

# How much Immunogenic are the Oral Polio Vaccines Sourced from the Central Cold-chain Facilities in South-Eastern Nigeria?

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## ABSTRACT

**Context:** There is need for continuous monitoring and validation of pharmaceutical products (including vaccines) in circulation in every country. Vaccines must be maintained in cold-chain from the manufacturer to the end user. **Aims:** This study aims to validate the Oral Polio vaccines sourced from the Central Cold-chain facilities and used for vaccination programmes in South-East, Nigeria. **Settings and Design:** The Study was an experimental in design and performed in laboratory. **Methods and Materials:** The immunogenicity test was done using Antibody Induction Method. This involved measuring the neutralizing antibodies in a control group (mice given Oral Polio vaccines stored at 37°C for 12 months) and test group (mice given Oral Polio vaccines sourced from South-east States) after 30 days using Enzyme-Linked-Immunosorbent-Assay technique. Statistical analysis used: One-Way Analysis of Variance (ANOVA), Dunnett's Tests of Multiple Comparison and Bartlett's test for equal variances were used. **Results:** All the vaccines used were within their shelf-life. The Mean  $\pm$  Standard Deviation of the temperature of the vaccines at point of collection was  $-19.60 \pm 0.56$ , before storage it was  $-13.00 \pm 3.74$  and at storage facility, it was  $-19.80 \pm 0.60$ . The mean antibody titres evoked by the Oral Polio vaccines from Enugu, Ebonyi, Imo, Anambra, Abia and then the control were 22.90, 23.18, 18.55, 17.12, 17.38 and 7.36 IU/mL respectively. One way analysis of variance shows that there is statistical difference (P value=0.0026) in the antibodies titres produced by the vaccine samples. The antibodies were enough to confer protection against the target diseases. **Conclusions:** This study showed that the oral polio vaccines from the central cold-chain facilities in south-eastern Nigeria were still in good

condition as at the time of sample collection and were immunogenic enough to induce protection. The cost of immunizing a child is heightened by the in-built cost of maintaining alternative power supplies to the national power grid. It is, therefore, recommended that vaccines not requiring cold-chain storage is urgently needed in resource-limited countries to reduce the high immunization cost brought about by cold-chain maintenance system. This will strengthen and enhance the process of achieving and sustaining the eradication of infectious diseases, especially polio, in developing countries.

**Key words:** Immunogenicity, oral polio vaccine, childhood immunization, vaccine potency, south-east Nigeria

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## Key messages

We evaluated the immunogenicity of oral polio vaccines sampled from cold-chain stores in South-eastern Nigeria using animal model. The vaccines were potent and immunogenic at the time of the vaccines sampling. Good cold-chain system is beneficial in maintaining vaccine potency even when the vaccine is close to its shelf-life.

## INTRODUCTION

Poliomyelitis is one of the vaccine preventable diseases (VPDs) that have raised public health concern. Often called polio or infantile paralysis, it is an acute and highly contagious viral disease caused by poliovirus a member of the *Enterovirus*-and transmitted via the faecal-oral route.<sup>[1]</sup> Before the introduction of the Global Polio Eradication Initiative (GPEI) in 1988, it was endemic in 125 countries and estimated to paralyze up to 1,000 children per day.<sup>[2]</sup> However, through immunization, the global incidence of the disease decreased by more than 99% though it has remained endemic in some countries.<sup>[2]</sup> Infection eradication is a challenging task-requiring high levels of population immunity in all regions of the world over a prolonged period, adequate surveillance and 100% immunization coverage.<sup>[3]</sup> High coverage with oral polio vaccine (OPV) has successfully eliminated type 2 poliovirus globally but types 1 and 3 elimination remain a global challenge.<sup>[4]</sup> Nigeria has focused on strategies to control, eliminate and eradicate some of the VPDs. In particular, special attention has been given to the global goal of ending poliovirus transmission, together with controlling the number of deaths due to infection with measles. For instance, by 2002, many states in southern Nigeria were reported to be polio-free<sup>[5]</sup> and in 2005, the country made considerable progress towards achieving the goal of ending poliovirus transmission.<sup>[6]</sup> In 2015, it was reported that Nigeria had stopped the spread of the wild virus<sup>[7]</sup> but it resurfaced in the northern part of the country the next year.<sup>[8]</sup>

Vaccines have been acclaimed one of the most equitable low-cost, high-impact public health measures, saving millions of lives and promoting

good health annually when vaccination programs are implemented on a national level.<sup>[9,10]</sup> Also, they have had the most profound effect on world health, especially of children, compared with any other public health measure with the exception of clean water.<sup>[11,12]</sup> However, according to the Centers for Disease Control and Prevention (CDC), vaccine storage and handling errors can result in the loss of vaccines worth millions of dollars, with subsequent administration of these mishandled vaccines potentially affecting large numbers of patients.<sup>[13]</sup> Failures in immunization programmes have been variously blamed on poor adherence to vaccine storage and handling protocols.<sup>[13-15]</sup> Patient confidence in vaccines and their providers is diminished when repeat vaccinations are required to replace invalid doses administered with potentially mishandled vaccines.<sup>[13]</sup> Therefore, for vaccines to be effective, a strictly maintained cold chain system must be put in place.

Cold chain - a system of transporting, storing and distributing vaccine in a potent state at recommended temperature until it is administered to a recipient is a vital link between the child and effective immunization against VPDs in children.<sup>[16]</sup> It is also vital for all immunization programmes in adults, children and animals. However potent a vaccine may be, if cold chain is not maintained from the manufacturer to the place of vaccination, the vaccine efficacy greatly suffers.<sup>[16-18]</sup> With the demand placed on the cold-chain system to maintain vaccine potency, safety and immunogenicity combined with the unreliable nature of electricity, one may wonder if the vaccines used for routine

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immunization in the country elicit sufficient protective antibodies and if the storage condition in the cold-chain stores is optimal. To help address these issues, this study was aimed at validating the Oral Polio vaccines utilized in childhood immunization programmes in South-East, Nigeria bearing in mind the vision of the Nigeria country to eliminate poliomyelitis. An animal model was used to quantitate the potency of these vaccines and thus determine if they would produce protective immunity. If the vaccines could elicit antibody response above a reliable protection level, then they are deemed to be potent and immunogenic.

## SUBJECTS AND METHODS

### The study area

The South-east zone of Nigeria is made up of 5 states, namely: Abia, Anambra, Ebonyi, Ebonyi and Imo. It has a population of over sixteen million<sup>[19]</sup> about 11.675% of the national population.

### Collection and storage of the vaccine samples

The vaccines, donated freely by the Ministries of Health of Ebonyi, Enugu, Imo, Abia and Anambra States, were collected between December 2011 and June 2013 from the central cold-chain stores in the respective states and transported in an insulated vaccines' carrier containing ice packs. They were subsequently stored in the storage facility of the immunization unit in Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi within 4 hours of collection. All studies were conducted within 1 month of vaccine collection, with all vaccines maintained in the facility at  $-19.7 \pm 0.7$  °C until use. The vaccines were used within their shelf-lives. Vaccines obtained from the "open-market" were stored at 37°C for 12 months to serve as a negative control. The ELISA Kits used in the study has an internal positive control (the cut-off point specified by the manufacturer of the ELISA kits).

### Typical storage conditions in these states

At the time of vaccine collection, the storage facilities (central cold-chain stores) in all the states had adequate power supply. National Electricity supply was supplemented with standby generators. The temperatures of the cold-chain stores were noted.

### Animals used for the study

Forty-two albino mice (weight range=18-30 g) accommodated in the animal house of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu under standard conditions (temperature:  $26 \pm 2$  °C, relative humidity:  $45 \pm 2$ %) and provided with standard pellet diet and water were used. The study was done in the Pharmacology Laboratory of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University in Agulu and in the Chemical Pathology laboratory of NAUTH, Nnewi. The guidelines given by the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) were followed.

### Selection of an adequate immunization dose

The modified Arciniega and Dominguez-Castillo<sup>[20]</sup> method was used. Briefly, oral polio vaccine of volumes 0.0125, 0.025, 0.05, 0.1 and 0.2 ml were used for oral administration to the mice. After 4 weeks, the animals were bled and the IgG antibody titers were determined using ELISA technique. One human immunizing dose (0.05 ml) was therefore chosen. The dose selected ensured the induction of an antibody response that can be measured accurately and precisely in the majority of animals immunized (above the Limit of Quantitation).

### Antibody development in the immunized animals, bleeding and serum extraction

Antibody Induction Method<sup>[21-23]</sup> was used but was modified by the

authors. Briefly, 42 albino mice of similar sex and weighing 18-30 g were evenly distributed into six groups of seven per group. The first five groups were immunized by oral administration with one human dose of the test OPV. The control group was immunized (by same route) with a human dose of OPV stored at 37 °C for 12 months. All the animals were well fed while the study lasted and their cages cleaned daily. The animals were checked daily for any abnormality before they were bled. At the end of a 30 days' observation period, the animals were bled using heparinized capillary tube inserted just below the eye-ball and their blood collected in sterile eppendoff tubes. After clotting, the blood was centrifuged (Universal 320, Hettich Zentrifuge, D/78532 Tuttlingen. Model: 2008, Type: 1401, Wreck number: 003014/02/00. Made in Germany) at  $2,683.2 \times g$  for 10 minutes and sera were carefully pipetted out, transferred into another sterile eppendoff tubes and preserved by freezing at -20°C until ready for use.

### Quantitation of the neutralizing antibody

#### Preparation of reagents

The ELISA kits used for OPV Immunoglobulin G (IgG) quantitation were manufactured by IMMUNOLAB Kassel, Germany. All reagents and samples were brought to room temperature before use. The wash solution was prepared by diluting 100 ml of the Wash concentrate with 900 ml of distilled water making sure that a homogenous solution was formed.

#### Assay procedure

This was done as reported in a previous study<sup>[24]</sup> with some modifications. All standards and samples were tested in duplicate. Each 10 µl was diluted with 1 ml of sample diluent before testing. Then, 100 µl of the diluted samples, the ready-to-use calibrators and controls were pipetted respectively into the 96 wells of a microliter plates previously coated with Polio antigens, leaving the first well of the plate empty for the substrate blank. The plate was covered with foil and incubated at room temperature for 60 minutes. The wells were aspirated and washed 3 times (to remove the unbound material) for 30 seconds with washing solution 300 µl/well using automatic microplate washer (Stat Fax-2600, model#:H009775). After blotting and drying by inverting the plates on absorbent material, 100 µl of anti-mouse IgG (whole molecule)-horseradish peroxidase antibody produced in rabbit (Sigma-Aldrich, Germany) were added into the wells, except the substrate blank well and incubated for another 30 minutes at 37 °C. The wells were re-aspirated, re-washed 4 times, re-blotted and re-dried followed by the addition of 100 µl Tetramethylbenzidine (TMB) Chromogenic solutions. A blue colour developed in the wells. After incubation for 20 minutes at room temperature, 100 µl of stopping solution (0.2 M Sulphuric acid) was added to each well which changed the colour to yellow. The intensity of the yellow colour is read at 450 nm wavelength using Stat Fax-2100 microplate reader (Awareness Technology, United States of America) and result recorded as the mean absorbance  $\pm$  standard deviation. This is proportional to the concentration of the IgG antibodies in the test serum.

#### Ethical issues

The work described in this article was approved by the Ethics Committee of NAUTH, Nnewi and the guidelines given by the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) was followed.

#### Statistical analysis

Both data analysis and graphical presentations were done using GraphPad Prism version 5.00 for Windows, GraphPad Software, Inc. San Diego California USA, [www.graphpad.com](http://www.graphpad.com). The inferential statistics used were One-Way Analysis of Variance (ANOVA), Dunnett's Tests of Multiple Comparison and Bartlett's test for equal

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variances. All data were presented as Mean  $\pm$  SD and the level of significance is taken as  $P < 0.05$ .

## RESULTS

The validity periods of the vaccines used for the study is as outlined in Table 1. It was observed that the vaccines from Ebonyi and Enugu have same lot number and expiry date while the rest have similar dates. The Mean  $\pm$  SD of the temperature of the vaccines at point of collection was  $-19.60 \pm 0.56$ , before storage it was  $-13.00 \pm 3.74$  and at storage facility, it was  $-19.80 \pm 0.60$  [Table 2]. Nevertheless, the vaccines were within the recommended storage temperature.

### The Oral Polio Vaccines antibody titers in the immunized mice

The mean antibody titers evoked by the vaccines from Enugu, Ebonyi, Imo, Anambra, Abia and then the control were 22.90, 23.18, 18.55, 17.12, 17.38 and 7.36 IU/mL respectively as shown in Figure 1.

Calculated OPV antibody Titer (in IU/ml) =

$$\frac{(\text{Samples' absorbance Value}) \times (\text{Concentration of the Cut-off Control})}{(\text{Cut-off absorbance Value})}$$

Note: Concentration of the Cut-off Control = 10 IU/ml

### The manufacturer's interpretation of results and recommendations

Manufacturer's Cut-off = 10 IU/ml means No reliable protection. Antibody level insufficient to confer protection; Booster vaccination is needed. The mean Oral Polio Vaccines antibody titers in the

immunized mice are as shown in Figure 1. The mean antibody titers were 22.90, 23.18, 18.55, 17.12, 17.38 and 7.35 IU/mL for the vaccines from Enugu, Ebonyi, Imo, Anambra, Abia and the control respectively.

The neutralizing polio virus IgG antibody titers in a control group (mice given OPV sourced from "open-market" and stored at 37 °C for 12 months) and test group (mice given OPV vaccines from each South-Eastern States of Nigeria) were compared after 30 days post-immunization using One-Way ANOVA, Bartlett's test for equal variances and Dunnett's Multiple Comparison Test. The mean antibody titers evoked by the vaccines from Enugu, Ebonyi, Imo, Anambra, Abia and the control were 22.90, 23.18, 18.55, 17.12, 17.38 and 7.36 IU/mL respectively. In comparison with the poorly stored vaccines, the differences in the means of the antibody titres were 15.54, 15.83, 11.20, 9.768 and 10.03 IU/mL respectively. By the interpretations of the manufacturers of the ELISA Kit used in the assay, the vaccines can produce the needed protection as they gave values well above the cut-off value of 10 IU/mL. On the contrary, the damaged vaccine (previously stored at 37°C for 12 months) gave a value less than the cut-off and is considered incapable of producing reliable protection.

## DISCUSSION

Vaccine immunogenicity refers to the vaccine's ability to elicit immune responses (humoral and/or cell-mediated) in the body of a human or animal recipient. In this study, we evaluated the immunogenicity of oral polio vaccines sampled from cold-chain stores in South-eastern Nigeria using an animal model. The vaccines collected from the states that are relatively far from the storage facility used in this study showed more rise in temperature just before storage. This is understandable because there is a positive correlation between time away from cold storage and temperature rise. Also, the logistics of road transport in the region has been a challenge due to bad roads and poor road network.

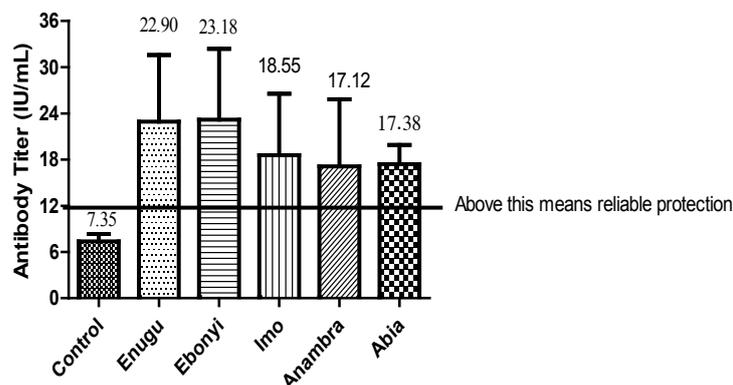
All vaccines collected from each state provoked a sufficient response that was enough for protective immunity against polio virus. The vaccines were still within the expected potency and immunogenicity margin. The vaccine from Enugu and Ebonyi States (similar lot number and expiry dates) evoked the highest titer while the poorly stored control evoked the least [Figure 1]. One-way ANOVA showed that the mean antibody titers produced by the vaccines differ significantly ( $P = 0.0026$ ). Bartlett's test for equal variances also shows that the variances were significantly different ( $P < 0.0001$ ). Compared to the vaccines poorly stored-control, Dunnett's Multiple Comparison Test of the mean antibody titers showed that vaccines from Ebonyi, Enugu and Imo states evoked significantly higher mean antibody titers ( $P < 0.05$ ) which was not so for the vaccines from Abia and Anambra States. However, the titers were higher than those of the poorly stored vaccines and were above the reliable protection limit. Even though the poorly stored vaccines and those from Imo, Anambra and Abia had similar expiration dates, the lot number was only the same for those vaccines from Abia and

**Table 1:** Information on the vaccines used

Vaccine	Lot #	Expiry Date	Source
OPV	113920	March 2013	Ebonyi and Enugu States
	2021412	July 2014	Imo and Anambra States
	J5385-1	July 2014	Abia State
	J5385-1	July 2014	Control ("Open Market")

**Table 2:** Temperature conditions of the vaccines

South-east States	Oral Polio Vaccines		
	Mean °C at collection	Mean °C before storage	Mean °C at storage
Ebonyi	-19	-10	-20.1
Enugu	-19	-14	-19.5
Imo	-20	-12	-18.9
Anambra	-20	-19	-20.1
Abia	-20	-10	-20.4
Mean $\pm$ SD	$-19.60 \pm 0.56$	$-13.00 \pm 3.74$	$-19.80 \pm 0.60$



**Figure 1:** Polio IgG Antibody titers in the immunized mice

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poorly stored control. The difference in the antibody titers evoked can therefore only be due to the storage/handling factors and neither due to batch number nor shelf-life. This is confirmed by a study in India where Deivanayagam *et al.*<sup>[25]</sup> conducted a field OPV samples study and found that about 77% of the OPV at vaccination points were potent. The rest were damaged. They attributed the loss in potency to breach in the cold-chain maintenance. Other studies also confirm that loss in potency is mainly due to cold-chain logistics.<sup>[13-15]</sup> A recent study in India<sup>[25]</sup> showed that administering one or two inactivated polio vaccine doses before two or one oral polio vaccine doses (OPV) is as good as using OPV alone for immunization programmes provided that good cold-chain system is maintained.

The Oral polio vaccines sourced from Ebonyi and Enugu States had the closest expiration dates at the time of the study but still elicited the highest antibody titers compared to those sourced from the other states. This shows that good cold-chain system can maintain vaccine's potency even when the vaccine is close to its shelf-life and that the benefit of good cool-chain system out-weighs that of a vaccine's shelf-life in maintaining vaccine safety and potency.<sup>[26,27]</sup> The poorly stored vaccines were not immunogenic even though it is still within the shelf-life.

Previous study in Maiduguri, Nigeria<sup>[28]</sup> and in Armed Forces Medical College, Pune, India<sup>[29]</sup> showed that OPV sampled had not been affected adversely. The Maiduguri study checked the total virus content and individual serotypes using L20B cell line while the Indian study estimated the composite virus content by viable count method in HEp2 (Cincinnati) cell lines. Oladejo *et al.*<sup>[5]</sup> reported absence of and low detectable protective polio neutralizing antibody in children and teenagers previously immunized with OPV. They strongly recommended repeat immunization and continuous monitoring of antibody titers in persons immunized with the vaccine. Antibody generation in a vaccine recipient is known to be one of the means of preventing infectious diseases.<sup>[30]</sup> In recent study, viral interference and innate antiviral immune mechanisms were shown to be "more important determinants of the immunogenicity of live-virus oral vaccines".<sup>[31]</sup> The present study was a one off study and did not get down to the immunization points. Continuous monitoring of the efficiency of the cold-chain maintenance as well as vaccine potency testing would translate to good vaccine strategy and successful childhood immunization program in Nigeria.

## CONCLUSION

This study showed that the oral polio vaccines from the central cold-chain facilities in south-eastern Nigeria were still in good condition as at the time of sample collection and were immunogenic enough to induce protection. The cost of immunizing a child is heightened by the in-built cost of maintaining alternative power supplies to the national power grid. It is, therefore, recommended that vaccines not requiring cold-chain storage is urgently needed in resource-limited countries to reduce the high immunization cost brought about by cold-chain maintenance system. This will strengthen and enhance the process of achieving and sustaining the eradication of infectious diseases, especially polio, in developing countries.

## Alphabetical list of abbreviations

ADDRF - African Doctoral Dissertation Research Fellowship, ANOVA - Analysis of Variance, APHRC-African Population and Health Research Centre, CDC - Centres for Disease Control and Prevention, ELISA - Enzyme-Linked-Immunosorbent-Assay, GPEI-Global Polio Eradication Initiative, HRP - Horseradish Peroxidase, IDRC - International Development Research Centre, IgG - Immunoglobulin G, NAUTH-Nnamdi Azikiwe University Teaching Hospital, NIH - National Institutes of Health, OPV - Oral polio vaccine, SD - Standard

Deviation, TMB - Tetramethylbenzidine, USA - United States of America, VPDs - Vaccine preventable diseases.

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## Conflict of interest

The authors are the natives of the region of Nigeria from where the samples were sourced. Neither the funders nor the vaccine donors had any influence whatsoever in the study design, data collection, manuscript writing nor decision to publish the work.

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