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Supplementary appendix 1

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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STUDY PROTOCOL

Version 3.2

TITLE: Cross-sectional study to assess persistence of immunity conferred by a single IPV dose administered in the EPI routine immunization schedule

Contents

List of Abbreviations

AE	Adverse Event
bOPV	Bivalent Oral Poliovirus Vaccine
CDC	Centers for Disease Control and Prevention
CRF	Case Report Form
cVDPV	Circulating Vaccine-Derived Poliovirus
EPI	Expanded Program on Immunization
ERC	Ethical Review Committee
GPEI	Global Polio Eradication Initiative
ID	Intradermal
IM	Intramuscular
IPV	Inactivated Poliovirus Vaccine
IRB	Institutional Review Board
IOM	Institute of Medicine
NHRC	Nepal Health Research Council
PID	Participant Identification Number
PCR	Polymerase Chain Reaction
SAE	Serious Adverse Event
SAGE	Strategic Advisory Group of Experts on Immunization
SOP	Study operating procedure
tOPV	Trivalent Oral Poliovirus Vaccine
VAPP	Vaccine-Associated Paralytic Poliomyelitis
VDPV	Vaccine-Derived Poliovirus
WHO	World Health Organization
WPV	Wild poliovirus

Project Summary

Background

To reduce the burden of paralysis caused by type 2 vaccine-derived poliovirus (VDPV), the Strategic Advisory Group of Experts in Immunization (SAGE) recommended the global switch from trivalent to bivalent oral poliovirus vaccine (OPV) that does not contain type 2 poliovirus. The SAGE also recommended the introduction of a single dose of inactivated poliovirus vaccine (IPV), to maintain population immunity to type 2 and mitigate the consequences of potential emergences of type 2 VDPVs shortly after the switch. Although a single dose of IPV induces detectable antibodies in 34% to 80% of infants, compared to >90% after three doses, clinical trials have shown that most of seronegative children (84-98%) have been "immunologically primed" by the first dose. Primed individuals will produce protective antibody levels in serum within one week of exposure to a new dose of IPV or OPV provided during an outbreak response. However, it is unknown whether seroconversion or priming responses persist, and for how long they persist, without boosting with additional polio vaccine doses. Furthermore, immunogenicity for IPV delivered in clinics in low-resource countries may be inferior to that observed in clinical trials because inappropriate cold-chain and other factors may decrease vaccine efficacy.

Following SAGE recommendations, Nepal introduced one dose of IPV in 2015, in advance of the global withdrawal of trivalent OPV that occurred in April 2016. However, Nepal, like many other countries had to stop vaccination by the end of 2016 because of a global shortage of IPV.

Research question and objectives

We propose to conduct a cross-sectional study in Nepal to determine whether the immune response provided by a single dose of IPV delivered through routine immunization services persists for more than a year. The primary objective of this study is to assess the proportion of infants vaccinated with one dose of IPV in routine immunization, who are seropositive or primed against type 2 poliovirus, either one month after vaccination (control group, recent vaccination), or more than 18 months after vaccination (expected to have waned response). Secondary objectives will include: 1) to determine the proportion of children seropositive to types 1 and 3 following a sequential bOPV-IPV schedule delivered through routine immunization services in a low-resource country; and 2) to assess the proportion of children who seroconvert or boost antibody titers for type 2 poliovirus after two doses of IPV provided > 1 year apart.

Methodology

Sample selection

This will be a cross-sectional phase IV study. Participants will be selected among children attending outpatient clinics for immunization or minor illness in study sites in Kathmandu, Nepal. Potential participants who have received the information about the ongoing study on IPV at Institute of Medicine through their preschool and female community health volunteers in the community and present to one of the study sites to learn about the study will also be enrolled if they fulfill the inclusion and exclusion criteria.

Infants will be enrolled if they meet inclusion/exclusion criteria and parents provide consent for participation.

Inclusion criteria: Birth after the switch from tOPV to bOPV and:

- Study Arm A: age >24 months and received one full dose of IPV between 3 and 6 months of age;
- Study Arm B: age 7-12 months and did not receive any IPV.

Exclusion criteria: acute or chronic conditions at the time of enrollment that would contraindicate venipuncture or IPV administration.

We decided to include children who had not received IPV in Arm B, instead of children who had received vaccine recently through routine immunization services, because, by the time of study implementation infants 7-12 months may have received IPV with a new schedule. The new schedule will include two fractional doses (1/5th of full dose) at 6 and 14 weeks of age as per the government plan for EPI program. The different schedules for children vaccinated recently or earlier would not allow an adequate answer to the main study objective.

Study procedures

All children will attend three study visits. During the first study visit, study staff will collect epidemiological information and immunization history from parents using a standard questionnaire; will collect a blood sample (baseline); and will administer one dose of IPV dose via intramuscular.
Children in study arm A (old vaccination) will attend the second and third visits 1 week and 1 month later, respectively. Children in study arm B (recent vaccination) will attend the second visit one month after the first visit, and the third visit, one week after the second visit. Procedures are summarized in the table below.

Study Arm	Inclusion criteria	1st visit	2nd visit	3rd visit
Arm A	- Born after 30 Apr 16 (age > 24 months) - IM IPV at 3-6 months	Day 0 - Blood sample - IPV challenge dose	Day 7 - Blood sample	Day 30 - Blood sample
Arm B	- Age 7-12 months - No IPV	Day 0 - Blood sample - IPV primary dose	Day 30 - Blood sample - IPV challenge dose	Day 37 - Blood sample

Blood samples will be centrifuged, and serum aliquoted and stored at -20°C at the IOM laboratory in Kathmandu until the end of the study. Serum aliquots will be shipped to Atlanta CDC for measurement of antibody titers to all three poliovirus types using a microneutralization assay.

Sample size and analysis plan

The main outcome for the analysis will be a comparison of proportion of children with detectable immunity to type 2 poliovirus (seropositivity or priming) in each age group. Seropositivity will be defined as antibody titers ≥ 1/8 at baseline; priming will be defined as antibody titers <1/8 at baseline and ≥ 1/8 one week after the challenge IPV dose. For secondary objectives, proportion of children who seroconvert or boost antibody titers to any poliovirus type after the second IPV dose will be assessed (boosting in titers defined as an increase above 4-fold in baseline titers). Seropositivity and median antibody titers to poliovirus types 1 and 3, adjusting for the number of IPV and bOPV doses received will also be assessed.

Using a one-sided test for differences between proportions with a continuity correction Z-test using pooled variance (PASS v14), and assuming a conservative estimate of 90% immune infants in the younger age group, it was estimated that 237 children would be necessary to detect a one-sided difference of ≥10% between the proportions of children immune (seropositive or primed) for type 2 poliovirus in each age group, with 90% power and 0.05 alpha. To account for potential 5% drop-outs, the sample will be rounded up to 250 per group or 500 children total.

Study implementation

The study will be implemented in three study sites in Kathmandu, Nepal, that provide immunization services and medical care to children. The study sites have previously collaborated in polio vaccine related studies with the CDC. We aim to complete implementation following protocol final approvals, and study site preparation during September 2018-March 2019.

Expected outcomes and benefits to the GPEI

This information will allow better estimations of children partially protected (primed) or fully protected against type 2 poliovirus depending on coverage and time since IPV vaccination. The new estimates will inform the GPEI on vaccine choices for responding to type 2 VDPV outbreaks and will help guide decisions on polio immunization schedules after cessation of all OPV types, for Nepal and for other countries.

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Background and Rationale

Importance of Poliomyelitis and Polioviruses

There are three poliovirus types, 1, 2 and 3, with minimal cross-immunity between them. Polioviruses are transmitted from person-to-person either through pharyngeal secretions or feco-orally. They enter the body through the mouth and replicate in the gastrointestinal tract for several days or weeks, causing no apparent illness or mild diarrhea, fever, and/or vomiting. In approximately one out of 200 infections (depending on the type of poliovirus), polioviruses invade the spinal cord or brainstem leading to paralysis of limb(s) and respiratory muscles. An estimated 5-10% of individuals with paralytic poliomyelitis die, while the remaining suffer from lifelong paralysis of one or more limbs. There is no cure for paralysis and poliomyelitis was the leading cause of permanent disability before vaccines became available [1, 2]. The presence of detectable antibodies in blood against each type protects against paralytic poliomyelitis, but intestinal immunity develops only when there has been exposure to live poliovirus (vaccine or wild), and individuals with antibody titers may be re-infected and shed poliovirus upon new exposure to live poliovirus, although the duration of shedding is usually shorter [2-5].

Poliovirus Vaccines

Oral poliovirus vaccines contain attenuated Sabin strains of poliovirus and induce both, humoral and mucosal immunity that protects against paralysis and re-infection. OPV can also immunize or boost immunity of close contacts through secondary spread and is inexpensive and easy to administer. Trivalent OPV (tOPV), with poliovirus types 1, 2 and 3, was the vaccine of choice for polio eradication [6]. The interference among the different serotypes and the lower efficacy of tOPV in tropical developing countries with high prevalence of diarrheal diseases and malnutrition, was compensated with provision of several doses in primary immunization schedules and with provision of several booster doses through vaccination campaigns of children up to 5 years of age [7, 8]. Between 2005 and 2010 mmonovalent vaccines for each serotype (mOPV1, mOPV2, mOPV3) and bbivalent OPV (bOPV) containing types 1 and 3, were licensed for use in campaigns because of their higher immunogenicity against the respective serotype [9, 10].

The inactivated poliovirus vaccine (IPV) contains the three poliovirus types inactivated by formalin and can be administered via intramuscular or subcutaneous. With the current formulation used since the 1980s, almost 100% of infants achieve high antibody levels against the three serotypes after 3 doses of IPV, depending on the time of the first dose [4, 11]. Maternal antibodies and short interval between doses may decrease the immunogenicity of IPV administered at the WHO schedule of 6, 10 and 14 weeks [12-15]. IPV is a very safe vaccine, with no serious adverse effects associated except for the potential risk of anaphylaxis in individuals allergic to the trace doses of antibiotics present in the formulation [4]. The major disadvantage of IPV is its limited effect on intestinal immunity, which makes it less effective for interrupting transmission of poliovirus. In addition IPV is substantially more expensive than OPV and more difficult to administer in campaigns [11].

Types of immune response and duration of immunity provided by polio vaccines

The immunological response to poliovirus vaccines is evaluated by measuring type-specific poliovirus antibodies using neutralization assays. Antibodies can be detected as early as 1 to 3 days after infection with WPV or receipt of OPV or IPV. Initially, most of antibodies consist of immunoglobulin (Ig) M, which peaks around 2 weeks after exposure and disappears after 2-3 months. IgG increases slowly, peaks about 4-6 weeks and then plateaus [16, 17]. The presence of detectable antibodies in plasma are known to

protect against paralytic disease. Secretory IgA antibody appears in the intestine about 1-3 weeks after infection with OPV or WPV, and induces intestinal immunity against infection [18]. Serum IgA can also be detected at low levels during the first months, but not in all individuals, and is not correlated with mucosal immunity [19].

Administration of additional doses of vaccine or new exposures to wild poliovirus, induces a quick rise in antibody titers, with a peak reached within one week after the dose, instead of several weeks [15, 20, 21]. This is the effect of induction of "priming" or immunological memory by the first dose, which consists of plasma B cells, predetermined for production of serotype-specific poliovirus antibodies. The presence of these memory cells allows the rapid increase of poliovirus-specific antibodies after a new exposure to a new vaccine dose or natural infection. As a result, individuals with no detectable antibodies but persistent "priming" after years of vaccination could be rapidly protected against paralytic polio with a new IPV dose in the event of a polio outbreak.

Because blood tests for determination of plasmatic cells with immunologic memory are difficult to conduct, we assess whether an individual has been "primed" by a previous vaccine dose, using a challenge with a new vaccine dose. Antibody titers are measured 1 week after the challenge dose. Those individuals, who demonstrate a change from a seronegative to seropositive status within a week of receiving the challenge vaccine dose, are considered as primed by the first vaccine dose that they received [15, 21-23].

Antibody titers usually decline in the first two years (10- to 100-fold reduction) and then plateau, persisting for many years [4, 24, 25]. Individuals with lower antibody titers to start with are more likely to reach undetectable levels in adulthood, in the absence of a booster vaccine dose or exposure to natural infection. Population surveys have found that a high proportion of individuals older than 40 and even 60 years of age who were vaccinated in infancy have detectable polio antibodies [26-28]. A considerable proportion of adults who are seronegative show immunological memory when they receive a challenge dose (i.e. rise in antibody titers one week after the challenge) [28, 29]. Although primary immunization series with OPV may induce lower antibody levels than primary immunization with IPV, duration of immunity is longer after primary vaccination with OPV than after vaccination with IPV [30].

Intestinal immunity that prevents infection and replication following a new exposure to OPV or WPV can only be induced by live viruses; is only partial, and appears to wane more rapidly than humoral immunity, with children being able to be re-infected with a challenge OPV a few years after the immunization series, although the re-infection results in a reduction in the duration of shedding and the amount of virus excreted [31]. However, although IPV alone does not induce any intestinal immunity, supplemental doses administered to children previously exposed to OPV, boosts humoral and intestinal immunity more effectively than a supplemental dose of OPV, especially in areas with lower effectiveness of OPV [31-33].

Changes in polio vaccine use with progress in global polio eradication

With the use of tOPV in routine immunization and campaigns, the Global Polio Eradication Initiative (GPEI) reduced polio cases from an estimated 350,000 in 1988 to 719 reported in 2000. The last case of type 2 WPV was reported in 1999, and the last type 3 WPV in 2012. Type 1 WPV circulation is now limited to a few areas in Afghanistan and Pakistan, where conflict limits access to intensive vaccination [34]. As WPV circulation was progressively eliminated throughout the world except for a few remaining countries, certain disadvantages of OPV became more apparent, especially in countries that had eliminated WPV circulation many years ago.

Sabin strains in OPV may cause paralysis in a small number of vaccinated children and their close contacts (vaccine-associated paralytic poliomyelitis or VAPP) [35]. Following WPV elimination from America and Europe, many countries in these regions felt the burden of paralytic cases related to VAPP and started to replace OPV with IPV in routine immunization schedules. In addition, Sabin strains can circulate among susceptible individuals for long time in areas where absence of wild poliovirus transmission and suboptimal coverage with routine immunization result in low population immunity. Prolonged person-to-person transmission of Sabin strains results in genetic changes and the emergence of circulating vaccine-derived polioviruses (cVDPV), which have the neurovirulence and transmissibility characteristics of WPV [36, 37] . Type 2 was responsible for about 40% of annual VAPP cases reported, and 85% paralytic cases caused by cVDPVs during 2000-2015 [38].

To decrease the burden of paralytic cases caused by type 2 vaccine strains and VDPVs, the Strategic Advisory Group of Experts on Immunization (SAGE) endorsed the phased cessation of OPV starting with the removal of type 2 by switching from tOPV to bOPV in primary immunization. The SAGE also recommended the introduction of "at least one dose of IPV in routine immunization" in order to: i) prevent poliomyelitis in IPV vaccinated individuals exposed to vaccine-derived poliovirus type-2 (VDPV2) or wild poliovirus type-2 (WPV2); (ii) improve the response to monovalent OPV type-2 (mOPV2) or an additional dose of IPV in a type 2 polio outbreak, (iii) reduce the transmission of a reintroduced type 2 poliovirus, and (iv) accelerate wild poliovirus eradication by boosting immunity to wild poliovirus types 1 and 3 [39].

Rationale for the study and expected outcomes and benefits for the GPEI

As explained above the introduction of one dose of IPV in routine immunization before the switch from tOPV to bOPV was recommended by the SAGE as a mitigation strategy, to reduce the extent of a type 2 cVDPV outbreak, whose risk of emergence is highest in the first two years after the switch [40]. Several recent studies have demonstrated that, although a single dose would result in seroconversion to type 2 for a limited number of infants (32% to 80% depending on the age of administration and study), a high proportion (>90%) of those infants who are seronegative, are actually "primed" by that single dose [15, 21-23]. As a result, 90%-100% of infants are either primed or seroconverted for type 2 after a single one dose. Although it is not clear whether priming may protect directly against paralysis if a type 2 cVDPV 2 outbreak emerges, primed children will be able to develop protective antibody levels quickly following a new OPV or IPV dose provided as a response to the outbreak.

Studies that assessed long-term immunity to IPV or OPV, were conducted in individuals who had received three or more doses of poliovirus vaccines and could have been exposed to circulating wild or vaccine poliovirus [4, 24-28]. It is unknown whether the proportion of children who were seropositive or primed following a single dose of IPV will stay positive or primed, and for how long this immune response will persist, without boosting antibody titers through additional doses or exposure to natural infection.

Furthermore, immune responses to IPV delivered in clinics and outreach sites in low-resource countries may be inferior to those observed in clinical trials, because inappropriate cold-chain, inappropriate dosing or schedule of administration may decrease final vaccine effectiveness [4].

To further complicate the situation, problems with IPV scale-up of manufacturing to deliver vaccine to more than 120 countries that were using OPV exclusively in 2015, have created a global shortage of IPV. As a result, many countries, including Nepal, did not receive IPV supply for 2016 and/or 2017. To stretch the supply, the SAGE recommended countries with supply shortages, to replace the single intramuscular

dose (0.5 mL) with two fractional doses (0.1 mL) administered at 6 and 14 weeks of age. The SAGE based this recommendation on a recent study in Bangladesh that showed that two doses of fractional IPV administered intradermally induced an immune response (priming plus seroconversion) that was non-inferior to that observed with a single dose of intramuscular IPV [15]. This new schedule with 2 fractional IPV doses will allow vaccinating more than twice the number of children that could be vaccinated with one full IPV dose [41]. UNICEF may deliver IPV supply earlier than predicted if countries replace full IPV with fractional IPV schedules. At the time of writing this, India, Bangladesh and Sri Lanka have introduced a fractional IPV schedule, and Nepal is planning introduction in 2018.

Reliable estimates of population immunity in a country or region based upon coverage and estimated immunogenicity of the type of vaccine and vaccination schedules received, are crucial to guide programmatic decisions and manage vaccine supply for outbreak responses to type 2 poliovirus, especially with the current global shortage of IPV and limited supply of mOPV2 [42-44]. Cohort clinical trials that will inform on the duration of immune response after a single IPV dose will not provide information until 2020.

Therefore, we propose to conduct a cross-sectional study to determine whether the immune response provided by a single dose of IPV persists for more than two years, by assessing the proportion of children born after the tOPV-bOPV switch and vaccinated with a single dose of IPV at 14 weeks in routine immunization who are still seropositive or primed at around two years of age. The study will also assess the proportion of children vaccinated with one dose of IPV through RI services, who will seroconvert following a second IPV dose provided > one year after the first dose. This information will allow better estimations of children partially protected (primed) or fully protected depending on coverage and time since IPV vaccination through routine immunization services, and on the expected population immunity reached for type 2 poliovirus with a supplemental IPV dose. These new estimates will inform the GPEI on vaccine choices for responding to type 2 VDPV outbreaks and will inform decisions on polio immunization schedules after cessation of all OPV types, for Nepal and for other countries.

Rationale for conducting the study in Nepal

Nepal polio immunization calendar included 3 doses of tOPV at 6,10 and 14 weeks of age, with booster doses at 9 months of age and twice a year through annual immunization campaigns targeting all children younger than five years of age. Following the SAGE recommendation, Nepal introduced one dose of IPV in September 2015, and replaced tOPV with bOPV in May 2016. The schedule proposed was bOPV administration at 6, 10 and 14 weeks of age, and IPV administration (intramuscular) at 14 weeks.

Nepal is an ideal country to assess the persistence of immunity following the WHO-recommended schedule with 1 dose of IPV at 14 weeks, because it has some of the risk factors that may decrease vaccine effectiveness, such as inadequate infrastructure to keep appropriate cold chain, or insufficient trained staff to complete vaccination schedules timely. The information provided by this study in Nepal will be very useful to complement results on persistence of immunity after a single full dose of IPV obtained from clinical cohort trials where the schedules and quality of vaccine administered are controlled.

Rationale for the age groups and study design

The age of study participants selected for this study is affected by potential changes in the vaccination schedule in Nepal. As explained above, Nepal introduced IPV in routine immunization in September 2015, but its administration was interrupted around September-November 2016 because of the global supply shortage, and the immunization program is planning (at the time of writing this protocol) to re-introduce IPV as two fractional doses around the 2nd -3rd quarter of 2018.

In order to assess the persistence of immunity to type 2 conferred by IPV about 1.5 years after vaccination we need to select children who are born after the tOPV-bOPV switch. The birth cohort born after April 30, 2016 (global switch) who may have received IPV before the stock-out will reach an age of above 24 months by August 2018, which is when the study is planned to be implemented. Delays in its implementation will limit the utility of the study in providing information to guide the selection of IPV schedules for the post-certification era.

Because it is important to have a control group who has received IPV recently, and maternal antibodies may mask the infant's immune response, we would need to select controls who had received vaccine recently but are older than 6 months of age (time at which maternal antibodies are expected to have disappeared from blood). However, by the time we implement the study in 2018, children 7-12 months of age may have received no vaccine or may have received two fractional doses (1/5th of full dose) at 6 and 14 weeks of age, instead of the single full dose at 14 weeks that older children in other study group have received. We cannot include children recently vaccinated through routine immunization services as a control group because they will have received a different schedule.

Therefore, to have an adequate study control group (Arm B), we will include children 7-12 months of age who have not received an IPV dose before. We will administer these children a full dose of IPV, and measure primary and secondary response after that dose. This control group may have a higher response than a control group including children vaccinated through the routine immunization system would have because: 1) administration of IPV is performed in a controlled setting where vaccine efficacy is expected to be higher; 2) children will receive IPV at an older age which will result in less interference from maternal antibodies on the immune response; 3) response will be measured within a short time after vaccination with less variation (i.e. 1 month instead of 1-3 months after vaccination). Selecting this control group will impose some limitations to our potential ability to determine the influence of waning versus other factors on the potential differences in immune response that we observe between study groups. On the other hand, the bias to a higher immune response in the control group may strengthen our conclusions, if we observe no difference in immune responses between the two study groups. In any case, we think that this study design is a better alternative than not having a control group of children recently vaccinated.

Objectives

The study has the following objectives

Primary**:** To compare the proportion of infants vaccinated with one dose of IPV after 14 weeks of age who are seropositive or primed against type 2 poliovirus, either > 21 months after vaccination (study group), or one month after vaccination (control group)

Secondary**:**

- To determine the proportion of children seropositive to types 1 and 3 following a sequential bOPV-IPV or bOPV alone schedule, delivered through routine immunization services in a low resource country.
- To compare antibody titers to type 2 among seropositive infants between children who received IPV > 18 months before blood collection (study group) and children who received IPV ~1 month before the blood collection.
- To assess antibody titers to type 1 and 3 following a sequential bOPV-IPV schedule according to the number of vaccine doses received and time since last dose.

Methods

Study Population

Inclusion Criteria

We will be including Nepali infants who fulfil the following criteria

1. Born after 30 April 2016
2. Healthy infant or mild illness
3. Age within one of the following age groups: 7-12 months or >24 months.
4. Receipt of one dose of IPV at 3- 6 months of age (if age >24months) or zero doses (if age within 7-12 months). IPV receipt must be validated through immunization card or registry book.
5. Parents that consent for participation in the full length of the study.
6. Parents those are able to understand and comply with planned study procedures.

Exclusion Criteria

1. Parents and infants who are unable to participate in the full length of the study.
2. A diagnosis or suspicion of immunodeficiency disorder either in the infant or in an immediate family member.
3. A diagnosis or suspicion of bleeding disorder that would contraindicate parenteral administration of IPV or collection of blood by venipuncture.
4. Acute infection or illness at the time of enrollment that would require infant's admission to a hospital.
5. Evidence of a chronic medical condition identified by a study medical officer during physical exam.
6. Known allergy/sensitivity or reaction to polio vaccines.

Discontinuation Criteria

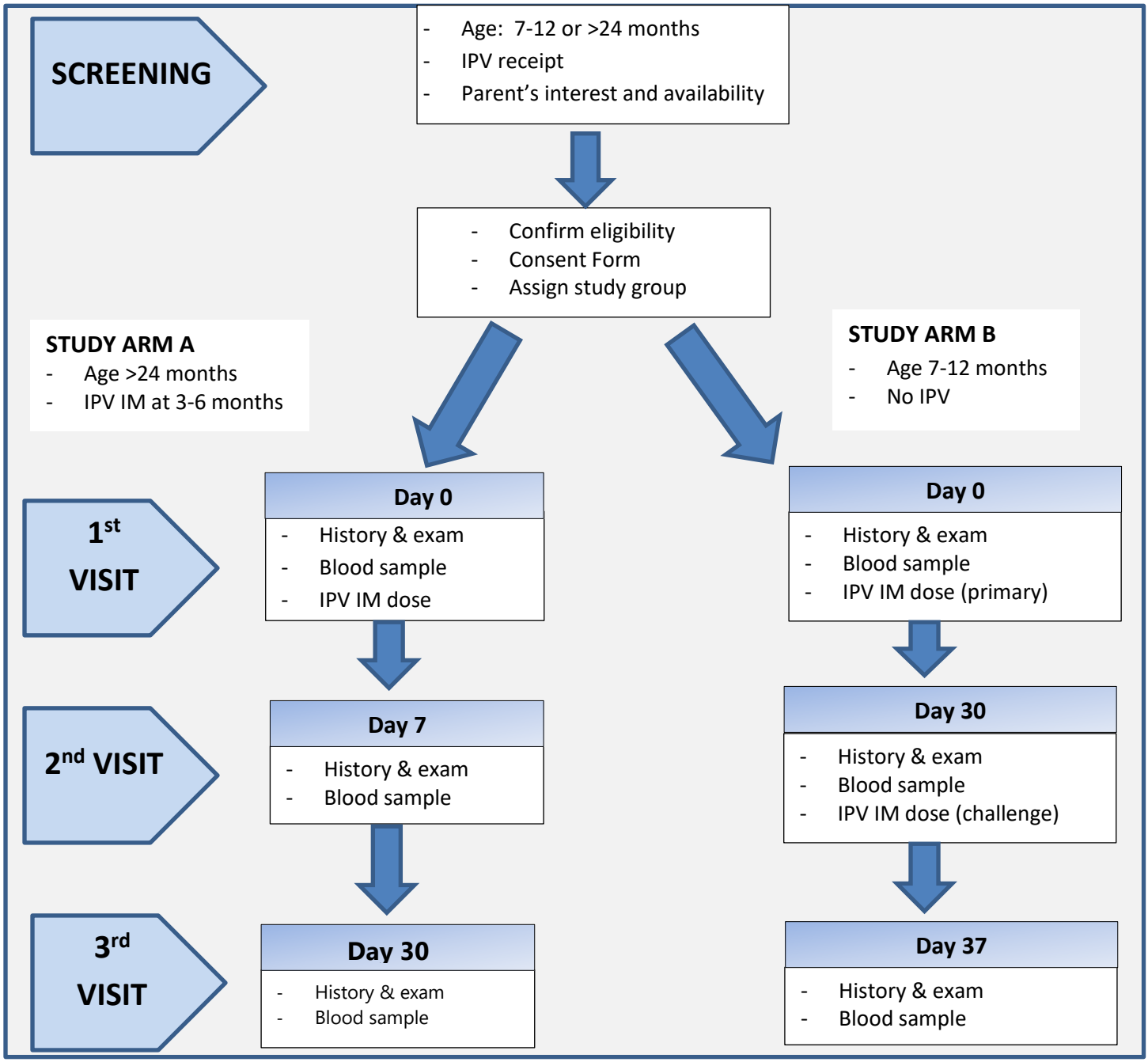
1. Withdrawal of consent for participation for any reason.
2. Request by parents of participant to terminate all study procedures.
3. Identification of immunodeficiency disorder, bleeding disorder or another medical condition for which continued participation, in the opinion of the principal investigator, would pose a risk to the participant to continue in the study.

4. Receipt of immunosuppressive medications.
5. Receipt of any polio (OPV or IPV) vaccine outside of study after enrollment (as per parent's report).
6. Allergic reaction to a dose of polio vaccine.
7. Unable to collect or obtain blood at enrollment.
8. Premature termination of the study.
9. Temporary discontinuation of study activities may occur if there is a mOPV2 campaign.

Study Design

This is an open-label phase IV clinical trial assessing immunogenicity to IPV. Study participants will be identified through screening of children who attend outpatient clinics at the study sites for well-child visits, immunization or minor illness. Infants who fulfill eligibility criteria and whose parents accept participation will be assigned to one of two study arms depending on their age (no randomization). Study procedures for each study arm are shown in the diagram below.

Figure 1. Study Design



Study participants will not be randomly assigned to study groups, because the assignment will depend on their age and IPV vaccination status. The study will not be blinded because both study groups will receive the same vaccine with different schedules. The main outcome of the study, serum antibody titers, is not expected to be affected by provision of placebo, and the pain of an extra injection to the study arm is not considered necessary to achieve the objectives of the study.

Study Vaccines

IPV will be administered as a full-dose (0.5 ml) intramuscularly by needle and syringe. Each dose of IPV contains the following vaccine strains and concentration: type 1 (Mahoney, 40 D antigen units), 2 (MEF-1, 8 D antigen units), and 3 (Saukett, 32 D antigen units).

A full dose at 14 weeks or older is recommended for routine polio vaccination in Nepal and other countries. Many countries provide three or more doses of IPV alone or in combination with other vaccines. An interval of at least one month between doses is recommended for obtaining adequate immune response. Administration of IPV is safe at any age, and there is no known interference in the immune response when IPV is administered with other vaccines used in the Nepalese routine immunization schedule.

Vaccine procurement from a WHO-approved manufacturer will be done in advance for the study, through UNICEF or direct purchase from the vaccine manufacturer. All vaccines will be shipped in reverse cold chain by the manufacturer or UNICEF. On arrival the study coordinator (or pharmacist) will make an inventory and acknowledge receipt of all shipments of study vaccines. The batch numbers, expiration dates and physical description will be present on the label. All vaccines will be stored in accordance with the instructions of the manufacturer (at 2-8 C in the IOM and study sites).

Stringent storage and handling practices will be maintained in the study sites, including daily monitoring and recording of freezer and ILR temperatures. Use of back-up generators will be arranged in all facilities to ensure 24-hour power supply. Because of low availability of single-dose IPV vials globally and in Nepal, multi-dose vials (5- or 10-doses) may be used for the study. Specific forms for each study site will be used to follow the number of study vaccine vials distributed, used, wasted and returned. The open vial policy for IPV will be applied strictly, as per WHO guidelines [45].

Disposal of Used Study Vaccines

The used vials of study vaccines will be stored and returned to the designated central storage facility periodically, for final storage in cold chain. This will be done to ensure availability of the used vaccine vials should a need arise, such as an investigation of adverse events. The used vaccine containers will be stored till the final vaccine accountability has been confirmed and documented by the monitors after the completion of all study visits, i.e. last subject last visit. The disposal will be as per EPI SOPs for disposal of used vials of bOPV and IPV.

Study Procedures

Screening

Nepali children in the eligible age groups will be identified by study personnel screening children presenting to the outpatient department clinic or immunization clinics at each study site. The screening will be undertaken only after the treating clinician has provided the required patient care. In addition, the site investigators will establish a system of identifying potential subjects from immunization registers of the clinics and approaching the perspective parents for study participation. All OPD clinicians will be provided a short orientation about the planned study and will be requested to sensitize parents with children in the required age groups about the study only after completing the clinical part of services. The clinician/support staff will guide the willing parents to the study staff placed in the OPD or to the study clinic set up in the near vicinity.

During the screening process, the study staff will assess for the presence of inclusion and exclusion criteria and fill out a screening Form (form 0). When a child fulfills the study criteria the study staff, will provide a description of the study verbally and will ask the caretakers to read the consent form or will make an appointment for the parents to come at a later date for the first study visit. Further assessment on inclusion and exclusion criteria, and other procedures will be undertaken by the study staff in the study clinic.

Additional strategies for screening:

Participant recruitment in arm A is likely to be slow as finding these children will be difficult. In case of slow recruitment rate, an advertisement will be circulated in preschools of Kathmandu to notify the parents of potentially eligible children about the study. An information leaflet for the parents to describe the study and the contact details for the study sites if the parents get interested has been prepared and is attached in appendix 4. The school authorities will be contacted, briefed about the study objectives and permission obtained before asking them to send the information leaflet to the parents along with the child's school communication book. The interested parents of the preschool children will be free to contact the study team to get further detailed information and decide to participate or otherwise. Interested parents will be called to the hospitals for potential enrolment of their child upon further evaluation.

During the progress of the study, investigators have realized that it has been difficult to recruit adequate number of participants in study arm A and we will be unable to recruit the desired number of participants in this arm during the stipulated duration of the study. Most of the children screened at the study sites lack confirmation of IPV receipt at 3-6 months of age for potential enrolment and these potential participants are not returning back. We have identified that most of these potential participants are going to some preschools; most parents are unwilling to leave three days of their work and are concerned about the child's 3 missed preschool days. We therefore want to spread out the message about IPV study in a small community setting through Female Community Health Volunteers who are currently conducting community visits for other unrelated studies.

Child Health Research project at the Institute of Medicine is currently undertaking some community-based studies on micronutrient supplementation in children and a study on Chronic Obstructive Pulmonary disease in adult populations. Female community health volunteers (FCHVs) who work for Bhaktapur Municipality's "Khowpa Public Health Centre" are assisting the field workers of the aforementioned studies as a part of the community program and visit households on regular basis.

We would like to ask the FCHVs to spread out the message about our ongoing study in the households when they visit these households for their other study related works. They will use the information leaflet prepared for the preschools for disbursing this information and direct the parents of the potential participants to talk to the study team if they want to.

The study team will then discuss with the potential participant's parents about the study, study procedures and methodology and ask the parents to come to one of the study sites for enrolment if they would like to participate in the study.

Once the parents of the potential participants present at the study site, the potential participant will undergo the same first study visit procedures as the participants screened in the study site's outpatient clinics. First study visit (Both Study arms)

During this visit, the following procedures will be conducted for participants in both study arms:

1. The study physician will conduct a short interview and clinical exam of the child to exclude medical contraindications for participating in the study and confirm child's eligibility.
2. If the child is eligible for the study, informed consent process will be undertaken by the study physician or the site investigator in the counseling room set up as part of the study clinic.
3. The study physician or the site investigator will explain the study procedures to the parents and will administer the consent form. If the caretaker has difficulty reading the consent, it will be read to them. Those who have the consent read to them will have an impartial witness present, and the caretaker will be asked to give a thumbprint if they cannot sign. The written consent will be obtained and documented as per the standard operating procedures and will take approximately 20 minutes.
4. Once the parents have signed the consent to participate in the study, the study physician or coordinator, will assign the participant identification number and the study arm according to the age group.
5. Then the study physician will record relevant demographic and clinical information in the corresponding questionnaire (Form 1), including education of parents, gender of baby, number of young children in the household, breastfeeding practices, vaccination history.
6. Study physician will also measure weight and height/length twice (to improve accuracy) and record the mean of the measurements.
7. Then a blood sample will be collected by venipuncture (1 mL)
8. A dose of 0.5 mL of IPV will be administered intramuscularly, using a needle and syringe.
9. The participant will be observed for 30 minutes to monitor for any adverse reactions from the blood collection or administration of study vaccines.
10. At the end of the study period the participant will receive any additional recommended routine childhood vaccinations, according to the age, if the parents agree.
    a. Study participants who have received less than 2 bOPV doses, will also be offered one bOPV dose at this time, to complete the polio vaccination schedule as per Nepal's schedule.
11. The study staff will provide compensation for study visit participation, information on where to call for potential clinical events or questions about the study and schedule the next visit for ~ 1 week (7+1 days) or 1 month later (30±3 days).

Second Study visit

<u>Arm A: Old vaccination: Study visit will occur on Day 7</u>

1. The study physician will conduct a short interview and clinical exam to assess whether there were any clinical events, or the child received any new vaccines and record this information in the corresponding questionnaire.
2. A blood specimen will be collected by venipuncture (1 mL)
3. The study staff will provide compensation for study visit, information on where to call for potential clinical events or questions about the study and schedule the next visit for day 30 post-baseline.

Arm B: Recent vaccination: Study visit will occur on Day 30

1. The study physician will conduct a short interview and clinical exam to assess whether there were any clinical events, or the child received any new vaccines. The clinical exam will also include measurement of weight and length/height twice. This information will be recorded in the appropriate questionnaire.
2. A blood specimen will be collected by venipuncture (1 mL)
3. A dose of 0.5 mL of IPV will be administered intramuscularly, using a needle and syringe.
4. The participant will be observed for 30 minutes to monitor for any adverse reactions from the blood collection or administration of study vaccines.
5. At the end of the observation period the participant will receive any additional recommended routine childhood vaccinations, according to the age, if the parents agree.
6. The study staff will provide compensation for study visit, information on where to call for potential clinical events or questions about the study and schedule the next visit for Day 37.

Third Study visit

Arm A: Old vaccination: Study visit will occur on Day 30

1. The study physician will conduct a short interview and clinical exam to assess whether there were any clinical events, or the child received any new vaccines and record this information in the corresponding questionnaire.
2. A blood specimen will be collected by venipuncture (1 mL)
3. The study physician will inform the parents that the study is complete and study staff will provide compensation for study visit.

Arm B: Recent vaccination: Study visit will occur on Day 37

1. The study physician will conduct a short interview and clinical exam to assess whether there were any clinical events, or the child received any new vaccines.
2. A blood specimen will be collected by venipuncture (1 mL)
3. The study physician will inform the parents that the study is complete and study staff will provide compensation for study visit.

Additional Follow-up procedures

- Before each study visit (24-48 hours), study staff will contact the parents through a phone call to remind them of the date of the next scheduled visit and confirm whether they will attend that day or the date needs to be changed.
- Between study visits and up to the date of the study termination the parents can phone the study staff or attend any of the study clinics to report clinical events.

Concomitant Medications and Vaccines

There will be no restrictions on the use of medications or treatments for concomitant diseases. If the participant needs to receive medications that cause immunosuppression, he/she will be discontinued from the study. If the participant receives a dose of either polio vaccines (OPV or IPV) outside of the study during the study period, the participant will be discontinued from the study. Parents will be encouraged and offered an opportunity to have their child vaccinated with routine childhood immunizations during study clinic visits, in accordance with Nepal EPI's vaccination schedule. Children who are missing bOPV doses will be offered the doses required to complete their schedule after completion of the study.

Specimen collection, handling and testing

Blood specimens (1 mL) will be collected during each study visit at the times specified in the table below

Study Visit	Study arm	Time	Collection Type	Collection Amount	Testing Purpose	Testing Location
Visit 1	A & B	Day 0 (at enrolment)	Blood	1ml	Polio neutralization	CDC-Atlanta
Visit 2	A	Day 7	Blood	1ml	Polio neutralization	CDC-Atlanta
	B	Day 30	Blood	1ml	Polio neutralization	CDC-Atlanta
Visit 3	A	Day 30	Blood	1ml	Polio neutralization	CDC-Atlanta
	B	Day 37	Blood	1ml	Polio neutralization	CDC-Atlanta

Following blood collection, the specimen will be placed at room temperature for 30 minutes and then at 2-8°C until centrifugation. The sample will be centrifuged within 24 hours of collection, either at the laboratory at the study site, or at the IOM laboratory, following specific standard operating procedures. Serum will be aliquoted into two cryovials (minimum 0.2mL) labeled with the PID, visit number, and date of collection. Sera can be stored up to 2 days at +4°C at the study sites before transport to the IOM laboratory. After 2 days sera samples must be stored at -20°C and shipped in cold chain conditions that maintains samples frozen. Samples will be sent periodically from study sites to the IOM laboratory for long-term storage at -20C in the designated freezer. When the last enrolled study patient has completed all study procedures, the co-investigators the laboratory IOM will organize and pack one aliquot of each serum specimen, for international shipment in dry ice to the CDC laboratory in Atlanta. The remaining serum will be stored at -20 degrees Celsius as a back-up for potential loss of samples during shipment

Determination of poliovirus antibodies in serum will be conducted at the Enterovirus Laboratory of the Division of Viral Diseases, CDC, Atlanta using a microneutralization testing that is not available in Nepal. The protocol for the testing is presented in Appendix 3. Presence of poliovirus neutralizing antibodies to all three poliovirus types will be assessed using microneutralization assay (Appendix 3). Antibody titers below 1:8 will be considered negative and the highest detectable titer will be 1:1448. Laboratory staff will be blinded to the study arm to which the study participants belong.

The back-up samples at IOM will be kept stored in cold chain till it is confirmed that CDC shipment has reached in good condition and the testing is satisfactorily completed. CDC will dispose of the samples at their end when the study is completed, and the results are published. This will be done under information to WHO and IOM counterparts. In any case, samples will not be kept beyond three years. Samples will be destroyed following the relevant guidelines applicable in the institutions.

Analytic Plan

Sample Size Calculations

The study will be powered to address the primary objective. Previous clinical trials showed that the proportion of children with detectable immunity to type 2 (seropositive or primed) about one month after one IPV dose was between 90 and 100% [15, 21-23]. The decline in seropositivity or priming after a single IPV dose has not been reported in other studies.

Using a one-sided test for differences between proportions with a continuity correction Z-test using pooled variance (PASS v14), and assuming a conservative estimate of 90% infants in the younger age group with detectable immunity to type 2, we calculated that we would need 237 children to detect a one-sided difference of ≥10% between the proportions of children immune (seropositive or primed) in each age group, with 90% power and 0.05 alpha. To account for potential 5% drop-outs, the sample will be rounded up to 250 per group or 500 children total. The number of bOPV doses and the time since last dose will be considered for secondary analysis but sample size will not be increased to allow stratification by number of bOPV doses because it is expected that bOPV cross-reactivity of the extra dose may play a very small role on type 2 immune response [15, 46].

Study outcome measures

Definition of outcome variables

To assess the immunogenicity of each study vaccine and vaccination schedule we will determine antibody titers against poliovirus types 1, 2 and 3 in sera extracted from blood. Based upon previous studies, we will assume that maternal antibodies have a half-life of 28 days and they should have disappeared from serum by the age of 6 months. The poliovirus antibodies detected in children by age of 6 months or older are, therefore, assumed to be the result of the infant's immune response induced by vaccination. Children 7-12 months of age with detectable antibody titers will be excluded from analysis

We will use the following definitions for outcome variables:

- **Seropositivity** will be defined as the presence of a reciprocal antibody titer of ≥ 8 at any determination time.
- **Priming** will be defined as change from seronegative at baseline to seropositive 7 days after the challenge dose, in children who were seronegative before the challenge dose.
- **Detectable immunity** against poliovirus will be defined as being either seropositive or primed against any type of poliovirus.
- **Boosting** will be defined as a four-fold increase in antibody titers following an IPV dose in children with positive antibody titers at baseline.

Primary Endpoints

- To compare the proportion of children with detectable immunity against type 2 poliovirus following one IPV dose administered 1-2 months before versus > 18 months before blood testing.

Secondary Endpoints

- To assess the proportion of children who seroconvert or boost antibody titers to type 2 poliovirus 30 days after a second dose of IPV, administered > 1 year after the first dose.
- To assess the proportion of children seropositive to types 1 and 3 polioviruses by age group, accounting for the number of bOPV doses received, and number of IPV doses received. It is

expected that all children will have received 3 doses of bOPV through the primary immunization series, even those who did not receive IPV because of the shortage. Children in study arm B may have received extra doses of bOPV through immunization campaigns.

- To compare the distribution of type 2 antibody titers between children who received a dose of IPV more than 24 months ago (study arm A) or recently (study arm B)
- To compare the distribution of type 1 and 3 antibody titers, accounting for the number of bOPV and IPV doses received.

The following tests will be used for describing and comparing outcomes

- One-sided Fisher's exact test will be used to compare the proportion of children with detectable immunity (primed or seropositive) to type 2 poliovirus after the challenge dose between the two study arms.
- Wilcoxon two-sample test, a non-parametric test, will be used to compare the distribution of antibody titers against type 2 poliovirus between children who had received IPV 1-2 months before and children who had received IPV > 18 months before.
- Secondary analyses to assess the influence of number of bOPV doses, number of IPV doses, and potential confounding factors (time since vaccination, presence of moderate-severe malnutrition) on the proportion of children seropositive to types 1 and 3 polioviruses at different study timelines will be conducted by both sub-population analysis (stratification) and logistic regression modelling.

The data will be analyzed by study investigators that include epidemiologists and statisticians using R and SAS statistical packages.

Analytical approach

Primary analytical approach

The primary analytical approach will be a modified intention-to-treat, because we need the participant to attend all study visits at a certain time in order to measure our immunity outcomes.

To evaluate the primary and secondary objective regarding type 2 immunity, the intention-to-treat analysis will be restricted to participants with the following criteria:

- The blood specimen after the challenge dose is collected 6-8 days after the date of administration of the challenge dose
- Have adequate amount of serum to perform serological analysis from the blood specimen collected at the time of administration of the challenge IPV dose
- Have adequate amount of serum to perform serological analysis from the blood specimen collected about 1 week after the challenge dose

Study participants who do not fulfill the above criteria will not be included in the analysis for primary outcomes.

Secondary analytical approach

In addition to the modified intention-to-treat analysis, we will perform a per protocol analysis for the primary outcome in the following study participants

- Aged 6 months or older at the time of receiving the challenge IPV dose

- For children in the younger age group, the primary dose was given within 30 ± 3 days before the challenge dose.
- The blood specimen after the challenge dose is collected 6-8 days after the date of administration of the challenge dose. The blood specimen after the primary dose is collected within 30 ± 3 days.
- Have adequate amount of serum to perform serological analysis from the blood specimen collected at the time of administration of the challenge IPV dose
- Have adequate amount of serum to perform serological analysis from the blood specimen collected 1 week after the challenge dose

To assess immunity against types 1 and 3 polioviruses (proportion of seropositive children and antibody titer distributions) depending on the doses of poliovirus vaccines received and time since last dose, we will perform an intention-to-treat in all study participants with adequate amount of serum collected in the baseline visit.

Data Safety Monitoring Plan (DSMP)

Records to be kept

Participant data will be collected and recorded in paper questionnaires. Personal identifiers will only be recorded on the paper-based screening questionnaire (Form 0) but will not be recorded on any other questionnaires, biological specimens or electronic records.

Participants will be identified by a patient identification number (PID), which will be provided after enrollment and randomization. The PID will be used to link information recorded in questionnaires and results from laboratory analysis of biological specimens. The screening form (Form 0) and informed consent form will be filed in specific locked cabinets at each study facility until the end of the study, with access restricted to select study staff and investigators

All other paper questionnaires will also be kept in locked cabinets at the study facilities, with access restricted to select study staff and investigators. At the end of the study all original documents will be transferred to the Institute of Medicine for final storage.

Data entry, cleaning and storage

Data collected in consent form and Form 0 (screening) will not be entered in any electronic database. Information collected in other questionnaires related to study visit procedures will be entered in an Access electronic database, using the PID for each study participant. The computers and databases used to enter data will be password protected and a restricted number of study staff and investigators will be authorized to access the digital records.

The database will be prepared before study initiation and will include data checks to identify and correct data entry errors. In addition, data entry for each study questionnaire will be done in duplicate. When discrepancies are detected, they will be checked and reconciled with the original forms. Data entry will be checked on a continuous basis, as new patients are enrolled, and procedures performed.

When the laboratory testing in the CDC laboratory in Atlanta is completed, laboratory and clinical datasets will be merged for analysis.

Data Ownership and Public Release

Study data will be jointly owned by the Institute of Medicine (Tribhuvan University), the WHO and the CDC. After completion of study activities and analysis, summary tables of results will be available on public websites of collaborating agencies, if available. Both websites will allow unrestricted public access to the summary tables.

Safety Monitoring

Potential risks and adverse events associated with study procedures

The potential discomforts from this study include having blood drawn and possible reactions to IPV.

Participants may experience pain and minimal bruising at the site of the blood draw. Bruising can be prevented or lessened by applying pressure to the draw site for several minutes. There is also a slight risk of infection associated with blood draws that will be minimized with the use of alcohol swabbing and sterile equipment.

IPV is a very well tolerated vaccine. Local side effects such as redness or induration are observed in less than 12% of infants and tenderness is observed in 14-29%. Systemic reactions, such as fever or convulsions are usually associated with other vaccines such as diphtheria-tetanus-pertussis, commonly given in association with IPV as separate vaccines or in combination [4]. Anaphylactic reactions to IPV are extremely rare but theoretically possible because IPV contains trace amounts of streptomycin and neomycin.

All participants will be observed for 30 minutes after vaccination to monitor for any immediate adverse reactions to the vaccine. Properly skilled medical personnel will be immediately available in the event of an unexpected adverse reaction. After the 30-minute observation period, the medical officer will record the presence of any potential reaction to the vaccine on the corresponding CRF. At the end of each study visit, the physician will provide the parents with a phone number to call if they have any questions and health facilities to attend if the child becomes sick after a study visit.

If a serious illness occurs while the infant is enrolled in the study (requiring a physician's visit or hospitalization), parents will be instructed to seek medical care immediately and to notify study staff as soon as possible. Medical care for study participants will be provided free of charge for minor illnesses that develop during the follow up period between study visits, and for any adverse outcomes judged to be possibly, probably or definitely related (detailed below) to the study vaccine or study procedures.

Recording, Monitoring and Reporting Adverse Events

During each study clinic visit, study staff will ask the parents about possible adverse events (AE) experienced by the participant since the previous study visit. If a potential adverse event is reported by the family member regardless of their relationship with the vaccine, the study physician will fill out the AE Form, will provide medical care, if appropriate, and will follow with the study participant's parents or guardians until it is resolved or no further change in status is expected.

The AE form will include a description of the event, time of onset, assessment of severity, treatment provided, and time of resolution/stabilization of the event. An AE will be considered a serious AE (SAE) if it meets any of the following criteria: 1) Death during the study period; 2) Hospitalization; 3) Paralysis or severe disability/ incapacity; or 4) Anaphylaxis associated with vaccine administration. Any AE considered serious will be reported within 24 hours to the PI, the WHO, and the Ethical Review Committee. Other AE not considered serious will be periodically reviewed by the PI and the Sponsor.

For each adverse event the PI (with endorsement by the DSMB) will determine the potential relationship of the adverse event and the vaccine. Interpretation of vaccine-relationship to AE will be based on the type of event, the relationship of the event to the time of vaccine administration or other procedure, the known biology of the vaccine and the investigators' medical judgment.

Safety Oversight

An independent Data Safety Monitoring Board (DSMB) composed of three individuals will provide safety oversight for the study, in addition to the WHO DSMB. The independent DSMB will independently review all adverse events, and thoroughly investigate those considered serious and unexpected in a timely fashion. The DSMB is expected to perform one interim analysis as well as one final analysis after study completion. In addition, the independent and WHO DSMBs will be informed of any serious AE within 48 hours of occurrence. The DSMB may request additional data and or interim analyses for adverse events.

Site Monitoring Plan

Prior to start of the study, Standard Operating Procedures (SOPs) will be developed by the PI and co-investigators in Nepal, CDC and WHO. Study staff will be trained in study procedures and human subjects management before starting the study. After the start of the study, monitoring of the study sites will be conducted periodically by the Principal Investigator at least three times during the study implementation, as follows.

1. Within one month of the study initiation
2. About 3 months after study initiation
3. Final monitoring visit: Within one month of the last study visit by participants

Additional visits, to address specific issues may also be done as decided by PI and the collaborating agencies.

During these visits, the monitors will observe study activities and talk to study staff, and will review the following study documents and procedures:

1. Participant records, including informed consent forms (with assistance of a medical officer)
2. Paper and electronic questionnaires for study visits
3. Adverse event forms (with assistance of a medical officer)
4. Laboratory specimen records
5. Vaccine and biological materials storage and records
6. Any additional medical records
7. Measures to ensure protection of study participants
8. Measures to ensure compliance with study protocol, and accuracy and completeness of records
9. Any regulatory files associated with the study will also be inspected to ensure all regulatory and reporting requirements are being followed

In addition to in-person visits the PI and collaborators will have periodic conference calls with co-investigators at the study sites to discuss updates of children enrolled and submission of completed study forms and logs, coordination of transportation of samples between study sites, potential adverse events, and questions regarding study procedures or study participants.

Site monitoring visits will be conducted at least once by an international monitor. The monitoring visit will encompass the same activities, but access to informed consents and participant records will be done with assistance of a medical officer to ensure that personal identifiers are not available.

Ethical Considerations

Justification for involving vulnerable patient population:

Children born after April 2016 will be the first birth cohorts in Nepal and other countries who used tOPV before the global switch to bOPV that will be protected against type 2 poliovirus only by a single dose of IPV. No boosters by additional doses of IPV or type 2 OPV or by exposure to wild poliovirus are expected to happen. Therefore, they are the only population that can be studied to determine the potential persistence of full or partial protection against type 2 poliovirus for the medium or long term. This information is crucial to make choices on long-term immunization schedules after global polio eradication, and on potential responses to outbreaks of type 2 VDPVs.

Possible Benefits for Study Participants

For study participants in the older age group, the extra IPV dose will enhance their protection against all poliovirus types compared to children of the same age in Nepal, without a significant risk for their health, as demonstrated with immunization schedules in Europe and the US. For study participants in the younger age group, the study will offer them assured access to IPV and protection against type 2 poliovirus against the unpredictable access to other children in the rest of the country because of the global supply deficit. They will also have the extra benefit on protection against poliovirus provided by two doses of IPV compared with the single dose or the two fractional doses that other children may receive in Nepal.

Parents will also be offered compensation for the cost of transportation to study sites for study visits and free clinical management of minor illnesses or immunizations that brought them to the clinic. Finally, for any participant who is found to be behind their immunization schedule, parents will be offered administration of the vaccine doses that are necessary as per Nepali guidelines.

Possible risks for participants including preventive or alleviative measures

The potential discomforts from this study will include some pain from blood collection and intramuscular injection of IPV. The risk of potential bruising or infection associated with blood collection will be minimized by using sterile equipment and adequate venipuncture technique by skilled laboratory technicians. In addition, only 1.0 mL of blood will be collected at each study visit.

The potential local and systemic side effects associated with IPV, other than pain, occur in a very small proportion of vaccinated infants. Study staff will minimize the potential risks of adverse effects by using appropriate sterile material and injection technique, by observing the study participants for 30 minutes after vaccine administration for immediate treatment of potential anaphylactic reactions, and by providing advice and contact information to parents on how to address expected minor local or systemic side effects after vaccination.

Compensation

To offset the cost of transportation and time off from work, study participants will receive 3000 Nepali rupees for attending the three study visits.

Provisions for protecting privacy/confidentiality

All data collected in this study will be kept confidential. Only the Screening Form and Consent Form will have identifier information. They rest of paper-based questionnaires and study logs, as well as all electronic databases and laboratory specimens will only include the PID, to maintain participant confidentiality.

All paper records will be stored in a locked cabinet and all electronic records will be stored in a password protected database, on a password protected computer. Access to paper and electronic records will be restricted to study staff and investigators. Blood samples that will be sent to CDC for processing and analysis will not contain any personal identification number, and results from laboratory testing will be entered into a secure database. WHO and CDC will only have access to paper-based study forms and records with a PID number and will have no access to the key that links the PID with the participant's identifiers.

De-identified data may be shared with vaccine manufacturers and regulatory authorities to conduct sub-analyses, both national and international, upon request and approval of WHO, IOM, and CDC collaborators/co-investigators. All three collaborating agencies will jointly own the data and will jointly decide on the extent of sharing the data.

Document safety and confidentiality will also be ensured during transport of questionnaires and logs between study facilities (i.e. from the study facility to the IOM coordinating study site), and long-term storage systems will be put in place.

Ethical Review

The Ethical Review Committees at the Nepal Health Research Council (NHRC) and the World Health Organization will be responsible for ethical and scientific review of the study protocol before implementation. The protocol, study forms and other associated appendices will be reviewed and approved by both committees before any participants are enrolled. Any modifications to the protocol will be submitted to the NHRC Ethical Review Committee for additional approval. The approved protocols will also be shared with the CDC IRB.

All participants will be below the legal age of consent. Parents of potential participants will be requested to sign an informed consent form before enrolling their children in the study. The informed consent form will describe the purpose of the study, procedures, confidentiality of information, and the risks and benefits of participation. Informed consent forms will be translated from English to Nepalese, the local language. A copy of the informed consent form will be given to the parent of the participant and noted in study records. A second copy of the signed informed consent form will be filed with study records. If the parent is not literate, the study physician or coordinator will read the consent to the participant in the presence of a literate witness that will verify that the consent form has been accurately read to the parent of the study participant.

Study Coordination

Study sites

The study will be implemented in three study sites in Kathmandu: TU Teaching Hospital, Patan Hospital and Kanti Children's Hospital.

The study sites will be required to have the following characteristics to allow study implementation:

- A large outpatient pediatric department and connection with satellite immunization clinics or other clinics that may refer potential study participants
- High daily patient volume of young children directly or in satellite clinics
- Staff capable of implementing study procedures after training
- Installations, furniture and equipment to conduct interviews, physical exam, and to store files and forms related to the study procedures (including key-locked cabinets for study patients forms and password-protected computers for data entry of electronic forms).
- Laboratory facilities where blood can be processed (centrifugation and serum extraction) and stored at 0-4 C for less than 3 days, or at -20C for longer than 3 days.
- Equipment for safe storage of study vaccine
- Availability of staff and equipment to provide clinical care of potential adverse events amongst study participants.
- Location close to the study coordination center to facilitate logistics for transport of samples and vaccine, coordination of data entry and monitoring of study activities.

The study coordination site where the local Principal investigator and co-investigators will be located will also require the following:

- Installations and equipment for electronic data entry and manipulation.
- Office installations and internet access to implement administrative tasks required to coordinate study activities in study sites and send reports to WHO and CDC.
- Office infrastructure and equipment for long-term storage of study documents
- Laboratory facilities with ability to conduct initial processing of blood samples and long-term storage for samples

To manage the implementation of the study procedures according to the schedule, the IOM will set up an organizational structure based upon the skills and capacity required to implement the study procedures expected in the study. We will also ensure the presence of adequate supervision and oversight to ensure that procedures are done according to the protocol schedule and required specifications, while fulfilling the requirements of Good Clinical Practices in Biomedical Research.

In the table below, we present the division of tasks involved in the preparation and implementation of the clinical trial among the study staff.

Table with tasks sub-tasks assigned to each personnel category

Tasks / sub-tasks	Staff involved
Review and modify study protocol and study instruments	PI and Co-PI
Submit protocol to IRB	PI and Co-PI
Develop manual of Standard Operating Procedures	PI, Co-PI, site co-investigators
Conduct training	PI, Co-PI, site co-investigators,
Screen and interview parents to assess eligibility	Medical officers, nurses
Conduct physical exam, parents interview and fill out study visit form for	Medical officers, nurses
Administer study vaccine	Nurse
Collect blood specimen and process blood to obtain sera	Laboratory technician
Transport blood specimens from study sites to the IOM laboratory	Ancillary staff
Management of adverse events	Study investigator, medical officers, nurse
Monitoring of vaccine adverse events	Data safety monitoring board, PI and co PI
Supervision of staff conducting study procedures	PI, co PI, site co-investigators,
Overseeing the quality of data collection	PI, co PI, site co-investigators,
Provide administrative support for filing study forms and logs, record and file invoices, and logistics.	Administrative and data entry staff

Study personnel and staff:

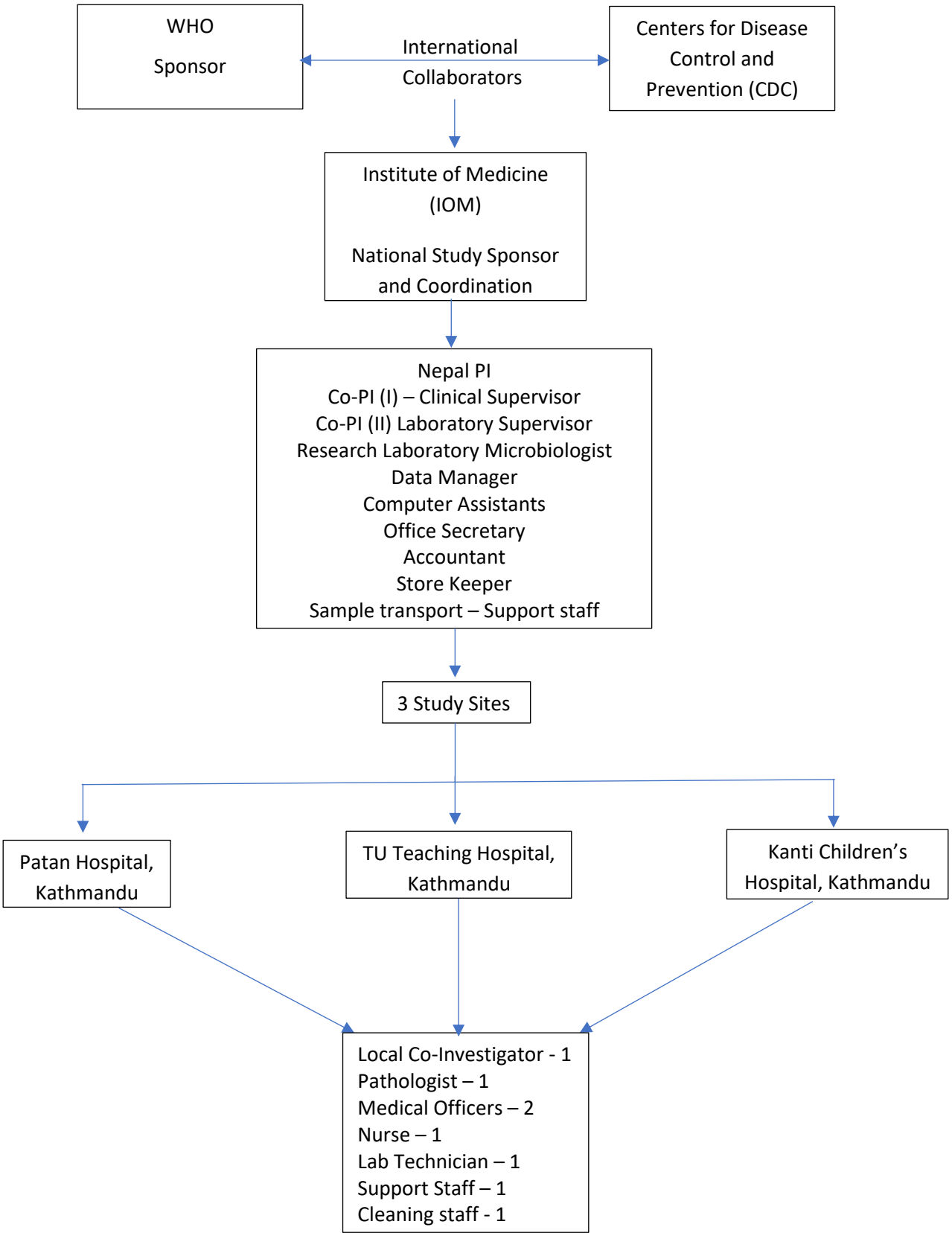
The following table summarizes the qualifications of the study personnel and staff executing the project.

Resource, Time and Role	Education, Training and Certifications	Summary of Experience and Qualifications
Principal Investigator	MD Pediatrics	• Extensive experience in clinical care of young children (22 years), currently heading the Department of Pediatrics • Extensive experience in clinical research and publications • Experience in managing/ supervising clinical trials, development of standard operating procedures for clinical research • Experience in executing multi-site clinical research with collaboration with different partners • Experience in ensuring the safety of the trial subjects. • Experience in maintaining the integrity of the data collected by overseeing all aspects of the study. • Experience in maintaining a schedule of events for conducting clinical trials. • Experience in development of training materials and training of field staff for clinical trials. • Experience of holding the executive Presidency of Nepal Pediatric Society
Co-investigator (1)	• Ph. D., Microbiology	• Extensive experience in microbiological techniques procedures and, currently heading the Department of Microbiology • Extensive experience in clinical research and publications • Experience in managing/ supervising clinical research, development of standard operating procedures for clinical research, • Experience in executing multi-site clinical research with collaboration with different partners

		• Experience in ensuring the safety of the trial subjects. • Experience in ethical principles/ guidelines of clinical research in Nepal being a former chairman of the institutional review board. • Experience in development of training materials and training of field staff for clinical trials.
Co-investigator (2)	• MD Pediatrics	• Extensive experience in clinical care of young children (12 years), currently working as a reader in the Department of Pediatrics • Experience in clinical research and publications • Experience in supervising clinical studies and development of standard operating procedures for clinical research • Experience in executing multi-site clinical research with collaboration with different partners • Experience in ensuring the safety of research subjects. • Experience in maintaining the integrity of the data collected, verification and data cleaning. • Experience in development of training materials and training of field staff for clinical trials.
Investigators at study sites	• MD Pediatrics	• Extensive experience in clinical care of young children, currently working as a reader in the Department of Pediatrics • Experience in clinical research and publications
Medical officers	• MBBS	• Completed basic medical education and certified by Nepal Medical Council for patient care. • Completed a rotatory internship in all clinical disciplines for 1 year. • Trained and experienced in collection of blood and other specimens from young children and administration of vaccines before the execution of the trial.

		• Trained in clinical management of adverse events related to vaccines and reporting of any vaccine associated adverse event. • Trained in proper execution of study procedures, including medical exam and parent's interviews, blood collection and vaccine administration, following the protocol schedules and guidelines, the Standard Operating Procedures and Good Clinical Practices for Biomedical Research. • Trained in proper recording of all patient data and events • Trained in proper collection of laboratory specimens, processing and, storage in the laboratory. • Trained in maintaining appropriate paper-based and electronics forms, maintaining data quality and accuracy as well as participants' safety and confidentiality for the study.
Nurses	• Registered nurse	• Completed basic nursing education and certified by Nepal Nursing Council for patient care. • Working as a nurse practitioner in immunization OPD for more than a year. • Experienced in storage of vaccine to be used in the study in appropriate cold chain. • Experienced in administering vaccine, monitoring of vaccine related and adverse event and reporting of any adverse event. • Trained in execution of study procedures as per Standard Operating Procedures and Good Clinical Practices for Biomedical Research.
Data manager	• Bachelor's degree in arts/ computing	• Certified with basic education in basic computing and data management • Experienced in data management, data cleaning and periodic generation of reports, creation of data logs and maintenance of logs. • Experience in handling data in database programs and ability to transfer data to CDC periodically

ORGANOGRAM


WHO
Sponsor
International Collaborators
Centers for Disease Control and Prevention (CDC)
Institute of Medicine (IOM)
National Study Sponsor and Coordination
Nepal PI
Co-PI (I) – Clinical Supervisor
Co-PI (II) Laboratory Supervisor
Research Laboratory Microbiologist
Data Manager
Computer Assistants
Office Secretary
Accountant
Store Keeper
Sample transport – Support staff
3 Study Sites
Patan Hospital, Kathmandu
TU Teaching Hospital, Kathmandu
Kanti Children's Hospital, Kathmandu
Local Co-Investigator - 1
Pathologist – 1
Medical Officers – 2
Nurse – 1
Lab Technician – 1
Support Staff – 1
Cleaning staff - 1

Laboratory Facilities at CDC

The Enterovirus laboratory of the Division of Viral Diseases at CDC, Atlanta has the necessary staff, infrastructure and equipment to determine levels of antibodies against poliovirus types 1, 2 and 3 using microneutralization testing. The testing of serum for polio antibodies requires specific training and equipment that is not currently available at any laboratory in Nepal.

Roles of Study partners

This study includes three partners with the following roles in the study:

1. World Health Organization, Geneva, Switzerland

- Design the study and develop the study protocol.
- Submit the protocol for WHO ethical clearance.
- Provide funding for study implementation.
- Review standard operating procedures, study forms and training material developed by the contractor to implement the study in Nepal.
- Monitor study implementation through visits to the study sites
- Lead data analysis, interpretation of results and writing detailed reports and manuscripts for publication in peer reviewed journals.

2. Centers for Disease Control and Prevention, Atlanta, GA, USA

- Design the study and develop the study protocol.
- Submit the final protocol approved by WHO and NHRC ERCs to the CDC IRB.
- Review standard operating procedures, study forms and training material developed by the contractor to implement the study in Nepal.
- Test serum specimens from study participants for the determination of antibodies against poliovirus and enter laboratory data into an electronic database (testing will be funded by CDC).
- Support data analysis, interpretation of results and writing detailed reports and manuscripts for publication in peer reviewed journals.

3. Implementation partner, Nepal

- Adapt the study protocol to local settings for implementation (i.e. include study sites and other local details)
- Submit the final protocol to the Nepal Health Research Council for scientific and ethical review
- Select study sites and study staff within the sites necessary to implement study procedures as per protocol.
- Develop a budget that includes the cost of equipment, supplies and human resources necessary to implement the study.
- Work with vaccine manufacturers/providers to obtain material transfer agreements for study vaccines.
- Develop standard operating procedures, study forms and training materials for the study
- Conduct training of the staff on study procedures and good clinical practices before starting the study
- Carry out implementation of the study procedures as per protocol schedules and specific guidelines for: screening of potential participants, obtain informed consent for enrollment from parents, interview and exam study participants, collection of blood specimens, administration of study vaccine doses, and scheduling of follow-up visits.
- Supervise study staff to ensure compliance with the protocol and with regulatory requirements.
- Coordinate logistics for sample collection, processing, storage and final shipment to the CDC laboratory.
- Coordinate study vaccine storage in each study site and administration to study participants as per schedule.
- Record and provide treatment for potential adverse events developed during the study.
- Ensure that documentation of study procedures, and handling and storage of study forms and documents is done using appropriate procedures to maintain participants' safety and confidentiality.

- Send periodically progress reports on study participant enrollment and study progress to the WHO/CDC counterparts.
- Participate in the analysis, interpretation and description of the results in study reports and manuscripts to be published in peer-reviewed journals.

Timeline

Year	Period	Activities
2018	May	Submission to Polio Research Committee for review
	June-July	Award of contract
		Submission of protocol for ethical reviews
	July-August	Protocol revisions, development of SOPs, training materials and database
	August-September	Finalize study tools and study site preparation
		Study sites preparation
		Staff training
2018-2019	Sept 2018-March 2019	Study implementation: Screening and enrollment of participants, collection of specimens and data, shipment of specimens to CDC laboratory
	April-June	Shipment and testing of specimens to CDC laboratory
	July-September	Analysis of results Co-investigators meeting for discussion of results
	October-December	Manuscript writing and dissemination of results

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Appendices

(In attachments)

Appendix 1. Consent Form

Appendix 2. Case Report Forms

Appendix 3. CDC microneutralization test for polio antibodies

Appendix 4. Advertisement: Information leaflet for parents

Appendix 4:

T U Teaching Hospital, Maharajgunj

Department of Pediatrics, Child Health Research Project, Institute of Medicine (IOM)

Request for Participation in Polio Vaccine Study

Polio is a serious disease that can cause paralysis. Government of Nepal is administering polio vaccine to prevent this disease in our children. Despite this, some children may be at risk of poliomyelitis. Department of Pediatrics, IOM is conducting a study on protection of children from polio by an injectable polio vaccine. If your child was born between 16th April 2016 and 15th September 2016, your child can be eligible for the study. If you are interested and want to know more about the study, please call the study phone numbers at the following hospitals.

TU teaching Hospital	9867069574
Patan hospital	9867069573
Kanti Children hospital	9867069572