries of stability study was also conventional that the developed vaccine is stable at room temperature. In future, surveys will be mandatory in route of its stability at room temperature, suitability & palatability of solution including its ingredients, protecting immunity, and welfare of the Sabin strains formulated live attenuated bivalent Oral Polio Vaccine (sbOPV) and the inquiries are still in advancement to achieve and to develop the vaccine effectively.

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