RESEARCH PROTOCOL OF VACCINE CLINICAL TRIAL

A PHASE III, RANDOMIZED, PARTIALLY DOUBLE- BLIND, ACTIVE CONTROL STUDY TO COMPARE THE IMMUNOGENICITY AND SAFETY OF A LIQUID FORMULATION OF ROTAVIN WITH THE CURRENTLY LICENSED FROZEN FORMULATION OF THE VACCINE (ROTAVIN-M1), IN HEALTHY VIETNAMESE INFANTS.

Protocol Number: CVIA 068

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Trial Registration Reference: NCT03703336 (ClinicalTrials.gov)

SUMMARY NAME: Phase III Bridging Study of Liquid Formulation of ROTAVIN

Principal Investigator: [redacted] **NIHE**

Co- Principal Investigator: [redacted] **NIHE**

Investigator's Institution: National Institute of Hygiene and Epidemiology, Vietnam

Sponsor: Center for Research and Production of Vaccines and Biologicals (POLYVAC), Vietnam

In Collaboration with: PATH, USA

Manufacturer: Center for Research and Production of Vaccines and Biologicals (POLYVAC), Vietnam

Authority Agency: Ministry of Health, Vietnam

Planned time: 4th quarter 2018

Total budget: From Government budget for Science and Technology: 0 VND

From PATH: Approximately 8.5 billion VND

Confidentiality Statement

STATEMENT OF COMPLIANCE

I am Principal Investigator of the study "A Phase III, Randomized, Partially Double- Blind, Active Control Study to Compare the Immunogenicity and Safety of a Liquid Formulation of ROTAVIN with the currently Licensed Frozen Formulation of the Vaccine (ROTAVIN-M1), in Healthy Vietnamese Infants". Signing below ensures that the study will be carried out on schedule, according to the content of approved protocol, and in accordance with Good Clinical Practice (GCP) as required by applicable rules of Vietnam: Decision No. 799/2008/QD-BYT, on "Guidance on Good Clinical Practice," Circular No. 03/2012/TT-BYT on "Guidance on Clinical Trial" and the Circular No. 44/2014/TT/BYT on "Medical Product Registration," Decision 1248/QD BYT on the National Guidelines on ethical issues in biomedical research; Decision 111/QD BYT on the guidelines on organizational and operational principles for IRBs; and Dispatch 6586/BYT-K2DT on the guidelines on recording/reporting SAE in clinical trial.

The study informed consent documents will embody the elements of consent as described in the Declaration of Helsinki.

All key personnel (those responsible for the design and conduct of this study) will have completed Human Subjects Protection Training, Responsible Conduct of Research (RCR) training and ICH-GCP training prior to interaction with any participants or to have access to their confidential study data.

Hanoi, Vietnam

Co- Principal Investigator

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Head, Center for Clinical Trials , NIHE

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LIST OF ABBREVIATIONS

AE	Adverse Event
AEFI	Adverse Event Following Immunization
°C	Degrees Celsius
°F	Degree Fahrenheit
BCG	Bacillus Calmette–Guérin
CCHMC	Cincinnati Children's Hospital Medical Center
CDC	Centers for Disease Control
CI	Confidence Interval
cm	Centimeter
CRF	Case Report Form
CRO	Contract Research Organization
D	Day
DCF	Data Clarification Form
DFID	The Department for International Development
DTP	Diphtheria, Tetanus and Pertussis
DTwP	Diphtheria, Tetanus, Pertussis (whole cell)
EDC	Electronic Data Capture
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
EPI	Expanded Program on Immunization
GCP	Good Clinical Practice
GMC	Geometric Mean Concentration
HepB	Hepatitis B
Hib	*Haemophilus influenzae type b*
ICF	Informed Consent Form
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICMJE	International Committee of Medical Journal Editors
IEC	Independent Ethics Committee
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IP	Investigational Product
IPV	Inactivated Polio Vaccine
IRB	Institutional Review Board
IU	International Unit
kg	Kilogram
L	Liter
LAR	Legally Acceptable Representative

mcg	Microgram
MedDRA	Medical Dictionary for Regulatory Activities
mL	Milliliter
Mm	Millimeter
MOH	Ministry of Health
MOP	Manual of Procedures
N	Number (typically refers to number of participants)
NICVB	National Institute for Control of Vaccine and Biologicals
NIHE	National Institute of Hygiene and Epidemiology
OPV	Oral Polio Vaccine
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PE	Physical Examination
PFU	Plaque Focus Unit
PI	Principal Investigator
PIDC	Post-Immunization Diary Card
POLYVAC	Center for Research and Production of Vaccines and Biologicals
PPMC	Provincial Preventive Medicine Center
SRC	Safety Review Committee
RBC	Red Blood Cell
RCR	Responsible Conduct of Research
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RV	Rotavirus
SAE	Serious Adverse Event
SD	Standard Deviation
SDV	Source Data Verification
SOP	Standard Operating Procedure
TCID	Tissue Culture Infectious Dose
US	United States
VND	Vietnamese Dong
VP	Viral Protein
WHO	World Health Organization
WIRB	Western Institutional Review Board

PROTOCOL SUMMARY

Title:	A Phase III, Randomized, Partially Double-Blind, Active Control Study to Compare the Immunogenicity and Safety of a Liquid Formulation of ROTAVIN with the currently Licensed Frozen Formulation of the Vaccine (ROTAVIN-M1), in Healthy Vietnamese Infants.
Description of Study Design:	• The study is designed as a phase III, randomized, double-blinded, active controlled study with two groups of infants receiving vaccines at the ratio of 2:1 (550 participants receiving the liquid formulation of ROTAVIN and 275 participants receiving the frozen formulation ROTAVIN-M1), to compare their immunogenicity and safety. Two doses of vaccine will be administered 8 (+2) weeks apart (minimum interval of 8 weeks and maximum of 10 weeks), with the first vaccine administration at 60-91 days (both days inclusive) of age. • Active surveillance for vaccine reactogenicity (solicited reactions) over the 7-day period after each vaccination, unsolicited AEs for 4 weeks after each vaccination and SAEs including intussusception over the period between first vaccination and four weeks after the last vaccination will be conducted for all infants. • Immunogenicity will be assessed in all subjects enrolled at one of the two proposed sites. Blood samples will be collected from all the participating infants from Quang Ninh site before the first and four (+2) weeks after the second vaccination to evaluate immune responses to rotavirus vaccine.
Hypothesis:	The study hypotheses are that: **Immunogenicity:** Liquid formulation of ROTAVIN manufactured under full MOH Regulatory compliance is as immunogenic as the currently licensed frozen formulation of the vaccine (ROTAVIN-M1) **Safety:** Liquid formulation ROTAVIN manufactured under full MOH Regulatory compliance is safe and well tolerated
Study Objectives	**Primary Objectives** **Immunogenicity** To demonstrate non-inferiority in the immunogenicity of the liquid formulation of ROTAVIN in comparison to currently licensed frozen formulation of the Vaccine (ROTAVIN-M1), 28 days after the last dose of the vaccine when administered to infants in a two-dose schedule starting at 60-91 days of age. **Safety** To evaluate the safety of liquid formulation of ROTAVIN in comparison with currently licensed frozen formulation (ROTAVIN-M1), in terms of solicited reactions after each dose of vaccine. **Secondary Objective** **Immunogenicity**

	To further characterize the immunogenicity of the liquid formulation of ROTAVIN in comparison with currently licensed frozen formulation of the Vaccine (ROTAVIN-M1). **Safety** To evaluate the safety of the liquid formulation of ROTAVIN in terms of immediate post-vaccination events, unsolicited adverse events and serious adverse events, including monitoring for intussusception.
Study Endpoints	**Primary Endpoint:** **Immunogenicity** Rotavirus vaccine immunogenicity will be tested on serum samples obtained in a subset of 450 participants before the first vaccination and four weeks after the second vaccination using a validated ELISA assay. The primary immunogenicity endpoint is as follows, • Geometric mean concentration (GMC) of serum anti-rotavirus IgA antibody concentrations 28 days after the second vaccination. **Safety**: Solicited post-vaccination reactogenicity (fever, diarrhea, vomiting, decreased appetite, irritability, and decreased activity level) during the 7 day period (Day 1-7) after each vaccination **Secondary Endpoint:** **Immunogenicity** • Percentage of participants with seroconversion in serum anti-rotavirus IgA antibody concentrations 28 days after the second vaccination. o For participants with a negative pre-vaccination IgA concentration (< 20 IU/ml), a positive post-vaccination IgA concentration (≥ 20 IU/ml); o For participants with a positive pre-vaccination IgA concentration, a post-vaccination IgA concentration at least 4 times as high as the pre-vaccination IgA concentration. • Percentage of participants with seropositivity at baseline and 28 days after the second vaccination. Seropositivity is defined as serum IgA antibody concentration ≥ 20 IU/ml. **Safety** • Immediate adverse events, within 30 minutes after each vaccination. • Unsolicited AEs from vaccination through four weeks after each dose. • Serious adverse events (SAEs) and intussusception from first vaccination through four weeks after the last vaccination.
Study Population:	Approximately 825 healthy male or female infants, aged 60-91 days (both days inclusive) at the time of enrollment.
Phase:	Phase III

Number of Sites:	2 site. The study will be conducted by National Institute of Hygiene and Epidemiology (NIHE) at Nam Dinh and Quang Ninh provinces, Vietnam
Study Duration:	Approximate duration of clinical phase of the study is estimated as 12 months whereas the total duration including analysis and reporting is approximately 18 months.
Participation Duration:	Approximately 3 – 4 months per participant
Description of Agent or Intervention:	**Test Vaccine** ROTAVIN liquid formulation: Live Attenuated human rotavirus vaccine at a dosage of ≥ $2x10^6$ Plaque Focus Units (PFU) / Dose of 2 ml. The vaccine can be stored and transported at 2-8 °C. The vaccine is for oral use. **Comparator vaccine** ROTAVIN-M1: Live Attenuated human rotavirus vaccine at a dosage of ≥ $2x10^6$ PFU / Dose of 2 ml. The frozen vaccine vial will be stored at -20°C. The vaccine can also be stored and transported at 2-8 °C up to 2 months. The vaccine is for oral use.
Estimated Time to Complete Enrollment:	Full enrollment for the study is anticipated within 7 months of first vaccination.

Table 1: Trial Schema- Schedule of Events

Visit	V1*		V2*	V3
Time point (Day)	D1		D57	D85
Interval	Screening	Post-enrollment	V1+ 8 (+2) weeks	V2+ 4 (+2) weeks
Information process and written informed consent	X			
Collect baseline demographic data	X			
Collect/review medical history	X			
Perform Physical examination and Vital examination	X[a]		X	X
Check inclusion/exclusion criteria	X			
Enrollment	X			
Randomization		X		
Collect blood for immunogenicity assessment[b]		X (Pre-dose)		X
Check withdrawal criteria			X	X
Check contraindications, warnings and precautions to vaccine administration			X	
Study vaccination		X	X	
Observe for immediate reactions for 30 minutes		X	X	
Perform post-vaccination Vital examination and targeted physical examination if required.		X	X	
Issue and instruct parent/LAR of participant on use of diary card		X	X	
Record solicited AEs within 7 days post-vaccination		X	X	
Safety follow-up by health worker[c]		X	X	
Record unsolicited AEs , including SAEs		X	X	X
Record any concomitant medications/vaccinations		X	X	X
Review interim medical history and record any intercurrent medical conditions			X	X
Participant completion of study				X

D = day; AE = adverse events; SAE = serious adverse event; Shading represents days of vaccination; V= Visit number.

* The study visits will be planned in advance such that the EPI vaccination days fall either 7 days before or after the study vaccination day. This will allow the participant to receive EPI vaccines in the window allowed by the protocol.

[a] Symptom based PE to be performed if screening is being repeated and there is any change in health since last screening.

[b] At Quang Ninh site

[c] This includes visit by the health worker to the participant's home on Day 2/58(+1) and 8/64(+2) after each vaccination and a telephone call on Day 5/61 (+2).

1 KEY ROLES FOR INDIVIDUALS AND INSTITUTIONS INVOLVED

Principal Investigator:	[redacted] Director, NIHE
Co- Principal Investigator	[redacted] Head, Centre of Clinical Trials, NIHE
Investigator's Institution:	National Institute of Hygiene and Epidemiology (NIHE)
Institutional Review Boards:	NIHE IRB, WIRB and MOH IEC, Vietnam
Manufacturer and Sponsor	POLYVAC Center for Research and Production of Vaccines and Biologicals 135 Lo Duc Street, Hai Ba Trung District, Hanoi, Vietnam. [redacted]
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Clinical Site	Nam Dinh and Quang Ninh provinces, Vietnam
Clinical and Specialty Laboratories:	Cincinnati Children's Hospital Medical Center (CCHMC) Division of Infectious Diseases Cincinnati, Ohio [redacted]
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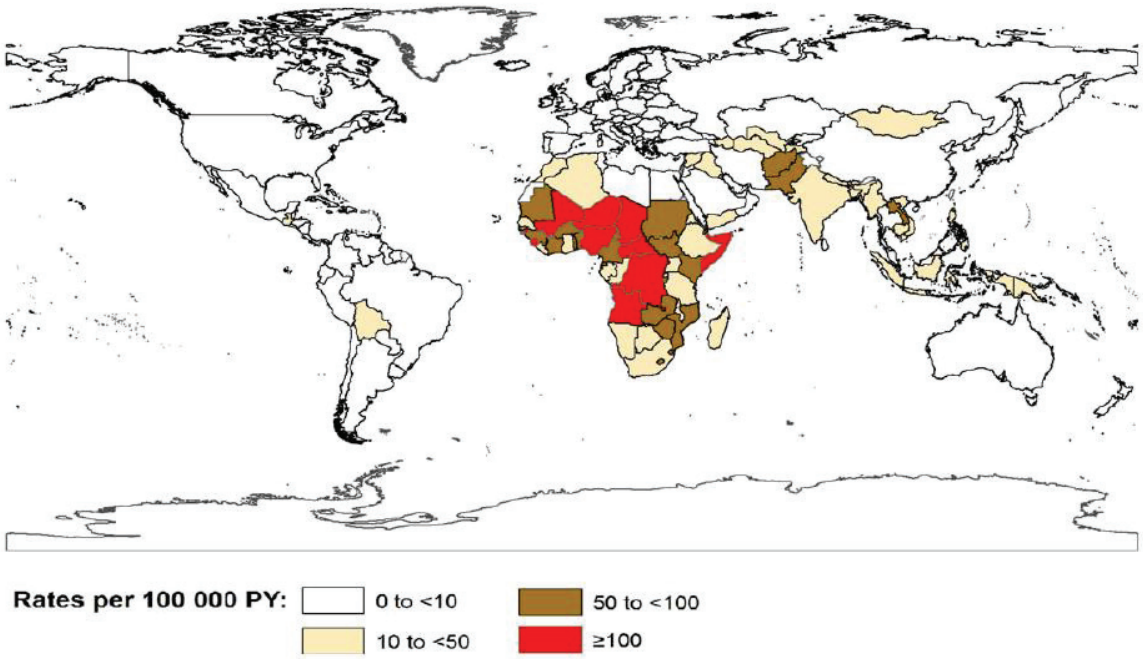
2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Overview of Rotavirus Diarrhea Disease

Diarrhea is a major cause of death among children under five years of age globally.[1] Rotavirus is the leading cause of severe diarrhea, resulting in an estimated 215 000 (range, 197 000–233 000) deaths in 2013 which corresponds to 3.4% of all deaths in children <5 years of age. More than 90% of rotavirus deaths occurred in low and low-middle income countries.

Figure 2.1: Mortality rate due to rotavirus disease among children <5 years of age, by country, 2013.



Rotavirus was identified in 1973 from the small intestine's epithelial cells of children who were affected by diarrhea.[1] Humans are mainly infected by rotavirus species A, B, and C and more than 90% of diarrheal infections occurs by species A.

Rotaviral genome (consisting of 11 segmented double stranded RNA) is shelled by three protein namely a core, inner membrane, and outer capsid proteins.[2] The outer shell that is used to characterize the rotavirus strain is formed by VP7 (a glycoprotein-G protein) and VP4 (a protease-cleaved protein—P protein). The combination of G and P proteins is mediated by VP4 and VP7 structural protein due to its independent nature of segregation both *in vivo* and *in vitro*. And more than 80% of severe rotavirus disease is caused by the mostly common four combinations (P[8]G1, P[4]G2, P[8]G3, P[8]G4) of G and P proteins.[3]

The exact mechanism of rotavirus infection in causing of diarrhea is relatively unknown within the public health community [4], particularly in developing countries.[5] Some researchers explained that the Rotavirus diarrhea is multifactorial, with malabsorption and secretion components, and may have other components suggested to be related to villus ischemia and intestinal motility.[6] The rotavirus mostly prefer to infect during cool and dry seasons with infection showing peaks in winter to cause diarrhea.[7,8]

It is suspected that clinical protection by natural infection probably involves mucosal (intestinal), systemic antibody response, and cell-mediated immunity however it is difficult to realize the mechanisms, protection as well as duration of protection against rotavirus infection.[9,10] The molecular mechanisms underlying the rapid induction of heterotypic protective immunity to RV, which provides the basis for the efficacy of licensed monovalent RV vaccines, have remained unknown. Epidemiologic and clinical studies demonstrate that heterotypic protective immunity is generated after a single RV infection[11] or immunization.[12,13]

Diarrhea is the fourth leading cause of child death in Vietnam. Up to half of diarrhea cases resulting in hospitalization are caused by vaccine-preventable rotavirus. In line with the world averages, in 2013 estimations, Vietnam reported a total of 2083 diarrhea related deaths of which 49.9% diarrheas were positive for rotavirus. This corresponds to 3.1% of all-cause mortality in children less than 5 years of age.[14] A recent publication on rotavirus surveillance in hospitalized children with diarrhea in Vietnam reported a decrease in rotavirus positive stool samples from approximately 55% in 2012 to 37% in year 2015. The study also noted that although rotavirus was detected year-round, but most rotavirus gastroenteritis cases occurred between December and May, corresponding to the rotavirus seasonality with the peak varying by regions in Vietnam.[15] G1P[8] was considered as the most common rotavirus strain causing rotavirus diarrhea. A downtrend in prevalence of isolation of G1P[8] was noted with this strain being isolated from approximately 82% samples in 2013 and 15% in 2015. However, G2P[4] was found in 5% of samples in 2012, 9% in 2013, 36% in 2014, and 28% in 2015 which indicated a shifting trend in the region.

Nearly all children, regardless of geographic location or economic status, are infected with rotavirus before their third birthday, but children in low-income countries are far more likely to be infected earlier in life and, because access to urgent care can be limited or unavailable in rural, impoverished settings, they are more likely to develop severe disease and die.[16]

2.2 Treatment and Prevention (Vaccine, Biological)

High rotavirus mortality rates still prevail in low-income countries in spite of the significant impact of oral rehydration therapy (ORT) and the general improvement in sanitary conditions. Such high mortality rates are likely associated with the elevated incidence of malnutrition, other co-morbidities and with limited access to appropriate medical care. In addition to causing loss of lives, rotavirus places a heavy burden on health care systems and on families with prohibitive medical care costs and loss of productivity for adults taking care of sick children.

Standard methods of sanitation such as antibacterial soaps are not 100% effective in killing the virus, and because low numbers of viruses can cause infection, transmission is common even with good hygiene practices. Thus, rotavirus vaccination is now recommended for use in every country in the world and should be a part of a comprehensive strategy to control diarrheal diseases with the scaling up of both prevention (promotion of early and exclusive breastfeeding, handwashing with soap, improved water and sanitation) and treatment packages (including low-osmolarity ORS and zinc).[17]

The public health impact of rotavirus vaccination has been demonstrated in several countries. For example, in the USA, a measurable decrease was seen in the number of rotavirus gastroenteritis hospitalizations accompanied by a suggested herd effect protecting older non-vaccinated children, while in Mexico a decline of up to 50% in diarrheal deaths in children < 5 years of age was attributed directly to the use of the vaccine. WHO continues to recommend that the first dose of

either RotaTeq™ or Rotarix™ be administered as soon as possible after 6 weeks of age, along with DTP vaccination. Apart from a low risk of intussusception (1 to 5 per 100 000 infants vaccinated)[18] the current rotavirus vaccines are considered safe and well tolerated. In low income countries, vaccine efficacy can be lower than in industrialized settings, similar to other live oral vaccines, however, due to the high rates of severe diarrhea, this modest efficacy still translates into high public health impact, thereby making rotavirus vaccination highly cost-effective in high-mortality settings. Rotavirus vaccine implementation in settings of high child mortality in Africa and Asia is just beginning to occur, and the real lifesaving potential of vaccination has yet to be realized. The scale up of rotavirus vaccines in developing countries is limited by inadequate availability of high quality vaccines at affordable prices.

Live attenuated oral rotavirus vaccines were first developed for high-income countries and have been shown to have modest efficacy in developing countries (in the range of 40% to 60%). [19-23] Six rotavirus vaccines, Rotarix (GSK), RotaTeq (Merck), ROTAVAC® (BBIL), ROTASIIL (SIIPL), Lanzhou lamb rotavirus vaccine (LIBP) and ROTAVIN-M1(POLYVAC) has been licensed in at least one country. Of these Rotarix, RotaTeq and Rotavac has been listed in WHO list of prequalified vaccines and can be exported to other countries. Although Rotarix and RotaTeq are considered effective in preventing severe gastrointestinal disease and are currently more widely available and have been demonstrated to be safe and effective in low-income, high-burden populations, they are not affordable in developing countries. The recent global recommendation by the World Health Organization (WHO)'s Strategic Advisory Group of Experts on Immunization (SAGE) for the inclusion of rotavirus vaccines in all national immunization programs and considered a priority particularly in countries in South and Southeast Asia and sub-Saharan Africa is expected to further increase demand, but economic barriers to access remain, and vaccine supply continues to be an issue for the countries in most need.[17] Two commercial rotavirus vaccines, Rotarix™ (GSK, Belgium) and RotaTeq® (Merck), have been tested in children in Vietnam and are available in the private market. The liquid formula of Rotarix™ when tested in two schedules, 1-month and 2-month interval between doses compared with placebo control in 375 children had a seroconversion rate of 63.3% and 81.5%, respectively.[24] RotaTeq® showed a seroresponse rate (number of participants with a three-fold or higher rise in from baseline) of 87.8% and an overall efficacy of 63.9% (72.3% in the first year and 64.6% in the 2nd year of follow-up) in a phase 3 efficacy trial in Vietnam.[21] However, neither of the two vaccines is currently available at an affordable price for the national program (e.g. Rotarix and RotaTeq costs around US $27-35 in the private market in Vietnam). Therefore, the candidate vaccine, ROTAVIN-M1, was developed in order to fill this need for a more affordable vaccine for Vietnamese children.[25] This vaccine is similar to Rotarix™, and was developed by selecting a common G1P [8] strain and attenuating it through serial passages and plaque purification in qualified Vero cells under GLP conditions. Further upstream processes towards manufacturing of the vaccine were conducted under GMP conditions. It is estimated that if a vaccine was introduced in the current childhood immunization schedule, it could reduce severe rotavirus disease by about 60% or more given current vaccine efficacy and coverage.[26]

Over the past decade Vietnamese government has encouraged local production of vaccines with the aim to achieve self-reliance and provide affordable supply for its own population. Several locally produced vaccines for poliomyelitis, cholera, Japanese encephalitis, and Diphtheria–Pertussis–Tetanus have contributed to the reduction in the prevalence of these diseases and in 2000 Vietnam announced that it had the status of poliomyelitis-free. Vietnam is one of a few countries in Asia which can produce vaccine itself. The national regulatory authority of Vietnam (NRA) was

prequalified by WHO and was recognized for meeting international standards for vaccine regulation in June 2015. This is a condition for Vietnam to export its vaccines. ROTAVIN-M1 was developed by POLYVAC and licensed in 2012.

2.3 Immune response to rotavirus vaccination

The availability of markers of rotavirus protection would significantly facilitate further vaccine development, however, in spite of many efforts, no clear correlates of protection have thus far been identified, and thus no assays are available as proxy of protection.[27] On the other hand, the lack of transmission of IgA antibodies from the mother to the fetus has allowed for clean measures of immune responses to rotavirus antigens using ELISA platforms. IgA ELISA tests have been successfully applied to measure vaccine responses in high resource countries, in which typically over 80% of the immunized participants are found to develop responses. When testing the same vaccines in resource limited countries, both the post-vaccination concentrations and seroresponse rates plummet to levels frequently lower than 1:50 concentrations or 40% response rates.[28] The drop parallels the observed diminished efficacy, however, the two phenomena are not necessarily linked, since in some studies vaccine efficacy has been greater than the observed IgA seroresponse rates. Nonetheless, IgA continues to be used as a practical indicator of vaccine effect and considered as the best marker of protection available, albeit "imperfect".[29] Thus far, ROTAVIN-M1 has only been tested for immunogenicity in Vietnam, where it showed a higher rate of immune responses (65-70%) and comparability to Rotarix® (Data on file).

2.4 Description of Study Vaccine

ROTAVIN-M1 is vaccine produced from a live attenuated G1P[8] strain (KH0118) developed from a rotavirus strain isolated in 2003 from an 6-month-old girl with diarrhea at the Khanh Hoa Hospital in Nha Trang, Vietnam. The virus was isolated, plaque purified and further passed in MA104 cells (6X) and later adapted to primary monkey kidney cells (12 passages) and eventually adapted to Vero cells. ROTAVIN-M1 is currently produced in Vero cells from working seed viruses G1P[8] also grown in Vero cells. ROTAVIN-M1 was approved by Vietnam MOH for production in 2007 and it was licensed in May 2012. The vaccine needs to be stored frozen at -20 $^{\circ}$C. It can be stored at 2-8 ^{o}C for up to two months. To date more than 800,000 doses have been used for children of all 63 Provinces of Vietnam so far.

The Liquid formulation of ROTAVIN was developed with the technical support from PATH. This formulation contains the same rotavirus strain (KH0118) as in Rotavin-M1. POLYVAC has changed a few excipients in the earlier formulation of finished product to increase the stability of the vaccine at 2-8 ^{o}C. The advantages with such liquid formulations include stability at 2-8°C which makes the vaccine amenable for easier storage and transport and makes it more suitable for programmatic application.

The finished liquid formulation of ROTAVIN is presented as an oral solution, containing not less than $2x10^{6.0}$ PFU of human rotavirus per dose. The vaccine is supplied as a 2 ml single dose presented in a glass vial with a silicon stopper, an aluminum cap seal. The formulation contains buffers to protect the viruses from gastric acid and a stabilizer.

2.5 Summary Results of Related Preclinical Studies

2.5.1 Preclinical Studies of ROTAVIN-M1

The oral live attenuated rotavirus vaccine ROTAVIN-M1 were successfully manufactured, tested and released by POLYVAC in 2008. Preclinical evaluation was conducted with all three lots of ROTAVIN-M1. The vaccine demonstrated good immunogenicity in monkey (*Macaca mulatta)* studies (quantitated by neutralizing antibody titer comparable to that induced by a comparator vaccine), and an acceptable safety profile in general safety studies following three immunizations.

General Safety studies in mice and guinea-pigs

Pre-clinical Toxicity studies in mice and guinea-pigs was conducted with single dose of 0.5 ml per animal. The animals were observed for safety parameters for 1 week after vaccine administration. Tables 2.1 and 2.2 below show that vaccine ROTAVIN-M1 in a dose of 0.5 mL was well-tolerated in mice and guinea-pigs. All studied animals were alive, gained weight and healthy at the end of study. The average weight gain was similar between vaccine and control group. The results demonstrated the safety of the vaccine in mice and guinea-pigs

Table 2.1: Weight of animals during safety study

ROTAVIN-M1 vaccine lot	Mice weight (gram)		Guinea-pigs weight (gram)	
	Pre injection	Post injection	Pre injection	Post injection
012008	12.6 ± 0.12	27.84 ± 3.09	289.9 ± 12.87	361.35 ± 10.39
022008	12.76 ± 0.25	25.78 ± 0.83	285.05 ± 4.03	340.3 ± 6.22
032008	13.08 ± 0.16	28.22 ± 2.93	271.35 ± 5.30	327.65 ± 8.41
Control	13 ± 0.22	27.42 ± 2.50	269.05 ± 20.29	332.8 ± 31.82

Table 2.2: Safety study in mice

	Vaccine group	Control group
Total number of mice	15	5
Death number during 24 hour	0	0
Death number after 24 hour	0	0
Number of mice loss weight	0	0
Total number of live and healthy mice	15	5

Table 2.3: Safety study in guinea-pigs

	Vaccine group	Control group
Total number of guinea-pigs	6	2

Death number during 24 hour	0	0
Death number after 24 hour	0	0
Number of guinea-pigs loss weight	0	0
Total number of live and healthy guinea-pigs	6	2

Safety studies in monkeys

A safety study was conducted in monkeys (*Macaca mulatta*). Tables 2.4 and 2.5 show the safety results in 8 monkeys including weight gain and body temperature, pre and post-vaccination. There were 3 monkeys in vaccine group (ROTAVIN-M1) receiving 2 mL of the vaccine, 3 monkeys in the control group (Rotarix vaccine), and 2 monkeys in placebo group. All monkys received three doses of the allocated vaccines one month apart. All monkeys (8/8) were alive and healthy, and gained weight at the end of study. The weight gain in the ROTAVIN-M1 group ranged from 200 – 360 grams, compared with 260 g/monkey in the Rotarix group, and 350 g/monkey in the placebo group. The body temperature at pre and post-injection were still in the normal body temperature range from 38.5-40ºC.

Table 2.4: Weight and body temperature of Monkey during the study

Vaccine	Weight (kg)		Temperature (oC)	
	Pre injection	Post injection	Pre injection	Post injection
ROTAVIN-M1	1.26 ± 0.05	1.46 ± 0.05		
41	1.3	1.4	38.7	38.3
42	1.2	1.5	38.7	38.1
43	1.3	1.5	38.9	38.1
Control	1.1 ± 0	1.45 ± 0.07		
53	1.1	1.5	39.3	38.7
54	1.1	1.4	39.1	38.2

Table 2.5: Results of safety study on Macaca mulatta monkey 6 – 17 months old

	ROTAVIN-M1	Control group	
		Rotarix	Placebo
Total number of monkeys	3	3	2
Death number during 24 hour	0	0	0
Death number after 24 hour	0	0	0

Number of monkeys got diarrhea	0	0	0
Total number of live and healthy monkeys	3	3	2

Immunogenicity results
Immunogenicity study for ROTAVIN-M1 was conducted in monkeys (*Macaca mulatta*). The results demonstrated the vaccine was immunogenic in the studied monkeys. All monkeys has a post-vaccination neutralizing antibody titer of ≥ 1:32 (2^5) and exhibited a seroresponse of at least a four-fold increase compared with baseline, equivalent with Rotarix vaccine.

2.5.2 Preclinical studies of liquid formulation of ROTAVIN

Since, the liquid formulation of ROTAVIN contain the same vaccine strain and dose level as ROTAVIN-M1 with the difference being addition of some excipients a pre-clinical study in Japan White Rabbits was conducted with the objective of determining the toxicity and local site reactogenicity of three doses of the liquid ROTAVIN formulation. Liquid formulation of ROTAVIN vaccine lot Rm-0118 and placebo were used in toxicology study. The vaccine lot is candidates for use in the phase 3 clinical trial.

Forty (40) specific pathogen free (SPF), males and females, Japan White Rabbits 3 - 4 months of age and weight of 1.5 - 2.5 kg/each were grouped. The rabbits received 3 doses of 2 ml of the vaccine or placebo at 2 weeks interval by oral route using a 22 French rubber catheter as described below:

Table 2.6: Summary of toxicology study design of liquid formulation of ROTAVIN vaccine

Group		Study product	Administration day	Blood drawn day	Necropsy day	Number of rabbit/group
1	Acute phase	Liquid formulation of ROTAVIN	1, 15, 29	-1, 3, 17, 31	31	10 (5 males, 5 females)
2	Recovery			-1, 3, 17, 31, 44	44	10 (5 males, 5 females)
3	Acute phase	Placebo	1, 15, 29	-1, 3, 17, 31	31	10 (5 males, 5 females)
4	Recovery			-1, 3, 17, 31, 44	44	10 (5 males, 5 females)
Total number of rabbits						**40**

During the quarantine and experiment phases, rabbits were kept in GLP standard rooms with closed, well-controlled environment and microclimatic conditions. Rabbits were cared for and fed under the POLYVAC procedure for laboratory rabbit. Consumption of food and drinking water are measured daily during the course of experiment.

Clinical Observations
- The animals were observed twice daily (AM/PM), animals were examined cage side for signs of acute toxicity, morbidity, and mortality.

- Pre-dose, 30 minute post-dose, and weekly, rabbits were removed from the home cage and the following (at a minimum) were be examined:
  - Skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet,
  - Any symptom of respiratory and circulatory systems (cough, dyspnea, cyanosis)
  - Digestive system (anorexia, abdominal distention, vomiting, diarrhea)
  - Autonomic effects such as salivation
  - Nervous system effects including tremors, convulsions, reactivity to handling, and bizarre behavior
  - The other abnormalities.
  - Fecal output and physical description (e.g., loose, soft, firm bolus) were also noted.
- **Monitoring food and water consumption:** Food and water consumptions were monitored daily during 5 days before administration and during the experimental period.
- **Monitoring body weight:** Rabbits were weighed on the receipt day (5 day before administration), pre-administration, 24 and 48 hours post-administration and then weekly during the experimental period.
- **Monitoring body temperature:** Rectal body temperature was measured in a pyrogenic testing room. The temperature was taken immediately before administration (T0-0 hour), and at T6-6 hours (±10 minutes), T24-24 hours (±20 minutes), and T48-48 hours (±30 minutes) after administration. If at any point the temperature was > 40ºC, additional measurements were to be taken until the values return to normal (baseline).
- **Blood draws:** Blood was collected before the study (day -1), and 2 days (48 hours) after each administration and necropsy day/at the end of the study (5 blood samples/rabbit). Serum was tested for heukocyte count (WBC), erythrocyte count (RCB), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT) and mean platelet volume (MPV). Blood biochemical testing included testing for alucose (GLU), urea nitrogen, creatinine (CREA), total protein, albumin, globulin, albumin/globulin ratio, cholesterol and total bilirubin.
- **Statistical analysis:** The collected data were analyzed by group, the mean values between groups were compared using T test. The SPSS software (version 16), one way ANOVA and T test were used to analyze data.

Study Results

The clinical observation demonstrated that all studied rabbits are alive, healthy and express normal physiology during the in-life phase, and there were no abnormal observations during the study period.

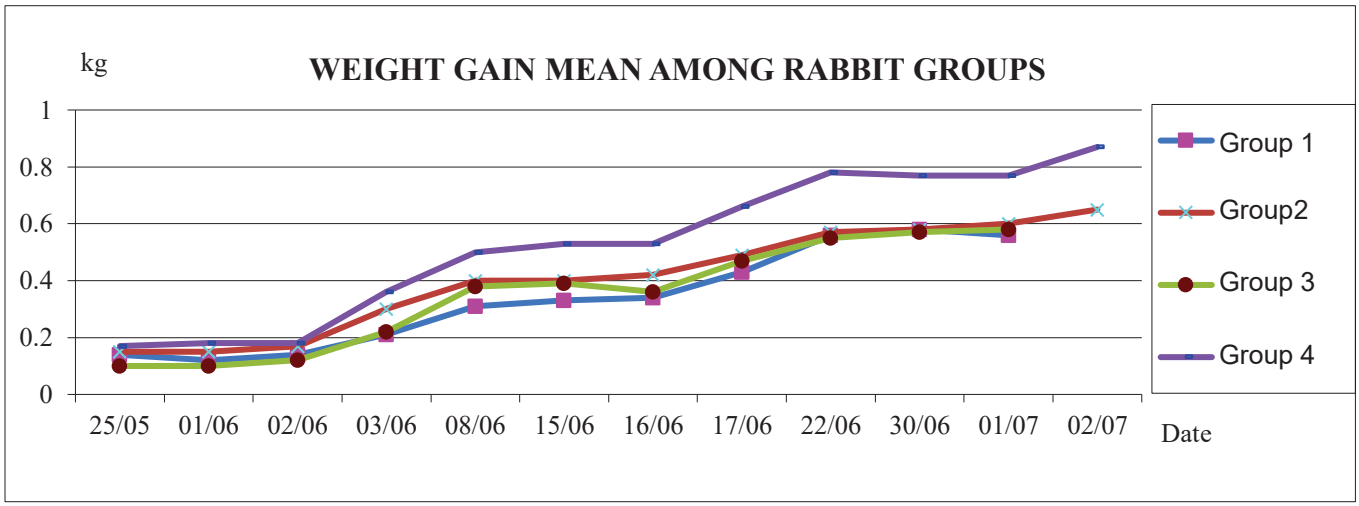
Food consumption: The data in Table 2.7 shows that the average food consumption per week per rabbit was approximately 120 g to 141 g. The food consumption did not change much over time or study product. Prior and post vaccination, there are no significant difference in food consumption level in both vaccine and placebo group ($p > 0.05$). The findings indicated that vaccine does not affect the food consumption of rabbit.

Table 2.7: Average food consumption per week per rabbit

Study group	Week	Average food consumption (g)		p value
		Vaccine	Placebo	
Acute phase	1	125	128	0.995
	2	125	120	
	3	131	126	
	4	128	130	
	5	130	130	
	6	138	135	
	7	135	128	
	8	130	136	
	9	137	131	
	10	140	135	
Recovery	1	121	120	0.999
	2	123	125	
	3	125	128	
	4	131	125	
	5	135	131	
	6	128	130	
	7	131	138	
	8	136	135	
	9	135	136	
	10	138	141	

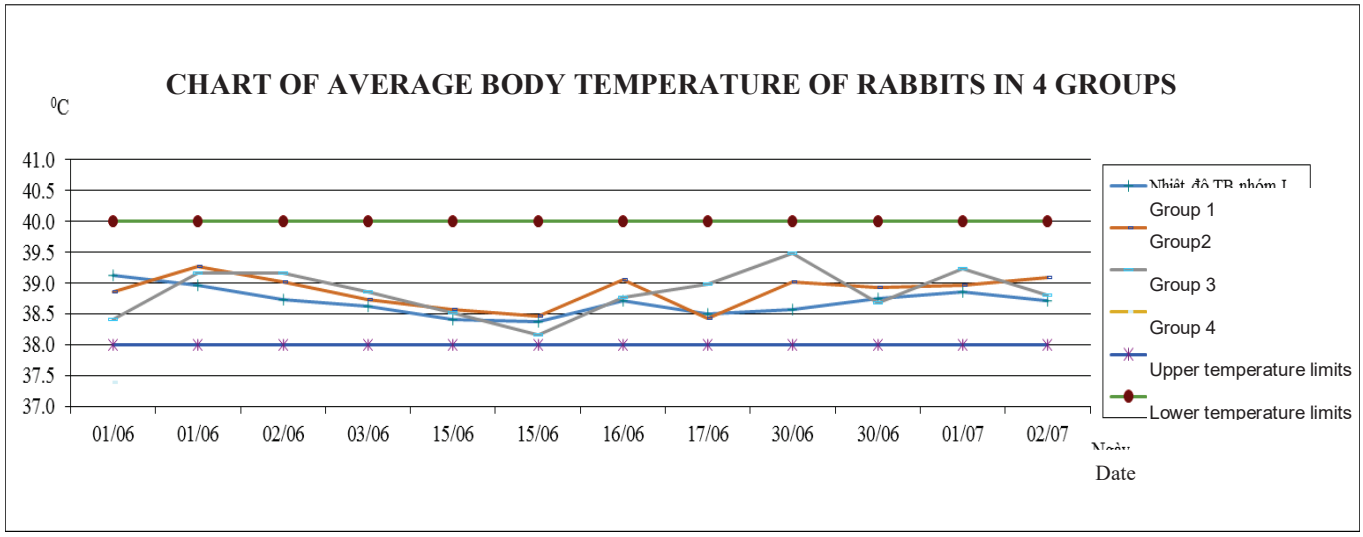
Weight gain: The findings from weekly monitoring shows that all studied rabbits gained weight compared to day 1. At the end day of study, the average weight gain was between 0.56 and 0.87 kg.

Figure 2.2: Chart of average weight gain of rabbit groups



Body temperature: The average temperature of the 4 groups was found to be relatively similar and within the limits.

Figure 2.3: Monitoring average body temperature rise of rabbit in 4 groups.



Hematology and Biochemistry findings

Blood samples were collected from all scheduled animals prior to study, 48 hours following each dose, and at each necropsy (Table 2.6).

Main study group:

The hematological results between vaccine and placebo group on the 4 collection days showed no statistically significant differences except differences in in percentage of neutrophils on Day -1 ($p = 0.04$), the percentage of lymphocytes of on Day -1 ($p = 0.04$) and Day 31 ($p = 0.01$), and MCHC value on Day -1 ($p = 0.03$). The differences are in the parameter of neutrophils, lymphocytes, and MCHC that are very variable in hematology testing and were not considered clinically significant.

Comparison of mean value for biochemical parameters between vaccine group and placebo group showed that there are no statistically difference with $p > 0.05$, exclude GLUCOSE findings on Day 17 ($p = 0.003$) and Day 31 ($p = 0.02$). However, the differences are in the reference value for rabbits that are very variable in testing and are not clinically significant.

Recovery group

Comparison of the hematological results between vaccine and placebo group on the 5 collection days showed no statistically significant differences for all of the above hematological parameter with $p > 0.05$ except for some cases as described below:

- The WBC value of Day -1 ($p = 0.01$)
- The percentage of neutrophils of Day -1 ($p = 0.005$); Test 2 ($p = 0.02$) and Test 3 ($p = 0.001$).
- The percentage of lymphocytes of Day -1 ($p = 0.004$); Day 3 ($p = 0.01$) and Day 17 ($p = 0.004$).
- The lymphocytes of Day -1 ($p = 0.0007$) and Day 17 ($p = 0.002$).

The differences are in the parameter of neutrophils, lymphocytes, and WCB that are very variable in hematology testing and are not clinically significant.

Biochemistry findings: Comparison of mean value for biochemical parameters between vaccine group and placebo group showed that there are no statistically difference with $p > 0.05$, except urea and protein findings at Day 17 ($p = 0.002$) and ($p = 0.009$), respectively. However, the differences are in the reference value for rabbits that are very variable in testing and are not clinically significant.

Histopathological findings

All surviving animals were killed for necropsy on days 31 (for main study group) and 44 (for recovery group. Selected organs/tissues from all animals were weighed and examined for histopathology. The histopathological findings showed that there were neither changes in anatomy, size and characteristics of the internal organs nor damage in tissues and cells of the examined organs. Further, there was no sedimentation, accumulation or degradation, or evidence of other disease in the examined tissues. The observed damage at lungs in some cases was not specific and distributed equally in both vaccine and placebo group. No other abnormality on histo-pathological examination was observed.

Conclusion

In comparison with the placebo group, after 3 doses of liquid formulation of ROTAVIN, it could be concluded that the vaccine was well tolerated: 100% examined rabbits were alive and healthy until the end of study, they gained weight, had normal consumption of food and no abnormal signs. The results of hematology and biochemistry shows normal data with no macroscopic and microscopic changes in examined tissues and organs.

2.5.3 Clinical Trials of ROTAVIN-M1

The clinical development of ROTAVIN-M1 vaccine started in the year 2009 with POLYVAC conducting an adaptive phase I study in adults with primary objective of safety, and a phase II study in infants with objective of assessing safety and immunogenicity of two different formulations of the vaccine, one with low titer ($10^{6.0}$ PFU/dose) and the second one with high titer

($10^{6.3}$ PFU/dose) of the virus. They were administered as either a 2-dose or 3-dose schedules to infants 6–12 weeks of age. A comparator group received lyophilized Rotarix™ vaccine, a licensed rotavirus vaccine in Vietnam. This phase II study was followed by a phase III study which also acted as a pivotal study and supported licensure of the vaccine in Vietnam. The protocol and consent form for the study were reviewed and approved by the Ethical and Scientific Committees of the National Institute of Hygiene and Epidemiology (NIHE) and of the Ministry of Health, Government of Vietnam, prior to initiating the study. The studies are described below.

Phase I study in adults

The phase 1 study was conducted in Thanh Son district, Phu Tho province of Viet Nam. For the study 29 healthy adult volunteers 18–49 years of age were enrolled after a thorough screening which included baseline blood sampling to test for hematological and biochemical parameters. All volunteers were administered 2 doses of the high titer vaccine, $10^{6.3}$ PFU/2ml at a 1-month interval. The volunteers were followed daily for 10 days for adverse events and for fecal sample collection after administration of each dose of the vaccine. Volunteers were followed up by phone for the next 20 days. Serum samples taken before and 30 days after the 1st and 2nd doses were tested for blood counts, BUN concentration and serum transaminase levels. During the 30 days post-vaccination of each dose, no diarrhea or severe adverse reaction was reported by any of the volunteers. No change in blood counts or BUN concentration was observed at any of the post vaccination sampling time points. Serum transaminase levels stayed below 40 IU/ml for >85% of volunteers or slightly elevated (42–56 IU/ml) in 10% of volunteers after 2 doses of vaccine. One volunteer had elevated levels of both SGOT and SGPT (71 and 48 IU/ml, respectively) before vaccination and the levels remained in this range after 2 doses of vaccine. Overall, two doses of ROTAVIN-M1 higher dose ($10^{6.3}$ PFU/dose) were well-tolerated in adults. No shedding of the vaccine virus occurred in these adults following vaccination. Data on the volunteers were reviewed by the Data and Safety Monitoring Board (DSMB). The DSMB judged the vaccine to be safe, permitting the phase II study to start in infants.

Phase II study in infants

The phase II study was a double-blinded, dose and schedule ranging study, conducted at 12 commune health centers in Thanh Son district, Phu Tho provinces from November 2009 through April 2010. Healthy infants 6–12 weeks of age were eligible for inclusion in the study if they were born at full term (38 weeks). Infants (n = 200) were randomly assigned to 5 groups (40 infants/group). Two groups received 2 oral doses of ROTAVIN-M1 at a titer of either $10^{6.0}$ or $10^{6.3}$ PFU at 6–12 weeks and 2 months later (groups 2L and 2H), respectively. These 2 vaccine titers were also given to infants on a 3-dose schedule, beginning at 6–12 weeks and subsequent doses one month apart (groups 3L and 3H, respectively). Rotarix™ was used as the vaccine control and was given to 40 infants at 6–12 weeks of age and 1 month later (Group Rotarix™)). The parents/guardians, the laboratory staff, and site team were blinded to the coding assignment of these groups. Other vaccines (BCG, oral polio vaccine, Diphtheria–Tetanus–Pertussis and hepatitis B) used in the country's Expanded Program of Immunization (EPI) were administered to these infants on different days (10–20 days before or after rotavirus vaccine was administered). All infants were followed for 30 days after each dose for clinical adverse events including diarrhea, vomiting, fever, abdominal pain, irritability and intussusception. Immunogenicity was assessed by IgA seroconversion and viral shedding was monitored for 7 days after administration of each dose. The AEs following vaccination were grouped into 3 time periods: immediate reactions (i.e. within 30 min), short term reactions (within 7 days post-vaccination) and longer term reactions (from 8 through 30 days post-vaccination).

No immediate reactions were observed after any dose of the vaccine. Irritability and fever were the 2 most frequently reported symptoms following administration of any dose of Rotarix™ or ROTAVIN-M1 but none of the differences were found to be significant. Fewer children reported any symptoms after dose 2 and dose 3, compared with dose 1. While analyzing the short term reactions it was noted that after the first dose, 3 children from group 3L (7.5%), 3 from group 2H (7.5%), 1 from group 3H (2.5%) and 1 from group Rotarix™ (2.5%) exhibited mild diarrhea. This difference was not found to be statistically significant and suggested that the vaccine virus had been adequately attenuated. All stool samples from cases with diarrhea were evaluated for rotavirus antigen which was isolated in fecal specimens from 1 case in each of the groups, Rotarix™, 3H and 2H during this period. From days 8–30, diarrhea episodes were reported by one case in Rotarix™ and 4 cases in ROTAVIN-M1 3H groups. Of these one case in group ROTAVIN-M1 3H was positive for rotavirus. While a few infants had mild diarrhea after administration of dose 2 or 3, only 1 case in group ROTAVIN-M1 3H (within 7 days after dose 2) and 1 case in group ROTAVIN-M1 3L (within 7 days after dose 3) were identified as rotavirus G1P [8]. Sequences of VP7 gene of these samples revealed that they were 100% homologous with the sequence of ROTAVIN-M1 or Rotarix™ (in respective groups). No elevation of levels of serum transaminase, blood urea, or blood cell counts were observed.

Vaccine rotavirus shedding was observed more in group of children who received Rotarix™ (65% after the 1st dose) vs. any group that received ROTAVIN-M1 (44–48% after the 1st dose). Furthermore, after the first dose, peak rotavirus shedding was observed 1 or 2 days earlier than shedding for ROTAVIN-M1. Little difference was observed in the shedding pattern between the 4 groups receiving ROTAVIN-M1. Viral shedding was significantly lower in all groups after dose 2 (6–20%) but slightly increased after dose 3 (30–37%) in both 3L and 3H groups.

The results of the immunogenicity analysis are presented below:

Table 2.8: Immunogenicity of ROTAVIN–M1 and Rotarix.

Group	Vaccine Titers (PFU)	No of Doses	N	Seroconversion % (95%CI)	GMC (95%CI)
2L	$10^{6.0}$	2	38[a,b]	61 (45, 76)	89 (58, 138)
2H	$10^{6.3}$	2	33[a,b]	73 (58, 88)	76 (44, 126)
3L (post dose 2)	$10^{6.0}$	3	39[a]	51 (36, 67)	94 (56, 162)
3L (post dose 3)			36[b]	56 (39, 71)	71 (35, 141)
3H (post dose 2)	$10^{6.3}$	3	36[a]	61 (45, 77)	61 (43, 87)
3H (post dose 3)			32[b]	63 (46, 79)	83 (55, 126)
Rotarix™		2	40[a,b]	58 (42, 73)	82 (52, 135)
[a]Number of participants for immunogenicity analysis (intention-to-treat). [b]Number of participant for immunogenicity analysis (per protocol).					

The highest rotavirus IgA seroconversion rate (73%, 95%CI:58–88%) was achieved in group 2H (2 doses – $10^{6.3}$ PFU/dose, 2 months apart). The 2 dose schedules performed slightly better than

the 3 dose schedules and the higher titer doses performed slightly better than the lower titer doses. These rates of seroconversion were similar to that of the Rotarix™ group (58%, 95%CI (42–73%).

Based on the data obtained from this study, another study was planned which assessed the safety, immunogenicity and efficacy of ROTAVIN-M1 (2 doses at $10^{6.3}$ PFU/dose) in a larger cohort of infants.

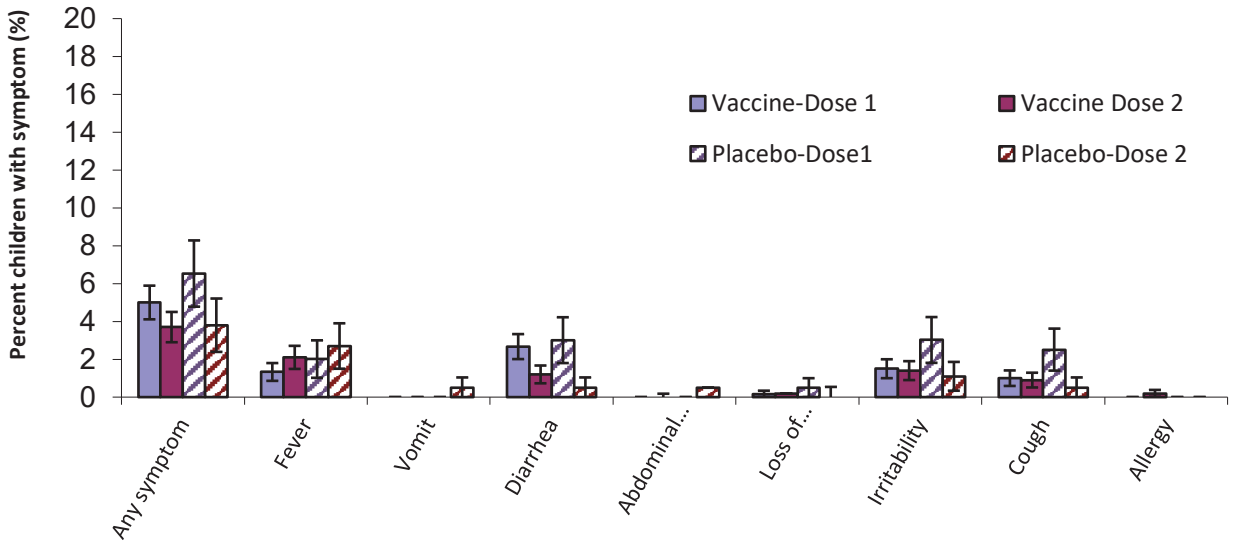
Phase III study in infants

The phase III study was a double blinded study conducted in Phu tho and Nha Trang provinces of Vietnam in the years 2010 to 2012. The main objective of the study was to evaluate safety, reactogenicity and immunogenicity of the study vaccine ROTAVIN-M1. A total of 800 infants were enrolled from the two provinces in Vietnam. After screening, all eligible infants were randomized at a ratio of 3:1 to one of the two groups receiving either two doses of ROTAVIN-M1(N=600) or two doses of Placebo (N=200). The first dose was administered at the time of enrollment and the second dose was administered 2 months after the first dose. ROTAVIN-M1 with a titer off $10^{6.3}$ PFU was used for the study. Only the staff administering the vaccine and staff holding the infants knew which product the infants received. The parents/guardians, the laboratory staff, and site team were blinded to the coding assignment of these groups. Other vaccines (BCG, oral polio vaccine, Diphtheria–Tetanus–Pertussis and hepatitis B) used in the country's Expanded Program of Immunization (EPI) were administered without any change in their schedule to these infants on different days (10–20 days before or after rotavirus vaccine was administered). All infants were followed for 30 days after each dose for clinical adverse events including diarrhea, vomiting, fever, abdominal pain, decreased appetite, cough, irritability and intussusception. Immunogenicity was assessed by estimating IgA and IgG antibodies before the first vaccination and 30 days after the last vaccination. The AEs following vaccination were grouped into 3 time periods: immediate reactions (i.e. within 30 min), short term reactions (within 7 days post-vaccination) and longer term reactions (from 8 through 30 days post-vaccination). For the study 400 children each were enrolled in Phu Tho and Nha Trang-Khanh Hoa provinces. At each site, 300 children were exposed to ROTAVIN-M1 vaccine and the other 100 children were administered the placebo vaccine.

Safety Assessment

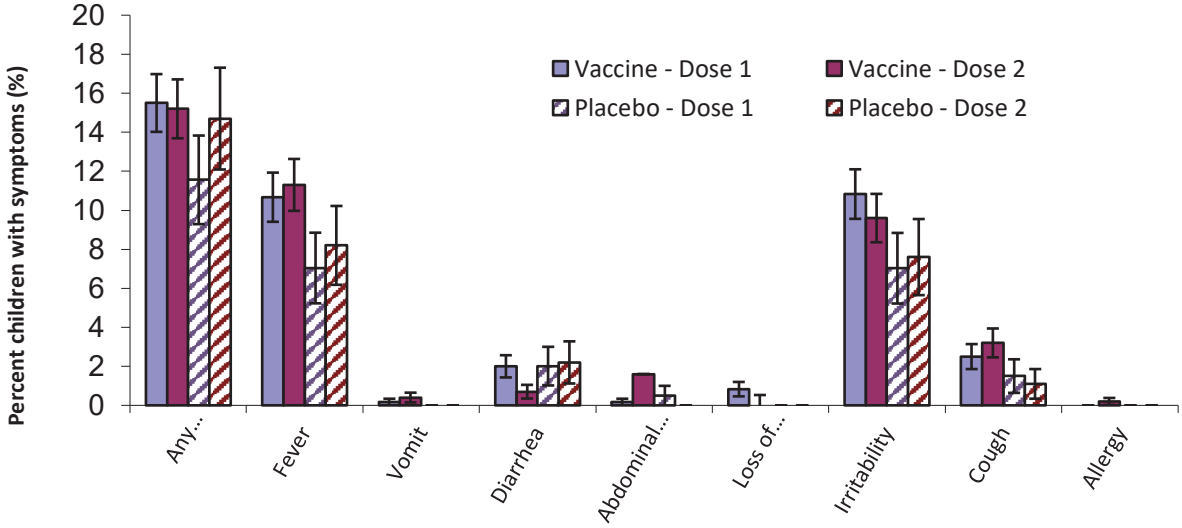
The incidence of solicited AEs within 7 days was low, with approximately 5% of participants in vaccine group and 6.5% participants in the placebo group reporting an event after the first dose and approximately 3.8% of participants in both arms reporting an event after dose 2. In the ROTAVIN-M1 group diarrhea, fever and irritability were the most commonly reported solicited AEs and were reported by less than 3% of the participants. The study did not report any difference in the solicited AEs between the vaccine and placebo groups within 7 days of vaccination.

Figure 2.4: Solicited adverse events within 7 days after each vaccine dose.



Adverse events were also evaluated between Days 8 and 30 post vaccination. During this period fever and irritability was the most commonly reported AE in the vaccine group, both after 1st and 2nd dose of the vaccine. Routine childhood vaccine including DTP were administered to participants during this period, and may be the reason for higher incidence of these events reported during this period.

Figure 2.5: Adverse **events between Day 8-30 after each dose.**



Three SAEs were reported in the study due to hospitalization of children with the diagnosis of encephalitis, pneumonia and bronchiolitis. All the cases occurred after 40 days of vaccination, and all infants recovered and were discharged from the hospital after recovery.

Immunogenicity assessment

A blood sample was collected prior to vaccination (pre-vaccination) and 1 month after second dose (post-vaccination) to analyse immune responses at these timepoints. Immune responses were

measured in terms of serum IgA and IgG antibodies at pre-vaccination and 1 month post vaccination time point.

Anti-rotavirus IgA was measured as it is considered the most reliable tool to estimate immune response to rotavirus vaccines whereas IgG antibodies are generally considered as transferred from mother through the placenta with high concentration at the time point of vaccine administration (when the children were at 6-12 weeks of age)..

IgA Response

Prior to participating in the study, RV-IgA antibody was not detected (concentration <20) in 94.3% (92.3% in Thanh Son and 96.3% in Thai Binh) of the enrolled population, suggesting that most children were not exposed to the virus at the time of vaccination (Table 2.9). For children with detectable antibody concentrations the concentration was mostly at the cut-off level (concentration = 20). There was no difference in RV-IgA antibody between the vaccine and placebo groups. However, significant difference was observed on comparing the pre-vaccination concentrations between the two study centres Phu Tho and Thai Binh ($p < 0.05$).

Table 2.9: Proportion of children with RV-IgA antibody prior to administration of vaccine/placebo

Site		Vaccine		Placebo		Total
	N	n	% Positive (95% CI)	n	% Positive (95% CI)	% Positive (95%CI)
Thai Binh	400	11	3.67 (1.84 – 6.47)	2	2.0 (0.24 – 7.04)	3.3 (1.7-5.5
Phu Tho	399	23	7.67 (4.92 – 11.28)	3	3.03 (0.63 – 8.6)	6.8 (4.5-9.7)*
Total	799	34	5.67 (3.96 – 7.83)	5	2.51 (0.82 – 5.77)	5% (3.6-6.8)

* $p<0.05$, chi-square =4.7

Seroconversion was defined as a post-vaccination serum anti-rotavirus IgA antibody concentration of at least 20 if a baseline concentration is < 20 or a post-vaccination serum anti-rotavirus IgA antibody concentration of ≥ 4-fold baseline level if a baseline concentration is ≥ 20. Post-vaccination IgA seroconversion rates of the vaccine and placebo groups for the entire cohort was was 80.7% for vaccine and 13.3% for placebo. The seroconversion rate for the vaccine and placebo groups in Thai Binh were 84.3% and 15.2%, respectively and for Phu Tho they were 77.3% and 11.1%, respectively. The difference between 2 groups of vaccine and placebo was statistically significant with $p < 0.00001$ (Table 2.10). The rate of IgA antibody conversion in the placebo group was anticipated to be because of natural exposure to rotavirus in some children. There was no difference in the probability of natural infection with the virus between Thai Binh and Phu Tho.

Table 2.10: Conversion rate of IgA antibody at 1 month after the second oral dose

Site	Vaccine	Placebo	P
Thai Binh	84.3% (79.1-88.6)	15.2% (8.6-24.2)	p<0.00001*
Phu Tho	77.3% (71.6-82.3)	11.1% (5.2-20.1)	p<0.00001*
Total	80.7% (77-84.1)	13.3% (8.6-19.3)	p<0.00001*

* T-test

Post-vaccination antibody concentrations showed a remarkable increase with the vaccine group which achieved geometric mean concentrations of 85.9 (Phu Tho- 68.2 and Thai Binh - 109.1). This translate to a 20-fold increase in Thai Binh and 11.4 times in Phu Tho (Table 2.11).

Table 2.11: IgA Geometric Mean Concentration (GMC) before and after the administration of vaccine/placebo at study sites

Site		Vaccine		Placebo	
		Pre-vaccination	Post-vaccination	Pre-vaccination	Post-vaccination
Phu Tho	n	300	255	99	81
	GMC (95% CI)	6.0 (5.6 - 6.4)	68.2 (55.8 - 83.2)	5.9 (5.2 – 6.7)	7.7 (5.9 - 10.1)
Thai Binh	n	300	248	100	92
	GMC (95% CI)	5.5 (5.2 - 5.7)	109.1 (89.3 - 133.3)	5.2 (5.0 – 5.5)	8.2 (6.5 – 10.2)
Total	n	600	503	199	173
	GMC(95% CI)	5.72 (5.5-5.9)	85.9 (74.5-99.1)	5.6 (5.2-6.0)	8.0 (6.7-9.4)

Another approach to evaluate the immunogenicity of vaccine was to study the distribution of antibody concentrations. The vaccine induced IgA antibody concentration at 80 or higher in 65.8% of children in Thai Binh and 54.5% in Thanh Son, Phu Tho. Meanwhile, there were still 6.5% of children in Thai Binh and 10.6% in Phu Tho with low antibody levels [20-40], and 14.1% of children in Thai Binh and 20% in Phu Tho did not induce IgA antibody response (<20). A small number of children in the placebo group also induced the concentration levels of 20-640, presumably due to a natural infection.

IgG response

Before participating in the study, the RV-IgG was detected in children enrolled in the study (100% in Thai Binh and Thanh Son). The persistence level of maternal antibody was quite high, approximately 320-5120 (accounting for 91.3-92.6% of samples). There were some samples with the antibody concentration of 10240 or higher (0.36-2.4%) (Table 2.12). Also, the geometric mean IgG antibody concentration before participating in the study of children in Thanh Son was higher than that of children in Thai Binh (p <0.01).

Table 2.12: Comparison of IgG antibody concentration levels of children before administration of vaccine/placebo in two study sites

Indicator	Thai Binh	Phu Tho
n	285	205
GMC	1175*	1521
IgG concentration level		
<20	0.0%	0.5%
[20-320)	5.6%	3.9%
[320-10240)	92.6%	91.3%
>=10240	1.8%	4.3%

* Significant difference ($p<0.01$)

In this study, the IgG-GMC of the placebo group decreased 7.6 times in Thai Binh and 8.7 times in Phu Tho. This was consistent with the nature of IgG half-life of 3-4 weeks. Meanwhile, the IgG-GMC of the vaccine group increased 2.1 times in Thai Binh, or decreased (but not significantly in Thanh Son-Phu Tho (Table 2.13), showing that the vaccine enabled to induce the IgG immune responses even when the pre-administration antibody level was high.

Table 2.13: IgG Geometric Mean Concentration (GMC) at examination times

Site	IgG	Placebo		Vaccine	
		Before administration (MO)	After administration (M2)	Before administration (MO)	After administration (M2)
Phu Tho	n	50	40	155	135
	GMC (95% CI)	1450 (1129- 1863)	167 (124 – 229)	1562 (1313 – 1857)	1320 (1098 – 1587)
Thai Binh	n	69	65	215	180
	GMC (95% CI)	1274 (997 – 1627)	170 (117- 246)	1181 (1027- 1358)	2454 (2094 – 2876)
Total	n	119	105	370	315
	GMC (95% CI)	1345 (1130-1602)	169 (131-218)	1328 (1190-1481)	1881 (1661-2130)

Since the baseline concentrations were high and there was a confounding factor of their decrease because of half life of maternally derived IgG antibodies the study used the ratio of IgG concentration with pre-vaccination concentration and a cutoff of 0.5 to define seroconversion. It could be seen that 72.8% of children in Phu Tho or 85.3% of children in Thai Binh who were administered with vaccine induced IgG antibody response (the ratio of M2/M0 ≥ 0.5). Similar to the IgA antibody conversion rate, a small number of children in Thai Binh (2.4%) receiving

placebo induced IgG response, suggesting that a natural exposure to the virus occured among children participating in the study in this site.

Persistence of antibodies at 1 year after last vaccination

The immune response to ROTAVIN-M1(2h group) and Rotarix were evaluated in participants of Phase II study, 3 month and 12 months after vaccination. For the evaluation, only the group that received two doses at higher concentration of ROTAVIN ($10^{6.3}$ PFU per dose) and Rotarix were assessed.

Table 2.14: RV-IgA antibody concentrations in ROTAVIN-M1 and Rotarix group at different time points (prior to study enrollment – M0, 3 and 12 months after the first dose (M2 and M3)

Study groups	GMC of RV-IgA antibody		
	Before administration of Vaccine-Mo (95% CI)	3 months after the 1st dose-M2 (95% CI)	12 months after the 1st dose-M3 (95% CI)
Rotarix Group (n=31)	5.64 (4.42 – 7.21)	27.80 (16.87 – 45.81)	31.28 (16.57-59.02)
ROTAVIN-M1Group (n=30)	5.18 (4.93 – 5.44)	36.78 (21.85 – 61.89)	34.82 (19.07-63.57)

After one year of vaccination, the RV-IgA GMC for the Rotarix group and ROTAVIN group did not change significantly compared to that of one month after the two doses of the vaccine There was no differences in the mean concentration between the two groups receiving these two vaccines.

Table 2.15: Rate of participants with Rota IgA antibody positive one year after the first dose

Vaccine group	% (95% CI)			
	N	%	95% CI	P
Rotarix	17	54.84	36.03 – 72.68	>0.05
ROTAVIN -M1 Group	20	66.67	47.19 – 82.71	

Table 2.15 shows that the antibody conversion of the ROTAVIN-M1 vaccine group and Rotarix vaccine group one year after vaccination was 66.67% and 54.84%, respectively, about 5% decrease in each group compared to the seroconversion at 1 month after the last oral dose (72.7% and 59%, respectively). This difference between two groups was not statistically significant.

Thus, the results of the clinical studies of the ROTAVIN-M1 vaccine indicate that the vaccine is safe and immunogenic when administered 2 months apart when administerd as two vaccine doses containing $10^{6.3}$ PFU/dose of virus titres. Based on these results the vaccine was licensed in Vietnam in 2012.

2.6 Dose and Study Rationale

ROTAVIN-M1 is the frozen formulation of the vaccine which needs to be stored and transported at -20°C. ROTAVIN-M1 was approved by NICVB for production in 2007 and it was licensed in May 2012. POLYVAC the manufacturer of ROTAVIN-M1 has also been working on a second generation formulation which may be more stable and can be stored at 2-8°C (liquid formulation of ROTAVIN) through the entire shelf life which will widen its usability and make it more suitable for programmatic application.

This study has been designed to compare the liquid formulation of ROTAVIN, manufactured under full compliance with the Vietnamese MOH regulations with the frozen formulation of the vaccine (ROTAVIN-M1) of the vaccine that had undergone several clinical trials which have shown it to be safe and immunogenic. The clinical trials of ROTAVIN-M1 included adults and infants of approximately 2 months of age and supported licensure of the vaccine in Vietnam. Since, the new liquid ROTAVIN vaccine has the same seed virus in the same quantity i.e. $\geq 2x\ 10^6$ plaque focus units (PFU) / dose as in Rotavin-M1 and enhanced stability is being targeted only by modifying existing excipients or adding new excipients and stabilizers. Preclinical studies have been conducted to identify any toxicity because of this new formulation and a the phase III bridging study has been planned in target age group. Similar design using immunogenicity bridging directly in the targeted population (without age de-escalation) with licensed first generation vaccine has been accepted by many regulatory authorities worldwide for the licensure of second generation liquid vaccine of Rotarix. WHO recommends that the first dose of rotavirus vaccine be administered as soon as possible after 6 weeks of age, along with DTP vaccination. Infants will receive under blind two doses of either vaccine 8 (+2) weeks apart, starting at 60-91 days of age. EPI vaccines will not be allowed within 7 days before or after study vaccination to prevent overlap of post vaccination AEs of these vaccines. . The levels of anti-rotavirus IgA antibodies induced by the two vaccines will be compared four weeks post the second vaccination in order to demonstrate non-inferiority of the new product with the current product.

2.7 Potential Risks and Benefits of Rotavirus Vaccine

All potential risks and known benefits are detailed below and will be provided and explained to study participant's parent/LAR. Before participating in the study, parent/LAR will be given the information sheet to read and will be invited to attend a meeting to learn more about the trial. During the study, if there are any changes related to the research plan or related to the product that may affect the parent/LAR's decision to continue their child's participation in the study, the parent/LAR will be notified in writing of such information.

2.7.1 Potential Risks to Participant Well-Being

Risk of vaccination with live attenuated rotaviruses include development of fever, diarrhea or vomiting as a consequence of the growth of the virus in the child's intestine, however, all the animal and animal-human rotavirus reassortants tested to date at similar or higher titers to those used with ROTAVIN formulations have seen adverse events like fever, diarrhea, cough, vomiting, irritability, otitis media, nasopharyngitis, bronchospasm, crying and rash being reported as common adverse events. In babies, natural rotavirus infection can cause diarrhea, vomiting and fever. It is possible that enrolled participants may have a few looser than usual stools, cry or have a slightly higher than usual temperature for a day or two after vaccination with the attenuated

rotavirus vaccine. These events are similar to those reported in other rotavirus vaccine clinical trials. These will be explained to parents in the consent form.

Serious or allergic reactions also may be possible. This risk is addressed by trying to screen out any children with known allergic reactions to vaccines in the past and who may have an allergy to one of the components of the vaccine. Should an allergic reaction occur with vaccination, the study clinic will follow the guidance/regimen issued by MOH of Vietnam for handling medical emergencies and have supportive medicines in place, in addition to the trained staff.

Initial large phase III studies conducted for RotaTeq and Rotarix suggested no association of rotavirus vaccines with intussusception but recent post-marketing studies suggest that intussusception may be associated with the licensed rotavirus vaccines but at much lower rates than earlier anticipated. A recent review of rotavirus vaccine studies concluded that the attributable risk for intussusception of the rotavirus vaccine could be between 1-7 per 100,000 vaccinated infants which gives it a favourable risk benefit ratio as the benefits of vaccination outweigh the small potential risk of intussusception.[30]

Collection of blood specimens may cause some discomfort to participants. Venipuncture is sometimes associated with discomfort, pain, bleeding, bruising, redness, swelling, local hardness, and/or infection at the puncture site. This risk will be mitigated by ensuring that only study staff members adequately trained in safe drawing of blood, conduct this procedure.

To minimize risk to the participants and early identification and treatment of side effects the following assessments are planned in the protocol.

- Conduct of study procedures like administration of vaccines, safety assessment and blood draws by staff trained in these procedures and in this age group.
- Monitoring of participants closely for 30 minutes after vaccination and providing emergency care for any immediate reactions. If medical issues arise that cannot be managed by the clinic performing the study, the study doctor will refer the participant to district or provincial hospital which would have specialists and more advanced treatment modalities.
- Telephonic call and visit of heath care worker to infant's home twice within 7 days of each vaccine dose.
- Regular follow up of all infants with severe and serious adverse events.

If any study product related adverse event occurs during the study period, POLYVAC will ensure coverage for the full cost of treatment according to the laws of Vietnam for research participants. The study site will inform the nearby medical centers of the study who will provide treatment at no cost (according to the insurance policy for children under 6 years of age) to the participant for reactions that may not be life-threatening, but nonetheless warrant medical observation or care.

Potential Risks to Participant Privacy: Personal identifiers, including name, birth date, sex, and location/address of residence will be collected and recorded on some study data collection forms. As a result, a potential risk of "loss of confidentiality" exists. To avoid this risk, study participant will be assigned a unique study participant number that will be used to identify the participant and link, using a master linking document, an individual to his/her study data and/or biological specimens. Whenever feasible, use of identifiers will be avoided and the unique study participant numbers will be used instead. Case report forms (CRF) to be sent to the CRO selected for data management and will contain only unique study participant numbers to identify the participant. Paper-based records will be kept in a secure location and only be accessible to authorized personnel involved in the study. Computer-based files will only be made available to personnel involved in

the study through the use of access privileges and passwords. Individual participants will not be identified in any study related reports. Study staff will treat all study data, including participant identifiers and laboratory testing results, as confidential and not to be shared with anyone unauthorized to view such data.

Biological specimens will be identified by study participant number and no personal identifiers will be utilized on any biological specimen. Biological specimens will be stored in the laboratories of Centre for Disease Control (CDC). The specimens will continue to have no personal identifiers even if they are stored at another laboratory. Likewise, laboratory reports will utilize only study participant numbers.

2.7.2 Known Potential Benefits

Potential participants will have aspects of their health status screened by qualified clinicians. This screening will be free of cost to the participant's family and may provide important health information to the screening candidates.

The study will provide the opportunity for some of the participant to be tested for anti-rotavirus antibodies, a test which is not commonly available. Results will be reviewed with them and shared with their personal physician, if so desired by the participant's parent/LAR.

The information sheet and the physician will educate the family about rotavirus diarrhea and its prevention and treatment.

Protection from rotavirus diarrhea is a possible benefit, although because the vaccine is experimental it cannot be guaranteed. Participants may not benefit personally through study participation. By participating in this study, participants will contribute information on the safety and immunogenicity of this new vaccine against rotavirus in Vietnam. Development of new rotavirus vaccine, such as this new formulation of ROTAVIN-M1, that is locally produced, more stable and potentially less expensive, would be a significant contribution to public health in Vietnam and regionally.

There are no other known potential benefits to the participant for participation in this trial. Given the history of good safety profiles of rotavirus vaccines, the risk-benefit ratio is considered favorable, especially considering that all participants will be under the observation of qualified medical personnel.

3 STUDY OBJECTIVES AND OUTCOME MEASURES

3.1 Study Objectives

3.1.1 Primary Objectives

Immunogenicity

To demonstrate non-inferiority in the immunogenicity of the liquid formulation of ROTAVIN in comparison to currently licensed frozen formulation of the Vaccine (ROTAVIN-M1), 28 days after the last dose of the vaccine when administered to infants in a two-dose schedule starting at 60-91 days of age.

Safety

To evaluate the safety of liquid formulation of ROTAVIN in comparison with currently licensed frozen formulation (ROTAVIN-M1), in terms of solicited reactions after each dose of vaccine.

3.1.2 Secondary Objective

Immunogenicity

To further characterize the immunogenicity of the liquid formulation of ROTAVIN in comparison with currently licensed frozen formulation of the Vaccine (ROTAVIN-M1).

Safety

To evaluate the safety of the liquid formulation of ROTAVIN in terms of immediate post-vaccination events, unsolicited adverse events and serious adverse events, including monitoring for intussusception.

3.2 Outcome Measures

3.2.1 Primary Endpoint

Immunogenicity

Rotavirus vaccine immunogenicity will be tested on serum samples obtained in a subset of 450 participants before the first vaccination and four weeks after the second vaccination using a validated ELISA assay. The primary immunogenicity endpoint is as follows:

- Geometric mean concentration (GMC) of serum anti-rotavirus IgA antibody concentrations 28 days after the second vaccination.

Safety

Solicited post-vaccination reactogenicity (fever, diarrhea, vomiting, decreased appetite, irritability, and decreased activity level) during the 7 day period (Day 1-7) after each vaccination

3.2.2 Secondary Endpoint

Immunogenicity

- Percentage of participants with seroconversion in serum anti-rotavirus IgA antibody concentrations 28 days after the second vaccination.
  - For participants with a negative pre-vaccination IgA concentration (< 20 IU/ml), a positive post-vaccination IgA concentration (≥ 20 IU/ml);
  - For participants with a positive pre-vaccination IgA concentration, a post-vaccination IgA concentration at least 4 times as high as the pre-vaccination IgA concentration.
- Percentage of participants with seropositivity at baseline and 28 days after the second vaccination. Seropositivity is defined as serum IgA antibody concentration ≥ 20 IU/ml.

Safety

- Immediate adverse events, within 30 minutes after each vaccination.
- Unsolicited AEs from vaccination through four weeks after each dose.
- Serious adverse events (SAEs) and Intussusception from first vaccination through four weeks after the last vaccination.

4 STUDY DESIGN

The study is designed as a phase III, multicenter, randomized, partially double-blinded, active controlled study enrolling 825 infants 60-91 days of age. The objective of the study is to compare

the safety and immunogenicity of the liquid formulation of ROTAVIN with already licensed vaccine ROTAVIN-M1.

The study will be conducted at commune health centres in Nam Dinh and Quang Ninh provinces in Vietnam. The study visits will be planned in advance such that the EPI vaccination days fall either 7 days before or after the study vaccination day. This will allow the participant to receive EPI vaccines in the window allowed by the protocol. Prospective participants, whose parent or legal guardian sign an informed consent form will be assessed for eligibility to participate in the study. Screening for eligibility will include solicitation of medical history, assessment of vital signs and physical examination. If the participant is found to be eligible, the infants will be allocated to one of the two groups at a ratio of 2:1 to receive the study vaccine with 550 participants receiving the liquid formulation of ROTAVIN and 275 participants receiving the frozen formulation (ROTAVIN-M1). The study will be conducted according to a partially blinded study design in which the vaccine administerer will be considered unblinded whereas the parents, and rest of the study team including endpoint accessor will be blinded to the treatment received by the child. Two doses of vaccine will be administered 8 (+2) weeks apart (minimum interval of 8 weeks and maximum of 10 weeks), with the first administration given at 60-91 days of age (both days inclusive). Childhood vaccines as per the Expanded Program for Immunization of the Government of Vietnam will not be allowed within 7 days before or after study vaccine administration.

To evaluate the Rotavirus vaccine immunogenicity, blood samples will be obtained from all the participating infants from Quang Ninh site before the first and four (+2) weeks after the second vaccination. Immunogenicity will be assessed in all infants who provided blood samples at the selected site. Serum Anti-rotavirus IgA antibodies will be analyzed at Cincinnati Children's Hospital Medical Center (CCHMC), Cincinnati, Ohio, USA using a validated ELISA which uses strain 89-12 as a substrate.

Vaccinated participants will be observed for immediate adverse events for 30 minutes after each vaccination. Active surveillance for vaccine reactogenicity (solicited reactions) over the 7-day period after each vaccination, unsolicited AEs for 4 weeks after each vaccination and SAEs including intussusception over the period between first vaccination and four weeks after the last vaccination will be conducted for all infants.

This protocol will have extensive safety monitoring in place. At the performing site level, the study site investigators and their clinical staff will be responsible for continuous close safety monitoring of all study participants and for alerting the protocol team if unexpected concerns arise. An team of vaccine experts, Safety Review Committee (SRC) composed of a group of physicians which include but not limit to the designated investigator from study team and the Medical Officer(s) from POLYVAC and PATH, a Pharmacovigilance/Medical officer from the CRO and an independent vaccine expert will be closely following the study for safety oversight. The SRC may seek independent expert medical opinion as dictated by the occurrence of certain events.

The SRC will have provision of expedited meeting that could be triggered by reporting of predefined AEs. The SRC may also seek independent expert medical opinion as dictated by the occurrence of certain events.

Study Sites

The study will be conducted at one district each of Nam Dinh and Quang Ninh provinces in Vietnam.

1. Nam Dinh Province:

- The study activities will be conducted at approximately 12 Commune health centers in Vu Ban district.
- Data entry/CRF storage at the Nam Dinh Provincial Preventive Medicine Center (PPMC).

2. Quang Ninh Province:
   - The study activities will be conducted at approximately 12 Commune health centers in Quang Yen district.
   - A blood sample will be collected from all infants participating in this study at this site.
   - Data entry/CRF storage at the Quang Ninh CDC.

National Institute of Hygiene and Epidemiology (NIHE) will collaborate with staff of the Commune Health Centers, District Health Center, as well as the staff of the Provincial level for the study. These locations are satellite sites for several vaccine clinical trials conducted in collaboration with NIHE. The staff of the district health center, as well as the staff of the provincial centers in the Nam Dinh province has experience in implementing vaccine trials and observational studies on various infectious diseases and in collaboration with contract research organizations (CROs), and with local and international sponsors. The staff at Quang Ninh and Nam Dinh will be appropriately trained on clinical study procedures before study start.

According to the health system in Vietnam, the district hospitals and their corresponding district health centers; provincial general hospital and CDC/Provincial Preventive Medicine Center are under the same management of Provincial Health Services; so it is easy for coordination of care between them should a serious adverse event occur. The commune health centers conduct routine vaccinations in the community and have the capacity to handle vaccine reactions, in the rare event that these would occur. They also have adequate infrastructure and trained personnel to take care of low grade events. The district hospitals also have a good capacity in terms of both personnel and equipment that meet requirements for medical care, as well as emergency care. These district health centers and their adjacent hospitals are only about 30-40 kilometers (one hour drive) from provincial general hospitals which would be engaged for higher grade adverse events requiring hospitalization or referred events requiring consultation with a specialist. This will assure rapid patient transport and referral to tertiary care hospitals at provincial levels in case such referral is necessary. The study documents will be stored at the provincial level where data entry will occur, and will be moved to the particular commune health center when a study visit is occurring there. The study samples will also be stored at provincial level.

The total time for conduct of the research is approximately 18 months. Recruitment and screening is expected to take approximately 7 months. Once enrolled, each participant will be in the study for approximately 3-4 months [4 (+2) weeks for vaccination and 4 (+2) weeks for follow up).

Schema for randomization

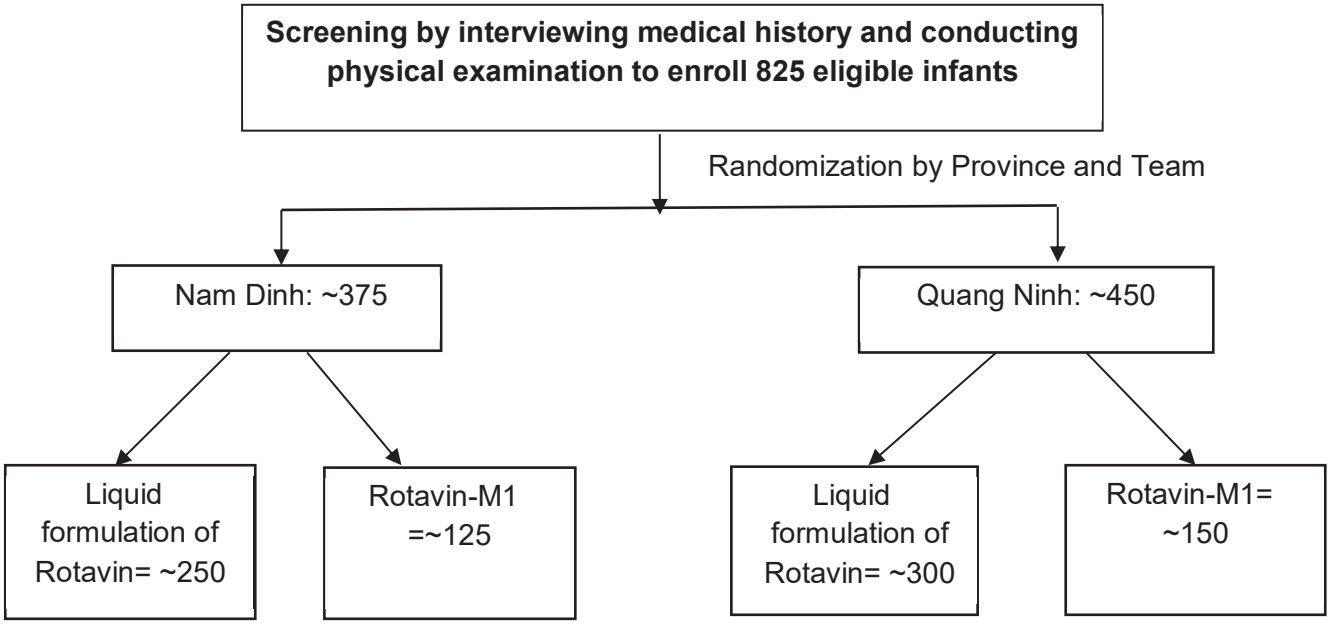
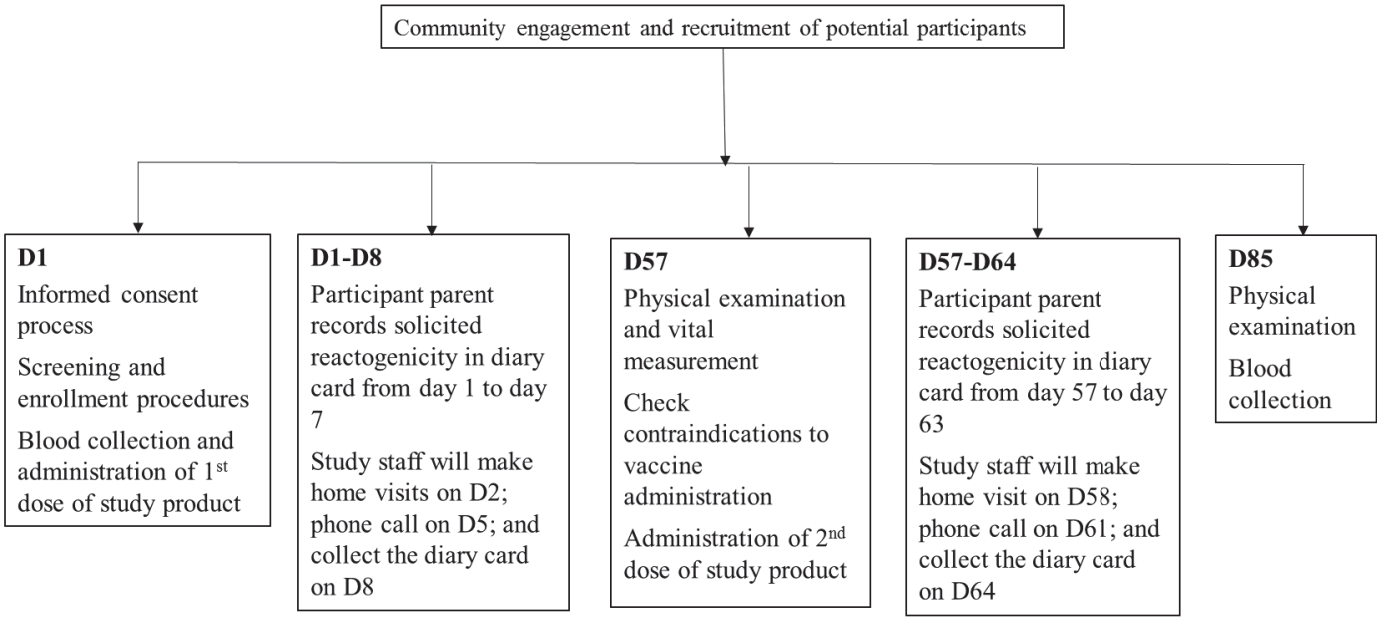


Diagram of trial activities schedule



5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Description of Participants, Source of Participants

Approximately 825 healthy male and female infants, 60-91 days of age will be enrolled in the study from the two provinces in Vietnam.

Dissemination meetings and discussions are ongoing to inform the community leaders on clinical trial background and principles, Rotavirus disease burden/prevention, common Adverse event following Immunizations (AEFIs), overview of vaccine development and any misunderstanding about the vaccines and clinical trials. Post approval of the protocol, commune staff together with population collaborators/village health workers will go household to household and use information from the information sheet of ICF to highlight the broad concepts of the study and eligibility criteria. They will assess interest of potentially eligible families and create a list from their household visits. These people will be invited to an information meeting to learn more about the study and then investigator/designee will go through the consent sheet again with every single participant's parent/LAR and answer the question if any in the private room. Participant's parent/LAR will sign the consent form, if they are interested.

5.1.1 Participant Inclusion Criteria

The following criteria must be met before a participant may be enrolled for participation:

1. Healthy infants as established by medical history and clinical examination before entering the study.
2. Age: 60-91 days (both days inclusive) at the time of enrollment.
3. Parental/LAR ability and willingness to provide written informed consent.
4. Parent/LAR who intends to remain in the area with the child during the study period.

5.1.2 Participant Exclusion Criteria

Participants meeting any of the following criteria will be excluded from participation:

1. Presence of diarrhea or vomiting in the previous 72 hours or on the day of enrollment (temporary exclusion).
2. Presence of fever on the day of enrollment (temporary exclusion).
3. Acute disease at the time of enrollment (temporary exclusion).
4. Concurrent participation in another clinical trial at any point throughout the entire timeframe for this study.
5. Presence of significant malnutrition (weight-for-height z-score < -3SD median)
6. Presence of any systemic disorder (cardiovascular, pulmonary, hepatic, renal, gastrointestinal, hematological, endocrine, immunological, dermatological, neurological, cancer, or autoimmune disease) as determined by medical history and / or physical examination which would compromise the participant's health or is likely to result in nonconformance to the protocol.
7. History of congenital abdominal disorders, intussusception, or abdominal surgery.
8. Known or suspected impairment of immunological function based on medical history and physical examination.
9. Household contact with an immunosuppressed individual or pregnant woman.

10. Prior receipt of rotavirus or an intent to receive this vaccine from outside of the study center during study participation.
11. Prior receipt of EPI vaccination during past 7 days or plan to receive them within next 7 days.
12. A known sensitivity or allergy to any components of the study vaccine.
13. History of allergy to antibiotic kanamycin.
14. Clinically detectable significant congenital or genetic defect.
15. History of persistent diarrhea (defined as diarrhea that lasts 14 days or longer).
16. Receipt of immunoglobulin therapy and / or blood products since birth or planned administration during the study period.
17. History of chronic administration (defined as more than 14 days) of immunosuppressants including corticosteroids. Infants on inhaled or topical steroids may be permitted to participate in the study.
18. Any medical condition in the parent/LAR or infant which, in the judgment of the Investigator, would interfere with or serves as a contraindication to protocol adherence.

5.1.3 Continued Eligibility Confirmation for subsequent vaccination

The following events constitute absolute contraindications to rotavirus vaccination and all participants should be evaluated for these before administration of second dose of the study vaccine. If any of these events occur during the study, the participant must not receive additional doses of the vaccine but should be appropriately followed up for safety by the Investigator.

- Hypersensitivity reaction following the administration of the study vaccine.
- Any uncorrected congenital malformation of the gastrointestinal tract (such as Meckel's diverticulum) which is diagnosed after the first vaccination and that would predispose for intussusception.
- Infants with any history of intussusception.
- Severe combined immunodeficiency (SCID).
- Detection of one or more of the exclusion criteria during dosing period.

The following events constitute contraindications to administration of the study vaccine at that point in time; if any of these events occur at the time scheduled for vaccination, the participant may be vaccinated at a later date or withdrawn at the discretion of the Investigator.

- Acute disease and / or fever at the time of vaccination.
    - Acute disease is defined as the presence of a moderate or severe illness with or without fever. All vaccines can be administered to persons with a minor illness such as mild upper respiratory infection without fever.
    - Fever is defined as temperature ≥37.5°C (99.5°F) on axillary setting.
- Gastroenteritis within 72 hours preceding the study vaccine administration.

5.2 Treatment Assignment Procedures

5.2.1 Randomized Procedure

This is a partially double-blind, randomized controlled trial with an enrollment target of 825 infants who will be allocated to one of the two groups at a ratio of 2:1 to receive the liquid formulation of

ROTAVIN or the frozen formulation (ROTAVIN-M1). Permuted block randomization will be used to generate the randomization schedules for the study. The randomization will be stratified by study site and study team and the block size for each part will be chosen based on the number of treatment groups, randomization ratio and anticipated enrollment size at each study site. Infants will be randomized sequentially in the order that they are enrolled at the site by the team.

The randomization list that contains a participant identification number and the corresponding randomization assignment will be generated using computer software by an independent statistician who is not involved in conduct or analysis of the study. Prior to study initiation it will be provided to the data management team to setup the randomization system

Each participant will be assigned a unique screening number by the investigator after signing the informed consent. He/she will be assigned a participant identification number once he/she is enrolled. The Investigator will maintain the screening/enrollment log. The log will contain essential information including screening number, date of screening, gender, date of birth, whether or not the potential participant meets eligibility criteria, whether participant is enrolled, participant identification number (if he/she in enrolled) and if not, reason for not enrolling.

Once a participant identification number has been assigned to a participant, it will not be used again. Additional participants may be randomized into the study at the discretion of the sponsor in the case of any participant who is randomized but does not receive any study vaccine.

5.2.2 Blinding and Unblinding Procedures

The study will be conducted according to a partially blinded study design since the colour of the two vaccines is different. With the exception of the designated unblinded site personnel described below, all study site personnel, the PI, PATH, POLYVAC and the CROs, will remain blinded to subjects' treatment assignments until first database closure and study unblinding. The randomization list for participants will be developed by an independent statistician and will be used by an unblinded team at POLYVAC to label study vaccine vials and then it will be immediately sealed. During conduct of the study, a limited number of unblinded site personnel will be responsible for preparing and administering study vaccines, performing vaccine accountability, and maintaining the security of the treatment assignments. The unblinded site personnel will not be involved in the safety assessment of the subjects or in any other aspect of the study. All other site personnel, including those who perform the clinical evaluations (such as but not limited to assessment of medical history, vital signs assessment, and PE), will be blinded with respect to the identity of the vaccine administered to the subjects.. The parents will also remain blinded to the treatment received by the child, throughout the study duration. To maximize blinding the two formulations of ROTAVIN vaccine vials will be packaged and labeled in such a way that they have similar appearance. Also, in addition to the original vial label of the vaccines designed by POLYVAC in compliance with the drug labeling regulations of the Ministry of Health, an identical study label designed for study vaccines containing only the study product code will be pasted over the original vial label to ensure implementation of blinding. The labeling will be done by an unblinded team at POLYVAC before study vaccines are shipped to the study site.

Study product administered to each participant will be recorded using the exact allocation code for each product received by each participant. The allocation codes which link treatment identification with each participant via participant identification numbers will be maintained in a secure location and managed by individuals who are not directly implementing or monitoring the trial. The

allocation code listing will not be opened or linked to the clinical trial database until after the database is locked.

The CRO will assign blinded monitor(s) and unblinded monitor to visit the site (including all field sites) during the study period. The blinded monitor(s) will be responsible for all aspects of the clinical trial related to subjects, the blinded site staff, and regulatory and audit readiness. The unblinded study monitor will visit the site (including few field sites) during the study period to assess and verify activities of the unblinded site personnel, review appropriate documentation, and provide a report to the CRO and Sponsor of ongoing activities and issues requiring resolution. The unblinded study monitor will be responsible for review of treatment assignments, vaccine storage and accountability, and dosing-related matters.

Any unblinding of additional project team personnel required to resolve issues will be clearly documented in the TMF. Of note, all reports to blinded personnel by the unblinded CRO monitor will be constructed in order to maintain the blind during the trial. No report that would break the blind will be released into the TMF until after database lock.

All blinded study staff will remain blinded throughout the trial. If any participant experiences an SAE possibly related to receipt of study treatment, treatment allocation to the participant may be communicated to the investigator only if that information is deemed necessary to properly treat the participant for the SAE. Written procedures for blinding, storage, and opening of study codes, in compliance with the approved protocol, will be developed to ensure the study is not unnecessarily unblinded.

6 STUDY PRODUCTS

6.1 Study Product Descriptions

The vaccine, ROTAVIN-M1, manufactured by POLYVAC, Vietnam, was developed from a G1P [8] strain recovered in 2003 from stool sample of a child hospitalized for the treatment of acute gastroenteritis at Nha Trang city (KH0118-2003). G1P[8] strain is the most common strain circulating both in Vietnam and in the world. The master seed of this vaccine were produced under GLP conditions using qualified Vero cells and reagents at the US Centers for Disease Control and Prevention (CDC). The vaccine lot was produced by one passage in Vero cells from the working seed, which was provided by the Japanese Polio Research Institute and approved for vaccine production by WHO. Two formulations manufactured from the same final bulk of Rotavirus vaccine manufactured by Center for Research and Production of Vaccines and Biologicals (POLYVAC) will be used in the study. Both vaccines are monovalent vaccines containing suspension of G1P [8] $\geq 2x\ 10^6$ plaque focus units (PFU) / Dose. Both vaccines are ready to use (no reconstitution or dilution is required). Details of the vaccines are provided below.

6.1.1 Acqusition

POLYVAC will provide both the study vaccines.

6.1.2 Formulation, Packaging and Labeling

Study Formulation

Test Vaccine: ROTAVIN liquid formulation

This is a live attenuated human rotavirus vaccine at a dosage of ≥ 2x 10^6 plaque focus units (PFU) / Dose of 2 ml. The vaccine can be stored and transported at 2-8 °C. The vaccine is for oral use.

ROTAVIN liquid formulation has a sterile, yellow, clear liquid, free of visible particles, to be used after shaking well. The vaccine contains Kanamycin as the residual antibiotic during the culture process. The future package insert will describe the vaccine as follows:

Product name:	Rotavirus vaccine
Trademark:	A formal trademark has not been received. In reference to this study it will be called ROTAVIN.
Active substance:	Live attenuated human rotavirus strain, G1P[8]
Formulation:	≥ 2x 10^6 plaque focus units (PFU) / Dose Dulbecco's Modified Eagle Medium (DMEM), Sucrose, buffers (citric acid, zinc chloride, calcium chloride, sodium citrate, potassium phosphate dibasic anhydrate), Hydrolyzed gelatin, and, Kanamycin sulphate and phenol red.
Product form:	Live attenuated human rotavirus vaccine
Pharmaceutical form:	Vaccine for Oral Use
Administration route:	Oral
Dosage:	2 mL, 2 vaccinations, 2 months apart
Packaging:	single dose of 2 ml in glass vials
Storage:	The liquid vaccine vial will be stored at 2-8 °C. .

**Because of the need for blinding of the two formulations, packaging in this trial may be different.*

Comparator vaccine: ROTAVIN-M1

Live Attenuated human rotavirus vaccine at a dosage of ≥ 2x10^6 plaque focus units (PFU) / Dose of 2 ml. The vaccine is for oral use. ROTAVIN liquid formulation has a sterile, pink clear liquid, free of visible particles, to be used after shaking well. The vaccine contains Kanamycin as the residual antibiotic during the culture process.

The frozen vaccine vial will be stored at -20°C. The vaccine can also be stored and transported at 2-8 °C upto 2 months. For this study ROTAVIN-M1 will be supplied monthly by the manufacturer with storage and transport at 2-8 °C at the site. The vaccine is for oral use.

ROTAVIN-M1 formulation has a sterile, pink clear liquid to be used after shaking well. Details of the vaccine are as follows:

Product name:	Rotavirus vaccine
Trademark:	ROTAVIN-M1
Active substance:	Live attenuated human rotavirus strain, G1P[8]
Formulation:	≥ 2x 10^6 plaque focus units (PFU) / Dose Sucrose, Dulbecco's Modified Eagle Medium (DMEM), Kanamycin sulphate and phenol red
Product form:	Live attenuated human rotavirus vaccine
Pharmaceutical form:	Vaccine for Oral Use
Administration route:	Oral
Dosage:	2 mL, 2 Vaccinations, 2 months apart
Packaging:	single dose of 2 ml in glass vials
Storage:	The frozen vaccine vial will be stored at -20°C. The vaccine can also be stored and transported at 2-8 °C upto 2 months. The vaccine should not be refrozen once it is stored at 2-8 °C.

Packaging

Liquid formulation of ROTAVIN and ROTAVIN-M1 will be supplied in glass vials at a dose volume of 2 ml, and covered with a pharmaceutically acceptable silicon stopper, and aluminum cap seal according to the local NRA requirements. Packaging will be done to assure that the vials are intact and vaccine remains of high quality. A continuous temperature data logger will be placed inside each carton box to monitor product temperature during the process of transportation, storage, and delivery of the product.

Labeling

The carton boxes will also have "product to be used for clinical trial purposes only" label and information on the product storage temperature (from +2°C to +8°C). The sample label is below:

- Template label for liquid formulation of ROTAVIN (2 ml)

- Template label for ROTAVIN-M1(2 ml)

The study product must not be used if the package or labeling appears to be tampered with, the label is illegible, or the physical properties (color and transparency) are altered. These labels will be overlabeled with a blinded randomization code, similar to what is shown below.

- Template blinding lable
-

6.1.3 Stability and Storage

Study product must be stored at a temperature between 2 - 8 °C. The shelf-life of liquid formulation of ROTAVIN-is 24 months in that condition whereas ROTAVIN-M1 is stable only for 2 months after transferring to 2-8 °C. Storage temperature must be monitored daily and documented on an appropriate form. Back-up power or storage must be available in case of primary power failure. Study vaccines must never be frozen in clinical trial conditions. Their temperature will be monitored at the manufacturer, during transport, and at the clinical trial site.

In case of accidental disruption of the cold chain, the products may not be administered and the investigator or the responsible person should contact POLYVAC to receive further instructions. In such cases, the investigator must receive written consent (through facsimile or emailed scanned copy) from POLYVAC before any study product may be used.

6.1.4 Lot Number, Expiry Date, and Quality Control Results of Study Vaccine Lots

One lot of each of study vaccine will used in this study. The study vaccine lots will be used only after they pass quality control testing performed by the National Institute of Control Vaccine and Medical Biologicals, Vietnam and a copy of the results will be kept in the study file. To maintain blinding the lot number and expiry date will not be mentioned on the blinding label.

6.2 Dosage, Preparation, and Administration of Study Products

6.2.1 Dosage and Schedule

Each dose of 2 mL of either of the two vaccines will be administered orally. The first dose will be administered at 60-91 days (both days inclusive) of age and the second dose will be administered 8(+2) weeks after the first dose.

6.2.2 Precautions and Warnings

1. The vaccines are for oral use only and should under no circumstances be injected.
2. The study vaccines are only to be administered to healthy infants participating in this study and as per study protocol. It is forbidden to use the vaccine for any other purpose.
3. Strict compliance with the regulations of the MOH on immunization is required (Decision No.12/2014/TT-BYT, dated 20/03/2014).
4. The investigational products must not be mixed with other medicinal products.
5. Study product vials that have been frozen after supply from POLYVAC should not be used.
6. ROTAVIN-M1 and the liquid formulation are homogenous pink and yellow coloured solutions, respectively. Do not administer if the study product vial appears to have unusual content or if the liquid in the vials has changed color.
7. Only unblinded health workers who have been trained in the framework of this research may administer study product to participants participating in the research, assuring proper and completed administration of the study dose.
8. Blinded researchers will observe for post-vaccination reactions in participants.
9. Study participants must be monitored during vaccination and 30 minutes afterwards.
10. During study vaccinations, there must be adequate facilities for monitoring and treating any reactions. Drugs to treat anaphylaxis must be available, as must specialist doctors for such an emergency.

6.2.3 Preparation and Administration

To prepare and administer study product, vaccination facilities and investigators must comply with Ministry of Health regulations (No.12/2014/TT-BYT, dated 20/03/2014). The step-by-step instructions for the investigator are as follows:

1. Only unblinded staff of team members will be involved in preparation and administration of the study vaccines.
2. Make sure you have emergency drug and equipment kits in both the vaccination area and the observation area.
3. Wash hands. Choose the appropriate dosing vial.
4. Shake well
5. Inspect visually for any foreign particulate matter and / or abnormal physical appearance prior to administration. In the event of either being observed, discard the vaccine vial.
6. Check the vial has not been damaged nor is already open.
7. Use a covered syringe and a needle to withdraw exactly 2 ml of the vaccine from the vial. Remove the needle from the syringe.
8. The withdrawn study vaccine should be used promptly.

9. Confirm the participant identity. Seat the child leaning slightly backwards. Administer the dose by gently placing the syringe into the infant's mouth towards the inner cheek and gently expelling the contents until the syringe is empty.
10. Record the date of vaccination and vaccine vial number on the participant's vaccination card. Do not note the actual treatment received but record vaccine only as rotavirus vaccine.
11. Ask the parent/LAR of participant to wait for 30 minutes so that the child could be observed.
12. While the participant is resting, explain parents/LAR how to use the Diary Card for recording symptoms during next seven days.

If a child spits up or regurgitates most of the vaccine (based on investigator's discretion) within 5 minutes of administration, a single replacement dose will be administered and the event will be recorded in the source documents and eCRF. The participant should continue to receive remaining doses in the recommended series.

6.3 Modification of Study Product for a Participant

There is no dose adjustment for the product in the study.

6.4 Accountability Procedures for Study Product

- POLYVAC will supply sufficient quantities of study vaccines (ROTAVIN-M1 and the liquid formulation of ROTAVIN) to allow completion of this study only after the MOH EC approval of final protocol. The study vaccines will be kept in a secure place, in cold storage, and segregated from other products at the Provincial Preventive Medicine Center (PPMC). During the study, the unblinded person in charge of research product management will record information related to the delivery of vaccines to the trial site, conduct inventory at the PPMC, check the number of doses given to the participants, number broken, check the number of unused doses and return the unused doses to POLYVAC upon completion of the study. The unblinded CRO monitor will visit the site (including field sites) periodically throughout the trial to review and verify vaccine accountability records, as well as to ensure compliance with all trial procedures by the unblinded site personnel. After final vaccine accountability is completed by the unblinded CRO monitor, any used or unused vials of study vaccine will be destroyed as per the instruction of the sponsor POLYVAC. Due to the need to maintain blinding, no vaccine accountability records will be sent to the Sponsor or included in the trial master file (TMF) until after database lock.

At the Vaccination Site

- On scheduled vaccination days, study vaccines will be transported to the local site with proper temperature control and monitoring by unblinded staff members.
- Standard procedures will be followed at the trial site to maintain proper transport, receipt, storage, and return of study products.
- Following vaccination, the vaccine vials will be labeled with the participant ID and date of vaccination using prepared stickers.

- In the case of interruption of the cold chain (i.e., the temperature is out of acceptable range), the Principal Investigator or qualified designated staff member must contact POLYVAC to get further instructions. The investigator must receive written consent (through facsimile or emailed scanned copy) from POLYVAC before clinical trial products can be used.
- The person who administered the vaccine and the time and date of vaccine administration will also be documented in an appropriate vaccine accountability log on the day of vaccination.

6.5 Assessment of Compliance with Use of the Study Products

Compliance with use of the study products will be closely monitored during the trial by the unblinded research team and trial monitors.

6.6 Concomitant Medications/Treatment

Childhood vaccines as per the Expanded Program for Immunization of the Government of Vietnam [Diphtheria, Tetanus, Pertussis, Haemophilus influenzae type B and Hepatitis B vaccine (DTwP-Hib-HepB) and OPV] and Pneumococcal vaccines will be allowed during the study period but they should not be administered within 7 days before or after study vaccine administration. This is required to avoid any overlap of safety event due to study vaccine and EPI vaccines. The study participants may receive additional doses of OPV as required during special immunization rounds irrespective of the timing of study vaccine.

All non-study vaccines administered during the study will be reported in the "Past and Concomitant Vaccination" section of the CRF.

6.7 Unauthorized Products

The following products are not authorized to be used during the study:

1. Any concomitant medicine or biologic specifically prescribed for the treatment of a condition which is an exclusion criterion for participation in the trial.
2. All non-study vaccines or biologics (including blood products) not included in section 6.6 above.

Other concomitant medications will be reviewed on a case-by-case basis by the medical monitor during periodic review of the listings, if the study staff are unclear.

Parent/LAR of the participants will be requested not to take analgesic or antipyretic drugs in a preventive way (before or soon after vaccination), as such medications might change the reactogenicity profiles of study vaccines.

The parents of the children should inform the PI or designee about intake of any drug taken for the treatment of an illness occurring since first vaccination until end of follow-up period. Use of prescription medications and any treatments / procedures during the study period will be recorded on source documents and Concomitant Medication CRF.

During the trial, if a situation arises where there is an adverse reaction or AE requiring treatment and prescription of unauthorized products or products not stipulated by the protocol, then such products may be prescribed. However, POLYVAC must be informed of such an occurrence within 48 hours. Information on the products (trade name, dosing or change in dosing, indications, start date, and termination date) must be recorded in the CRF.

7 STUDY SCHEDULE; DESCRIPTION OF VISITS

The total expected duration of the study:

First participant enrolled in study	Day 1 of first participant
The last participant enrolled in study	Day 1 of last participant is estimated approximately 7 months after the Day 1 of first participant.
The last participant completes the trial	Day 85 of last participant
Lock database	16 weeks after Day 85 of last participant
Clinical trial report	6-8 months after Day 85 of last participant

Children will be seen at the study clinic on the day of screening/enrollment/first vaccination, visits for second vaccinations and follow up visit 4 weeks after the second. Below is a detailed description of study activities on each scheduled visit day.

7.1 Screening/Enrollment/ first vaccination Visit (Day 1)

Screening and enrollment will be done on the same day. Parent/LAR of all eligible participants will be provided an information sheet approved by IEC/IRB with details of the study and the study will be discussed with the family. If the family is interested in participating in the study the study physician will counsel the parent and signed consent will be obtained before enrollment. No study specific procedure would be conducted before signing the consent form by the parent/LAR. The investigator will record on the screening/enrollment log the screening IDs of all participants who enter screening; whether they entered the trial or failed screening with the reason for screen failure.

After obtaining written consent participants will be screened for eligibility through medical history review and general physical examination. To assess the eligibility, the following procedures will be conducted:

Demographic data: The parent will be interviewed to collect baseline demographic data including age, ethnicity, gender, date of birth, birth weight (kg) and the study staff will measure present weight(kg) and length (cm) of the baby. A copy of the child's immunization card or the immunization logbook kept at the commune health centre will be taken as a source document. Complete address of the parent will be recorded.

Medical History: A study clinician will interview the parent to collect detailed medical history. This will include history of participation in a drug research study/clinical trial, immunization history, any ongoing diarrhea or other illness. It will also include past medical history; vaccination history; surgical history; previous hospitalizations; history of any allergy to drugs or vaccines; current medication history; and any significant family history, including history of immunodeficiency in any household member.

Physical Examination: The study clinician will perform the general physical examination. Physical examination will include vital signs (axillary temperature, heart rate, and respiratory rate) and systematic examination of head and neck, skin, eye, ears, nose, and throat (ENT), cardiovascular system (CVS), respiratory system (RS), gastrointestinal system (GIS), genito-urinary system (GUS), central nervous system (CNS), and musculoskeletal system.

Participants who failed screening will be recorded on screening pages of the eCRF documenting reason for failure.

Enrollment procedures: The day of the child's first study vaccination is designated as study Day 1.The following procedures will be conducted on this day:

1. Randomization: Once eligibility is ascertained, the child would be assigned a randomization code which will assign him/her to receive the study vaccine.
2. Blood collection: Collect baseline blood sample of up to 2 ml for immunogenicity assessment at one selected site.
3. Vaccination: Oral vaccination will be done after the child comes back to normal state after blood collection. Unblinded study staff will administer the vaccine and the study product will be masked so that the participant's parent/LAR will remain unaware of product allocation and administration. The participants will receive vaccine with the same randomization code at the time of second vaccination. Vaccines will ONLY be administered if participant does not meet any contraindication to vaccine administration. During each vaccination visit, a section of study eCRF will be completed that records information about vaccines received, date and time. Proper accountability of all vaccines will be maintained by unblinded staff.
4. Concomitant Vaccination and Other Treatments: No other vaccine will be administered at this visit. Administration of other vaccinations and use of prescription medications (including name and duration of treatment) and any treatments / procedures during the study period will be recorded on source documents / eCRF, if available.
5. Immediate Post-Vaccination Reactogenicity Assessment: After each dosing, all participants will be observed at the clinic site for 30 minutes to check for any immediate AEs including any episodes of vomiting and allergic reaction to vaccine. After 30 minutes post-vaccination observation period, vital signs will be measured; if indicated, a targeted physical examination will be performed. If the participant experiences an immediate adverse reaction, he/she will be treated and clinically significant abnormal findings on targeted physical examination and vital signs will be reported in the CRF as an unsolicited AE. All procedures except those involving vaccination will be done by blinded staff members.
6. Provide digital thermometer and the post-immunization diary card (PIDC) to record, from the day of vaccination and daily for next six days, any solicited reactions, including diarrhea, fever, vomiting, decreased appetite, irritability, and decreased activity level and concomitant medications taken. The participant's parent/LAR will be instructed how to use the Diary Card and thermometer. All relevant explanations will be included in the Diary Card. The Diary Card will also have contact information for the investigators, should the participant have any questions. The participant's parent/LAR will be informed that a member of the investigator's team will visit the participant the next day (Day 2) and 7 days after the injection (Day 8) and will contact them by phone on Day 5 to check on the participant's completion of the Diary Card and participant's well-being.
7. Parents/LARs will be instructed that if the participant later experiences an AE requiring medical care, the parent/LAR of the participant should inform the investigator as soon as possible and seek medical care as appropriate. If the participant visits a health care provider, the parent/LAR should be sure to inform the health care provider of their child's participation in this study and provide the health care provider with the investigator's contact information.
8. Inform the parent on the next clinic visit after 56 days.

Safety Follow-Up

1. Participants parent/LAR will complete the Diary Card daily, reporting any systemic reactions experienced and medications taken from the evening of D1 through D7 after vaccination.
2. Study staff will make a physical home visit on Days 2 (+1 day) and Day 8 (+2 day) and a telephone call to the participants parent/LAR on Day 5 (+2) to determine the child's health status and to support completion of the PIDC. In case the child experiences any illness during this period, they will be referred to the study clinic for further evaluation and treatment.
3. Completed PIDC can be collected by the field officer at on Day 8 visit or the parent can bring it to the site at the next visit, if parents were not available during the home visit.
4. The parent/LAR will be instructed that if the participant later experiences an AE requiring medical care, the parent/LAR should inform the investigator as soon as possible and seek medical care as appropriate. If the participant visits a health care provider, the parent/LAR should be sure to inform the health care provider of participation in this study and give the health care provider the contact information for the investigator.
5. The data in the PIDC collected by the parents will be reviewed by the study physician/designee and entered in the CRF. Any solicited reaction extending beyond 7 days after vaccination will also be recorded on the PIDC/CRF with date of resolution (if available) and the highest severity during the occurrence. Also, unsolicited AEs will be captured up to 28 days after each vaccination and SAEs including suspicion of intussusception will be recorded at all times between first vaccination and last visits. These will be managed medically as needed.

7.2 Second Vaccination (Day 57; + 14 days)

The date for this visit will be calculated 56 days from the date of the first study vaccination. A maximum window period of +14 days will be allowed for this visit. Parents will be contacted by phone few days before the visit to remind them of the appointment. Participants who miss the scheduled visit date will be prompted to re-schedule for the missed visit. The following procedures will be carried out at this visit:

1. Study staff will confirm participant's identity.
2. Check for any adverse events since last visit and record them in the source note and eCRF if applicable. Update medical history including follow-up of prior AEs.
3. Collection and review of the diary cards if not already collected by field worker earlier.
4. Check for use/administration of any medication/vaccine since last visit. Any medication taken for the treatment of the illness or vaccine administered will also be recorded in the relevant section of the CRF.
5. Conduct physical examination and vital examination and record the information on the CRF. Results will be reviewed by study staff and discussed with the parent. Any AEs that have occurred will be recorded in the appropriate section(s) of the CRF.
6. Check for any contraindication to vaccination. Reschedule visit if the infant is experiencing an illness that is temporary contraindication to vaccination.
7. Vaccines will be administered by an unblinded study staff if participant does not meet any contraindication to Investigational Medicinal Product (IMP) administration. The participant will receive a dose of vaccine bearing the same randomization code as for visit 1. A section of study eCRF will be completed that records information about vaccines received, date and time.
8. Procedures 5-7 under section 7.1 above will be repeated.

9. Remind the parent of the safety follow up visit and phone call by the field officer and advise him/her on the next clinic visit after 28 days.

Safety Follow-Up

1. Participants parent/LAR will complete the Diary Card daily, reporting solicited reactions experienced and medications taken from the evening of D57 through D63 after vaccination.
2. Study staff will make a physical home visit on Days 58 (+1 day) and Day 64 (+2 day) and a telephone call to the participant's parent/LAR on Day 61 (+2) to determine the child's health status and to support completion of the PIDC. In case the child experiences any illness during this period, they will be referred to the study clinic for further evaluation and treatment.
3. Completed PIDC can be collected by the field officer at on Day 64 visit or the parent can bring it to the site at the next visit, if parents were not available during the home visit.
4. The parent/LAR will be instructed that if the participant later experiences an AE requiring medical care, the parent should inform the investigator as soon as possible and seek medical care as appropriate. If the participant visits a health care provider, the participant parent/LAR should be sure to inform the health care provider of participation in this study and give the health care provider the contact information for the investigator.
5. The data in the PIDC collected by the parents will be reviewed by the study physician/designee and entered in the CRF. Any solicited reaction extending beyond 7 days after vaccination will also be recorded on the PIDC/CRF with date of resolution (if available) and the highest severity during the occurrence. Also, unsolicited AEs will be captured up to 28 days after each vaccination and SAEs including suspicion of intussusception will be recorded at all times between first vaccination and last visit. These will be managed medically as needed.

7.3 Final Study Visit (Day 85; + 14)

The date for this visit will be calculated 28 days from the date of last vaccination. A maximum window period of +14 days will be allowed for this visit. Parents will be contacted for the participants who miss the scheduled visit date. The following procedures will be conducted:

1. Study staff will confirm participant's identity.
2. Review / update address and contact details.
3. Check for any adverse events since last visit and record them in the source note and eCRF if applicable. Update medical history including follow-up of prior SAEs. Ensure all ongoing AEs are followed up as per the protocol.
4. Collection and review of the diary cards if not already collected by field worker earlier.
5. Check for use/administration of any medication/vaccine since last visit. Any medication taken for the treatment of the illness or vaccine administered will also be recorded in the relevant section of the CRF.
6. Perform physical examination.
7. Collect up to 2 mL of blood for immunological assay.
8. Review vaccination history and advise the parent/LAR on future vaccination of the infant.
9. Complete the study termination/completion form

7.4 Early Termination

Participant parents/LARs have the right to decline study treatment or procedures for any reason and at any time during the study. If a participant parent/LAR declines further vaccination or study procedures (but continues in the study for safety assessment) this will be recorded as a study deviation and the reason will be clearly documented in the source document. The participant parent/LAR will be encouraged to have their child complete the remaining applicable safety-related follow-ups. A blood sample will not be collected in infants who miss one of the vaccinations. If the participant parent/LAR does not wish to remain in the study by declining any follow-ups or procedures, the participant parent/LAR can choose to withdraw consent and be withdrawn from the study.

The participants may be withdrawn from the study for any of the following reasons:

- If parent/LAR of participant wishes to withdraw consent.
- If the Principal Investigator (PI) decides that withdrawal is in the best interest of the participant.
- Significant non-compliance with treatment regimen or trial requirements.
- Participant is lost to follow-up.
- The sponsor/manufacturer recommends to terminate the study.
- Governing IRBs recommends to terminate the study.

In all cases, where the participant is withdrawn from the study the reason for withdrawal will be documented in an appropriate section of the eCRF. However, the data collected up to the last contact will be part of the analysis. In the event of withdrawal from study, reasonable efforts should be made to conduct the following procedures:

- Review diary card if still in use prior to withdrawal.
- Updating any ongoing AE/SAEs that remain ongoing at time of participant's last visit prior to withdrawal.
- Query about AEs, SAEs and concomitant medications since the last visit.
- Conduct physical examination.
- Update contact information.

Strategies to maintain retention in the trial include treating them respectfully, making sure they understand the study and potential side effects, and close communication with each participant parent/LAR about how the study unfolds. Given the short duration of the trial and anticipated side effects of the study vaccines, participant withdrawal and loss of follow-up is expected to be low. To prevent delays in subject visits and lost to follow-up, the study team will ensure that valid contact details are obtained prior to enrollment so that the parents/LARs can be reminded by phone at least two day before their scheduled visit. In the event of a missed visit, the participant parent/LAR will be contacted by phone; if that fails, a home visit within 3 days of the scheduled visit will be made by the field worker. A participant who cannot be located after at least 3 documented contact efforts will be considered lost to follow-up. Efforts to contact will be documented in source documents. Any participant who failed to attend the final study visit will be classified as lost to follow-up. A participant who is not vaccinated within the window of Visit 2 may be continued in the study, vaccinated outside of the window at the discretion of the investigator, and followed up according to guidance in the protocol. These cases if any will be

considered as protocol deviations and will be notified to the IEC/IRB in the progress reports and also be included in the list of protocol deviations in the study report. Participants who discontinue after enrollment will not be replaced.

POLYVAC and PATH must be informed within 48 hours of all instances of the premature termination of a participant's participation in the trial.

If the participant develops a reaction to study vaccine which the investigator believes threatens the participant's well-being, the withdrawn participant must be treated or transferred to a treatment facility.

7.5 Unscheduled Visits

Interim contacts and visits (those between regularly scheduled follow up visits) may be performed at participant's parent/LAR's request or as deemed necessary by the investigator or designee at any time during the study. Parents will be asked to bring the child to the clinic at any time between visits if they have any condition that requires medical attention. All interim contacts and visits will be documented in participant's study records and on applicable case report forms as "Unscheduled visits".

7.6 End of Trial According to the Protocol

The end of the trial is defined as the date of the last contact of the last participant in the trial, according to the trial scheme.

8 STUDY EVALUATIONS

8.1 Clinical Evaluations

Definition and Categorization of AEs

The primary objective of this study is to compare the safety and immunogenicity of the liquid formulation of ROTAVIN with already licensed vaccine ROTAVIN-M1. An AE is defined as any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. Grading of AEs is also described in this section. Included below are brief listings of specific clinical safety measurements to be made. All clinical safety evaluations must be made by a qualified clinician (physician, physician assistant, or nurse practitioner) or will be self-reported by the participant's parent/LAR. Serious adverse events may occur at any time during the study. They are defined in Section 9.9.

Specific Clinical Signs and Symptoms of Interest

All clinical signs and symptoms must be documented. Specific signs and symptoms of interest, and their date of onset/occurrence and resolution, will be solicited and recorded for all participants from the time of signing ICF until termination of participation. These are listed in Section 9.7 as Solicited Systemic Reactions.

These evaluations will be made by a clinician on Days 1, 57, and 85 if not earlier. Evaluation must be performed both prior to and after administration of study product on Days 1 and 57. Reported

signs and symptoms will be recorded by the participant's parent/LAR on Diary Card from Days 1-7 and Days 57-63 in the study.

Medical History

At enrollment, medical histories must be thoroughly reviewed with the participant's parent/LAR. The following medical conditions, in particular, will be assessed:

- Presence of diarrhea or vomiting within 72 hours of enrollment or past history of persistent diarrhea.
- Presence of fever on the day of enrollment
- Any current acute disease.
- Clinically relevant history of cardiovascular, pulmonary, hepatic, renal, gastrointestinal, hematological, endocrine, immunological, dermatological, neurological, cancer, or autoimmune disease.
- Past or current history of congenital abdominal disorders, intussusception, or abdominal surgery
- Known or suspected immunologic impairment of any kind.
- Recent vaccination history.
- Recent receipt of immune globulin or other blood products, or injected or oral corticosteroids, or other immune modulator therapy since birth.
- Hypersensitivity of any kind.
- Seizures, including history of febrile seizures, or any other neurologic disorder.
- Medications taken in the 2 months (including trade name, dosing or change in dosing, indications, start date, and termination date).

Physical Examination

General Physical Examinations

A General Physical Exam is performed on each visit and anytime it is clinically indicated according to the medical judgment of the study staff. Qualified study clinicians will conduct a physical examination of all participants. This physical examination will include the following:

- Recording of general appearance.
- Physical examination of all organ systems. This includes the following:
  - Head & Neck
  - Eyes
  - Ears, Nose & Throat
  - Skin and subcutaneous tissue
  - Neurologic examination
  - Chest auscultation
  - Examination of lymph nodes (axillary and cervical)
  - Heart auscultation
  - Abdomen palpation (to check for liver size)
  - Genitourinary

- Musculoskeletal and connective tissue

- Measurement of the following vital signs:
  - Body temperature (axillary)
  - Respiratory rate
  - Heart rate

8.2 Laboratory Evaluations

To quantitate antibody concentrations elicited by the study vaccines, a baseline blood sample will be collected from all participants at one of the two sites, before the first vaccination and four weeks after the last vaccination.

Preparation, Processing, and Transport of Specimens

Collection of Blood

Following universal precautions, clinicians and experienced nurse/phlebotomists will be trained in appropriate sample collection methods. Appropriate aseptic procedures will be employed with appropriate needles and syringes in place for sample collection procedure. Following universal precautions, a maximum of 2 ml of blood, from a peripheral vein, will be collected by an aseptic technique into serum separator tubes (SST). A maximum of 3 attempts will be made for collection of the blood sample. Sampling attempt will not be repeated in case the team is not able to collect the sample on this day. A blood sample will not be collected for participant who reported a protocol deviation which might have influenced the immune response of the participant as determined by the PI or the medical monitor.

Processing of Blood specimen

Immediately after collection, the blood specimen tube will be gently inverted 4 or 5 times, (labeled with the study participant number, the protocol number and the blood specimen number), and will be stood upright to clot for at least 30 minutes at room temperature before transport to the laboratory at room temperature for processing. The specimen will be transported to the laboratory at PPMC where it will be centrifuged within 8 hours of collection.

Division of Sera

Within 8 hours of collection, clotted blood in the tube will be centrifuged at the PPMC laboratory to obtain serum. Designated laboratory personnel at the site will aliquot the samples into two aliquots as follows:

- 1st aliquot: 0.5 mL for IgA assay
- 2nd aliquot: remaining serum as backup

The study participant number, date and time of collection, blood specimen number, the date and time of division and volume of serum in each aliquot will be specified on a serologic specimen log form/ study worksheet. On this form, comments may be made on the quality of specimens (e.g., hemolyzed, contaminated, etc.).

Conditions for Transport and Storage of Sera

The two aliquots will be stored in separate areas of controlled- freezer maintained at or below -20°C until the time of shipment to the testing laboratory. These freezers will have backup power source in case of outage on the national power grid. All handling will be done to prevent unnecessary freeze-thaw cycles (i.e., back-up samples should not be thawed unless required for testing). Temperature monitoring will be done to assure maintenance of cold chain and specimen

quality. One aliquot will be sent to the testing laboratories (i.e., laboratory at CCHMC, Cincinnati, USA) at or below –20°C using validated packaging and temperature monitors. The testing laboratories will be responsible for testing for anti-rotavirus IgA antibodies and storing of samples till study completion.

Immunological Assays

The quantitative IgA assay is the most frequently used serologic test for determining immunologic response to rotavirus vaccination. Immunogenicity will be assessed in all participants enrolled at one of the two proposed sites. Testing will be conducted on serum samples obtained before the first vaccination and four weeks after the second vaccination using a validated ELISA assay in the selected participants. The laboratory will remain blinded to the treatment allocation to the infants being tested. Geometric mean concentration (GMC), seroconversion rate, and seropositivity rate of serum anti- rotavirus IgA antibody will be calculated and compared.

Estimation of serum IgA antibodies to rotavirus by ELISA

Serum anti-rotavirus IgA is used as a measure of immunogenicity of rotavirus vaccines. Quantification of anti-rotavirus IgA antibodies will be done by ELISA using 89-12 as the substrate and will be performed at Cincinnati Children's Hospital Medical Center (CCHMC), Cincinnati, Ohio using validated procedures. The method used is an antibody sandwich enzyme immunoassay in which plates coated with polyclonal anti-rotavirus rabbit antiserum are used to capture virus lysate and detect human serum IgA in samples using biotinylated rabbit anti-human IgA. SOP no 110: Quantitative Determination of Serum Anti-Rotavirus IgA by EIA describes the procedure to measure anti-rotavirus IgA in human samples. The quantity of specific IgA is determined by comparison of the net optical density from sample wells to a standard curve generated by a human plasma standard. Dilution corrected concentrations are reported for samples if results do not fall within the quantifiable range of the standard curve.

Biohazard containment

As blood-borne pathogens can infect through contact with contaminated needles, blood, and blood products, appropriate precautions will be employed by all personnel in shipping and handling of all specimens for this study as recommended. All biological specimens will be transported using appropriate packaging. All dangerous goods or materials, including diagnostic specimens and infectious substances, must be transported according to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations. Biohazard waste will be contained according to institutional, transportation/carrier, and all other applicable regulations. All dangerous goods or materials, including diagnostic specimens and infectious substances, must be transported according to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations. Biohazardous waste will be contained according to institutional, transportation/carrier, and all other applicable regulations.

9 ASSESSMENT OF SAFETY AND ADVERSE EVENTS

9.1 Adverse Events

Adverse Event: An adverse event is defined as any untoward medical occurrence in a participant after administration of the investigational vaccine and that does not necessarily have a causal relationship with the investigational vaccine. An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory findings), symptoms, physical examinations, or

disease temporally associated with the use of the investigational vaccine, whether or not related to the investigational vaccine. This definition includes exacerbations of pre-existing conditions. Stable pre-existing conditions which do not change in nature or severity during the study are not considered AEs; however, these should be reported as part of the medical history. AE's will be recorded from D1 after study vaccination up to last visit of the participant. An AE does not include:

- Medical or surgical procedures (e.g., surgery, endoscopy, transfusion), but the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for elective surgery, social and / or convenience admissions).
- Overdose of either study drug or concomitant medication without any signs or symptoms

Information to be collected on AEs includes event description, time of onset, clinician's assessment of severity, relationship to study product, and time of resolution/stabilization of the event. AE assessment should be made only by those with the training and authority to make a diagnosis.

Solicited AEs are pre-specific adverse events that are common or known to be associated with vaccination and that are actively monitored as indicators of vaccine reactogenicity. Investigators will not be required to assess causality of solicited adverse events if the onset is during the solicitation periods. Solicited adverse events with onset after the solicitation period will be captured as unsolicited AEs.

For this trial, immediate AEs will be assessed by study staff 30 minutes after each vaccination and solicited AEs will be assessed daily for 7 days after each vaccination by the participants. Participant parent/LAR will be provided a diary to record the presence or absence of solicited AEs, severity of the solicited AE and use of concomitant medication.

In this study only systemic solicited events of fever, diarrhea, vomiting, decreased appetite, irritability, and decreased activity level will be monitored. Local reactions to EPI vaccination should be reported as unsolicited AEs.

Unsolicited AEs are any AEs reported spontaneously by the participant's parent/LAR, observed by the study personnel during study visits or those identified during review of medical records or source documents. Unsolicited AEs are not specified for active monitoring, but spontaneously reported as untoward events occurring in a participant. All such events will be recorded on the 'Adverse event' pages of the eCRF.

SAEs are defined in Section 9.9.

9.2 AE/SAE Reporting Period and Parameter

The safety profile will be evaluated by the proportion of participants experiencing AEs, related or not related, under the following five categories:

- Number and percentage of participants with immediate adverse events within 30 minutes after each vaccination.
- Number and percentage of participants with solicited adverse events (fever, diarrhea, vomiting, decreased appetite, irritability, and decreased activity level) over the 7-day period (Days 1-7) post vaccination.
- Number and percentage of participants with unsolicited adverse events from first vaccination through four weeks after the last vaccination (Unsolicited adverse events are any untoward

medical occurrence in the participant, temporally related to receipt of the study products, whether or not considered related).

- All serious adverse events (SAEs) and Intussusception from first vaccination through four weeks after the last vaccination.

9.3 Severity of Event

Severity of solicited reactions will be graded as follows:

Reaction	Intensity grade	Parameter
Fever (°C)	0/Normal	Axillary temperature < 37.5°C
	1/ Mild	Axillary temperature ≥ 37.5 – ≤ 38.0°C
	2/Moderate	Axillary temperature > 38.0 – ≤ 39.0°C
	3/ Severe	Axillary temperature > 39.0°C
Diarrhea	0/Normal	0 - 2 looser than normal stools / day
	1/ Mild	3 looser than normal stools / day
	2/Moderate	4 - 5 looser than normal stools / day
	3/ Severe	≥ 6 looser than normal stools / day
Vomiting	0/Normal	Normal (no emesis)
	1/ Mild	1 episode of vomiting / day
	2/Moderate	2 episodes of vomiting / day
	3/ Severe	≥ 3 episodes of vomiting / day
Decreased appetite	0/Normal	Appetite as usual
	1/ Mild	Eating/breastfeeding less than usual / no effect on normal activity
	2/Moderate	Eating/breastfeeding less than usual / interferes with normal activity
	3/ Severe	Not eating/breastfeeding at all
Irritability	0/Normal	Normal (Behaviour as usual)
	1/ Mild	Crying more than usual with no effect on normal activity
	2/Moderate	Crying more than usual that interferes with normal activity
	3/ Severe	persistent crying and the child could not be comforted and that prevents normal activity
Decreased activity level	0/Normal	Behaviour as usual
	1/ Mild	Drowsiness easily tolerated

	2/Moderate	Drowsiness that interferes with normal activity
	3/ Severe	Drowsiness that prevents normal activity

The severity of all unsolicited AEs / SAEs occurring during the course of the study will be graded as per the guidance document by Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, corrected version 2.1, July 2017, of the US National Institute of Health as attached as Appendix 2 to the protocol. Severity of unsolicited AEs and SAEs not included in the grading system mentioned above will be graded as follows:

Grade 1 / Mild - Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated.

Grade 2 / Moderate - Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated.

Grade 3 / Severe - Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated.

Grade 4 / Potentially Life Threatening - Potentially life-threatening symptoms with intervention indicated to prevent permanent impairment, persistent disability, or death (the Investigator should not grade a reaction as life-threatening if had it occurred in a more severe form then it might have caused death).

Grade 5 / Death- All AEs leading to death are Grade 5 events.

Definitions:

Usual Social & Functional Activities: Activities that are age and culturally appropriate, such as social interactions, play activities, or learning tasks.

Medical Intervention: Use of pharmacologic or biologic agent(s) for treatment of an AE.

An AE that is assessed as severe should not be confused with the term SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. AEs characterized as intermittent require documentation of onset and duration of each episode.

AEs are graded with the worst severity grade during the illness/symptoms.

9.4 Relationship to Study Vaccines

The clinician's assessment of an AE's relationship to study product is part of the documentation process. The clinician must determine whether there is a reasonable possibility that the investigational product(s) caused or contributed to an AE. It is essential to have the best assessment possible as to whether adverse events are related to investigational products.

- Is there a temporal (time-based) relationship between the event and administration of the investigational product?
- Is there a plausible biological mechanism for the investigational product to cause the AE?
- Is there a possible alternative etiology for the AE such as concurrent illness, concomitant medications?
- Are there previous reports of similar AEs associated with the investigational product or other vaccines in the same class?

For this study, the clinician must classify the causality of the AE according to the categories defined below:

Related: There is a reasonable possibility that the product caused the event. "Reasonable possibility" means that there is evidence to suggest a causal relationship between the study product and the AE.

Not Related: There is not a reasonable possibility that the administration of the study product caused the event.

Assessment of causal relationship should be recorded in the AE and SAE forms.

Any solicited reactogenicity that occurs during the 7-day period post-injection is automatically regarded as related.

9.5 Guidelines for AEs

To improve the quality and precision of acquired AE data, the PI should observe the following guidelines:

- All AEs and SAEs will be recorded in the source document and eCRF for all participants throughout the study participation.
- A solicited symptom reported during the solicitation period as an SAE should be recorded on the PIDC and SAE forms.
- Whenever possible, use recognized medical terms when recording AEs on the AE CRF. Do not use colloquialisms and/or abbreviations.
- If known, record the diagnosis (i.e., disease or syndrome) rather than component signs, symptoms and laboratory values (e.g., record congestive heart failure rather than dyspnea, rales, and cyanosis). However, signs and symptoms that are considered unrelated to an encountered syndrome or disease should be recorded as individual AEs (e.g., if congestive heart failure and severe headache are observed at the same time, each event should be recorded as an individual AE).
- AEs occurring secondary to other events (e.g., sequelae) should be identified by the primary cause. A "primary" AE, if clearly identifiable, generally represents the most accurate clinical term to record. If a primary serious AE (SAE) is recorded, events occurring secondary to the primary event should be described in the narrative description of the case.

  For example:

  Acute Gastroenteritis→ Diarrhea→ Nappy Rash

  The primary AE here is acute gastroenteritis.
- Death is an outcome of an event. The event that resulted in the death should be recorded and reported on the SAE CRF.
- For hospitalizations for surgical or diagnostic procedures, the illness leading to the surgical or diagnostic procedure should be recorded as the SAE, not the procedure itself. The procedure should be captured in the case narrative as part of the action taken in response to the illness.
- While no routine safety laboratories will be performed under this protocol, any abnormal laboratory findings (e.g., clinical chemistry, hematology) or other abnormal assessments (e.g., electrocardiogram, vital signs) that the study team may be aware of are not per se reported as AEs. However, abnormal findings that are deemed clinically significant or are associated with signs and / or symptoms must be recorded as AEs if they meet the definition of an AE (and recorded as an SAE if they meet the criteria of being serious) as described previously. Clinically significant abnormal laboratory or other abnormal findings that are detected after study drug administration or that are present at baseline and worsen following the

administration of study drug are included as AEs (and SAEs if serious). The Investigator should exercise his or her medical judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant. Usually, the abnormality should be associated with a clinically evident sign or symptom, or be likely to result in an evident sign or symptom in the near term, to be considered clinically significant.

9.6 Immediate adverse Events

Immediate adverse events will be assessed 30 minutes after vaccination on Day 1 and Day 57. The 30-minute Reactogenicity Assessment may consists of vomiting, anaphylaxis etc.

Appropriate medical treatment must be readily available in case of an anaphylactic reaction following the administration of the study product. Immediate reactions will be assessed by a study physician or appropriately trained medical staff. All reactions that occur during this time will be recorded on the CRF. Any immediate reaction which meets the criteria for an SAE must also be documented on an SAE form.

Emergency medicine will be available at the study clinic in the event there are any adverse reactions or events occurring among participants participating in this research. If anaphylaxis occurs after vaccination, it must be treated in accordance with the applicable guideline issued by MOH of VN. Treatment for mild reactions after vaccination will be per "Guidance on the treatment of post- injection reactions" of the National Expanded Program on Immunization. The applied treatment methods must be recorded and kept in the participant file.

9.7 Solicited Reactions (Expected Reactions)

Specific systemic reactions will be solicited (specifically asked of participant's parent/LAR) while participants are in the study. These specific reactions, i.e. (fever, diarrhea, vomiting, decreased appetite, irritability, and decreased activity level) will be graded by the study team as per the guidance above.

Solicited Systemic Reactogenicity will be collected through study Day 7. If a solicited sign or symptom has started during the 7 days post vaccination and continues beyond the 7 days, it will continue to be reported as a reactogenicity symptom. Any solicited sign or symptom starting after 7 days post vaccination will be recorded as an "unsolicited AE".

9.8 Unsolicited Adverse Events

Unsolicited adverse events are any AEs that occur any time after the study vaccine is given (temporally related to study product), whether or not deemed "related" to the product, and are not solicited. Unsolicited AEs can be observed by study staff while the participant is at a clinic for a study visit or reported by the participant's parent/LAR at any time. Unsolicited adverse events will be recorded with onset from the time of vaccination up to 28 days after each vaccination. Any solicited sign or symptom starting after 7 days post vaccination will be recorded as an "unsolicited AE".

9.9 Serious Adverse Events

A SAE is any untoward medical occurrence that:

- Results in death,
- Is life threatening,

- Requires inpatient hospitalization* or prolongation of existing hospitalization*,
- Results in persistent or significant disability** / incapacity,
- Is a congenital anomaly or a birth defect,
- Medically important event[#]

NOTE: Investigator-confirmed cases of intussusception will qualify as SAE in the study and will be reported on the SAE form. The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

[#]Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

*Hospitalization is an official admission to a hospital. Hospitalization or prolongation of a hospitalization constitutes a criterion for an AE to be serious; however, it is not in itself considered an SAE. In absence of an AE, a hospitalization or prolongation of a hospitalization should not be reported as an SAE by the Investigator through a SAE form, examples of such situations include:

- A hospitalization or prolongation of hospitalization is needed for a procedure required by the protocol.
- Hospitalization or prolongation of hospitalization is part of a routine procedure followed by the center (e.g., stent removal after surgery). This should be recorded in the study file.
- A hospitalization for a pre-existing condition that has not worsened.
- Hospitalization for social reasons.

**Disability is defined as a substantial disruption in a person's ability to conduct normal life functions.

All SAEs reported from the time of first vaccination upto 4 weeks after last vaccination will be reported in the study. All SAEs must be reviewed and evaluated by a study clinician (SAE relationship to study vaccine must be evaluated as outlined in Section 9.4) and recorded on an SAE form and reported, as specified in Section 9.1010. All such SAEs should also be followed until satisfactory resolution or until the investigator deems the event to be chronic or the patient to be stable.

9.10 Reporting Procedures

Serious Adverse Events

All SAEs must be documented and reported to POLYVAC or its designate, even if the investigator considers that the SAE is not related to treatment. The study clinician will complete a **Serious Adverse Event Form** within the following timelines of such events:

- All SAEs, whether related or unrelated, will be recorded on the Serious Adverse Event Form and notified to local IRB and sponsor by fax or email within 24 hours of site awareness.
- All deaths and immediately life-threatening events, whether related or unrelated, will be recorded on the Serious Adverse Event Form and reported to the MOH by fax or email within 7 days of site awareness.

- SAEs other than death and immediately life-threatening events (i.e., events resulting in hospitalization or prolongation of hospitalization, persistent or significant disability or incapacity, or other significant events determined by the investigator to be SAEs), regardless of relationship, will be reported reported to the MOH via fax or email by the site within 15 days of becoming aware of the event.

POLYVAC will be primarily responsible for medical monitoring of serious adverse events documented by the investigator. Medical Officers from PATH serving as technical consultants will review all serious adverse events and provide guidance regarding SAE management, including classification and reporting. Details for review and reporting of serious adverse events and other unanticipated problems will be in an SOP drafted prior to study initiation.

Reporting of AEs

Collected SAEs will be reported to responsible ethical review committees according to their requested timelines. An SOP for reporting to the responsible committees will be developed with reporting requirements and timelines prior to study initiation. It will be the investigator's responsibility to assure that all reportable events are reported to the proper authority, or to POLYVAC, and/or its designate in a timely manner and according to existing SOP. PATH technical consultants may assist the investigator with regulatory reporting, per the finalized SOP.

Other Unexpected Issues/Unanticipated Problems

During study process, if there are any problems related to the trial (unanticipated problems), the Principal Investigator is responsible for reporting to POLYVAC and PATH to discuss reasonable ways of handling the problem. The Principal Investigator is responsible for reporting any unanticipated problems that affect the health, welfare, or rights of study participants, or that may impact the integrity of the study data to the ethics committees involved in the review of the research. The Principal Investigator should maintain written documentation of all unanticipated problems, their reporting, and resolution.

9.11 Duration of Follow Up for AE Resolution

Participants will be monitored throughout the study period for adverse events. AEs come to the attention of site clinicians through interim medical histories, physical examinations and laboratory testing conducted to investigate other illnesses or routine tests. The possible occurrence of any AE and SAE will also be asked during the scheduled study visits. In addition, the parents will be advised to contact study staff immediately at any time throughout the study period if their child experiences an AE. All adverse events will be closely monitored in the safety monitoring processes.

AEs will be managed in accordance with good medical practices by the clinical study site team who will assess and treat or refer the participant for medical care as appropriate. Where feasible and medically appropriate, the parent will be encouraged to seek medical care at the facility where the study clinician is based, and to request that the clinician be contacted upon their arrival. If needed to monitor or treat an adverse event, additional study visits may be conducted.

All AEs and SAEs will be followed until resolution, until the condition stabilizes, or until the participant's participation in the study ends, whichever is earlier. Once resolved or stabilized, the appropriate AE / SAE form(s) will be updated. Participants who have an ongoing study product-related AE/SAE at study completion or at discontinuation from the study will be followed by the PI or his designee until the event is resolved or determined to be irreversible, chronic, or stable by the PI. The Investigator will ensure that follow-up includes any supplemental investigations as

may be indicated to elucidate the nature and / or causality of the AE or SAE. This may include additional laboratory tests or investigations, histopathological examinations or consultation with other health care professionals.

POLYVAC or its designee may request that the Investigator perform or arrange for the conduct of supplemental laboratory analysis and / or evaluations to elucidate as fully as possible the nature and /or causality of the AE or SAE. The Investigator is obliged to comply with this request if justifiable. Site Investigators will make sure that the required diagnostic tools (e.g., ultrasound) and surgical treatment for intussusception are readily available for the study participants under his watch. If a participant dies during the study period or during a recognized follow-up period, attempts should be made to provide a copy of any post-mortem findings, including histopathology. The updated SAE form should be sent to the SRC within the time frames outlined in the SOP.

The outcome of adverse event will be assessed as at the time of last observation as per the following categories:

- Recovered/resolved without sequelae
- Recovered/resolved with sequelae
- Ongoing at the end of the study
- Stabilized
- Death
- Unknown. The outcome of the AE is not known

9.12 Safety Oversight

For this study, a diligent mechanism for review of safety of the participants will be planned. It will ensure safety oversight by the site PI and Safety Review Committee (SRC) with provisions for referring to infectious disease specialists in case a consultation is recommended by the SRC.

Routine Reviews by Principal Investigator

The study site Investigators will be responsible for continuous close safety monitoring of all study participants, and for alerting the protocol team if concerns arise or if criteria for expedited review of safety data are met.

Routine Reviews by Safety Review Committee (SRC)

An team of vaccine experts, the SRC, will be established to examine safety at periodic intervals. The SRC, a group of physicians which include but not limit to the designated investigator from study team and the Medical Officer(s) from POLYVAC and PATH, a Pharmacovigilance/Medical officer from the CRO and an independent vaccine expert will routinely monitor safety throughout the duration of the trial. The SRC will be chaired by PATH Medical Officer and may seek additional independent expert medical opinion as dictated by needs. The CRO statistician with assistance from the data management staff will prepare safety reports for review by the SRC. These reports will provide at a minimum the following information: 1) accrual and participant status data with regard to completion of study vaccinations and study visits; and 2) summaries of solicited and unsolicited adverse events during the review period 3) Reported SAEs.

The SRC safety review will be conducted by email/teleconference occurring approximately fortnightly to monthly (depending on rate of enrollment) during the vaccination phase of the study and as needed thereafter for the remainder of the study. An expedited safety review will be carried out within 36 hours of submission of the safety information for the safety events listed below:

Event and relationship to study agent*	Severity Grade
SAE, related	All grades
A case of Intussusception	All grades
Unsolicited AE, related	4 and above
* As assessed by investigator or Medical Monitor	

The designated investigator or an assigned medical monitor/officer will be responsible for informing the other members and convening an ad hoc safety review whenever it is aware of an SAE or adverse event that meets any of the criteria above. The SRC may seek independent expert medical opinion as dictated by the occurrence of certain events.

In addition to safety review, the SRC may elect to discuss trial conduct issues that impact study integrity and participant safety. These may include but not limited to data quality, critical monitoring findings, study product, research specimens, etc.

The reports and their analysis by the SRC will be submitted to the various ECs/IRBs, if needed. The Terms of Reference for the SRC will be developed.

10 MONITORING

10.1 Monitoring Plan

Sponsor monitoring responsibilities will be provided by the CRO. Individuals qualified by education, training, and experience will be assigned to monitor the study. The study monitors will periodically contact the site and perform on-site visits.

The CRO will assign qualified blinded monitor(s) and unblinded monitors to visit the site (including all field sites) during the study period. The extent, nature, and frequency of visits will be based on such considerations as study objectives, study design and complexity, and enrollment rate. Periodicity and nature of monitoring activities will be described in the Monitoring Plan that will be approved by POLYVAC and PATH in advance of monitoring. The monitoring activities will be done according to the CRO's SOPs with the goals to protect the rights, safety and well-being of study participants; and assure the study compliance, data quality and integrity. The Monitoring Plan will contain detailed report requirements and study progress for POLYVAC and PATH. Representatives of POLYVAC and PATH or designates may participate in monitoring visits or visit the study site on their own in order to provide proper oversight.

10.2 Initiation Visit

The study monitor/PATH will contact the site prior to the start of the study to discuss the protocol and data collection procedures with site personnel. Prior to enrollment of participants at the study site, specific regulatory documents must be available. These include approvals from the independent ethics committee (IEC) at the Vietnam Ministry of Health and from institutional review boards of the participating institutions. Curriculum vitae for key investigators must also be available. POLYVAC/ PATH will inform the investigator of any additional documents that need to be provided.

10.3 Routine Monitoring Visits

Monitoring will be conducted according to an agreed upon Site Monitoring Plan. Monitoring will be targeted towards issues critical to the rights and welfare of study participants as well as accuracy and integrity of the study data. Individuals responsible for monitoring the study should have access to all records needed in order to periodically ensure the ethical and safe conduct of the study and the integrity/validity of the recorded data.

During sites visits and contacts, the monitor will:

1. Assess if consent was properly obtained.
2. Assess adherence to the protocol eligibility criteria.
3. Look for evidence that randomization was followed.
4. Look for evidence that blinding was maintained.
5. Look for evidence that the product was administered correctly.
6. Check on study conduct and documentation of procedures/assessments related to the study endpoints:
   - Specimens obtained correctly.
   - Specimens labeled correctly.
   - Reactogenicity diaries completed and collected.
7. Check on study conduct and documentation of protocol-required safety assessments, including SAEs.
8. Ensure that there is documentation of withdrawals and deaths with reasons provided.

The blinded monitor(s) will be responsible for all aspects of the clinical trial related to subjects, the blinded site staff, and regulatory and audit readiness and its documentation. The CRO will also assign an unblinded study monitor, who will visit the site (including all field sites) during the study period to assess and verify activities of the unblinded site personnel including review of treatment assignments, vaccine storage and accountability, and dosing-related matters and their documentation. Both blinded and unblinded study monitors will provide a blinded report to the CRO and Sponsor/PATH of ongoing activities and issues requiring resolution. Any unblinding of additional project team personnel required to resolve issues will be clearly documented in the TMF. Of note, all reports to blinded personnel by the unblinded CRO monitor will be constructed in order to maintain the blind during the trial. No report that would break the blind will be released into the TMF until after database lock.

As part of study conduct, the Principal Investigator will have to agree to allow the monitor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to discuss findings and relevant issues. The study consent also makes participants aware that medical records relevant to events in the study may be accessed and viewed by people conducting and overseeing the study.

The Principal Investigator also agrees to allow representatives of POLYVAC and PATH or its designates to occasionally accompany the monitor during site visits.

10.4 Close-out Visit

Upon completion of the study, the study monitor and the investigator will conduct the following activities:

- Data clarification and/or resolution.
- Accounting, reconciliation, and destruction (if planned) at sites of used and unused vaccines.
- Review of site study records for completeness.
- Return of all study data to POLYVAC.

11 AUDITS AND INSPECTIONS

For the purpose of compliance with applicable regulatory guidelines, it might be necessary for POLYVAC, it's designates, or national or foreign regulatory authorities to conduct a site audit/inspection. This could occur at any time from site initiation to after conclusion of the study. If a regulatory authority requests an inspection, the Principal Investigator must inform POLYVAC and PATH immediately about this request.

The Principal Investigator should agree to allow the auditor/inspector to have direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

12 STATISTICAL CONSIDERATIONS

12.1 Overview and General Considerations

An outline of the planned statistical analyses is provided in the following sections. Prior to a database lock, a statistical analysis plan (SAP) that contains full details of all planned analyses will be created and finalized. All statistical analyses will be performed using SAS® software version 9.4 or later.

12.2 Sample Size and Power Considerations

The sample size for the study is determined based on the regulatory requirement to submit safety data for 500 vaccine recipients receiving the new vaccine. Considering a dropout rate of 10% the study will enroll 825 infants receiving vaccines at the ratio of 2:1 (550 participants receiving the liquid formulation of ROTAVIN and 275 participants receiving the frozen formulation ROTAVIN-M1).

The non-inferiority of the liquid formulation of ROTAVIN to currently licensed frozen formulation of the vaccine (ROTAVIN-M1) will be primarily assessed by GMC of serum anti-rotavirus IgA antibody concentrations 28 days after the second vaccination in all participants enrolled at one of the two study sites.

It is anticipated that at least 375 infants (250 ROTAVIN recipients and 125 ROTAVIN-M1 recipients) at one of the two sites will provide evaluable blood samples. Considering a non-evaluability rate of approximately 15%, at least 450 participants will be enrolled at this centre. Power to show that the ratio of the GMC in the liquid formulation of ROTAVIN group to that in the ROTAVIN-M1 group is at least 0.5 was calculated using a one-sided two-sample t-test with a significance level of 0.025. Table 12.1 shows the power corresponding to different sizes of evaluable participants and assumed standard deviations (SD) of log10-transformed IgA concentrations.

Table 12.1: Power to detect at least a ratio > 0.5 of anti-rotavirus IgA GMCs between ROTAVIN liquid formulation and ROTAVIN-M1 groups 28 days after the second dose, based on various group sizes and different assumed SD of log10 IgA antibody concentrations

Assumed SD of log10 IgA antibody concentrations	Number of evaluable participants *per* group (ROTAVIN liquid formulation vs. ROTAVIN-M1)	Power to detect at least a 0.5 ratio of anti-rotavirus IgA GMCs between ROTAVIN liquid formulation and ROTAVIN-M1
0.70	250 vs. 125	97%
0.75	250 vs. 125	95%
0.80	**250 vs. 125**	**93%**

With 250 evaluable participants in the liquid formulation group and 125 evaluable participants in ROTAVIN-M1 group and an assumed SD of the log10 IgA antibody concentrations of 0.8 which was estimated from a phase 3 clinical trial for ROTAVIN-M1 conducted in Vietnam, we will have 93% power to demonstrate the non-inferiority of ROTAVIN liquid formulation to ROTAVIN-M1 in terms of GMC using a one-sided two-sample t-test with a non-inferiority margin of 0.5 and a significance level of 0.025.

With the proposed sample size of 550 participants receiving the liquid formulation of ROTAVIN, this study is designed to have 95% power to detect at least one solicited post-vaccination reaction after each vaccination if true incidence is 0.55%.

The percentage of participants with at least one solicited post-vaccination reaction will be compared between the two vaccine groups after each vaccination. The minimum difference in percentage of participants with a solicited post-vaccination reaction between ROTAVIN liquid formulation and ROTAVIN-M1 groups that can be detected with 90% power is provided in Table 12.2, based on a two-sided Fisher's Exact test with a significance level of 0.05.

Table 12.2: The minimum difference in percentage of participants with solicited post-vaccination reaction after each vaccination between ROTAVIN liquid formulation and ROTAVIN-M1 groups can be detected with 90% power

Expected percentage of participants with a solicited post-vaccination reaction in ROTAVIN-M1 group	Number of vaccinated participants per group (ROTAVIN liquid formulation vs. ROTAVIN-M1)	Minimum difference in percentage of participants with a solicited post-vaccination reaction between the groups which can be detected with 90% power
1%	550 vs. 275	4.2%
2%	550 vs. 275	5.1%
5%	**550 vs. 275**	**6.8%**
10%	550 vs. 275	8.6%
15%	550 vs. 275	9.8%
20%	550 vs. 275	10.6%

With 550 vaccinated participants in ROTAVIN liquid formulation group and 275 vaccinated participants in ROTAVIN-M1 group, the study has 90% power to detect at least a 6.8% difference in percentage of participants with solicited post-vaccination reaction between the ROTAVIN liquid formulation and ROTAVIN-M1 groups after each vaccination, assuming that the rate of the solicited reactogenicity is 5% in the ROTAVIN-M1 group and a significance level of 0.05.

12.3 Analysis Populations

12.3.1 Enrolled Population

The enrolled population is defined as all screened participants who provide informed consent and are eligible for study participation, regardless of the participant's randomization and treatment status in the study.

12.3.2 Full Analysis population

The full analysis population is defined as all participants in the enrolled population who were randomized, received a study vaccination, and provided at least one evaluable serum sample. The analysis based on this population will serve as supportive results for all immunogenicity objectives.

Participants in the Full Analysis (FA) population will be analyzed "as randomized", i.e. according the vaccine a participant was designated to receive, which may be different from the vaccine that the participant actually received.

12.3.3 Per Protocol population

The per-protocol population is defined as all participants in the FA population who correctly received study vaccine per randomization with no major protocol deviations that are determined to potentially interfere with the immunogenicity assessment of the study vaccines. This population will serve as the primary analysis population for all immunogenicity objectives.

Due to unpredictability of some irregularities, the criteria for exclusion of participants from the Per Protocol (PP) population will be determined and defined in the statistical analysis plan (SAP) which would be finalized before the database is locked. Review of PP exclusions will be performed on blinded data by the clinical operation team, statistician and medical monitor at PATH and POLYVAC.

12.3.4 Safety population

The safety population is defined as all participants in the enrolled population who received a study vaccination and had any safety data available. Participants in the safety population will be analyzed as "treated", i.e. according to the actual vaccine received at the first dose. All safety analyses will be performed using this population. The denominators for different safety endpoints may vary according to the number of participants with available data for the specific endpoint. For instance, the solicited systemic adverse event endpoints will be based only on those who have the corresponding CRF data regardless of other safety follow-up data.

12.4 Analytical Methodology

12.4.1 Analysis of demographics and other baseline characteristics

Demographic and baseline characteristics (age, ethnicity, sex, length, and weight) will be tabulated by vaccine group on the FA population. If more than 10% of the FA population is excluded from the PP population, the description and comparability of the vaccine groups at baseline will be repeated on the PP population.

Continuous variables, such as age, length, and weight, will be described as number of participants, mean, standard deviation (SD), minimum, median, and maximum. Categorical variables, such as ethnicity and sex, will be described by number of participants and percentage for each vaccine group. Group comparison will be performed to confirm whether the vaccine groups are similar with regard to demographic and baseline characteristics, using t-test or Fisher's exact test as appropriate.

Medical history will be tabulated by Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC) and preferred term for each vaccine group. Medications taken since birth will be summarized by anatomical therapeutic chemical (ATC) classification and preferred drug name coded by WHO Drug Dictionary (WHO DD) for each vaccine group.

12.4.2 Analysis of primary objectives

Analysis of primary immunogenicity objective

The GMCs of serum anti-rotavirus IgA antibody concentrations at 28 days after the second dose of ROTAVIN or ROTAVIN-M1 will be calculated along with its two-sided 95% CI, by exponentiation of the corresponding log10-transformed mean and its two-sided 95% CI limits.

To compare the immunogenicity of ROTAVIN and ROTAVIN-M1 at 28 days following the second dose of the study vaccine, the following hypothesis will be tested,

H_0: $GMC_{ROTAVIN} / GMC_{ROTAVIN\text{-}M1} \leq ½$

H_1: $GMC_{ROTAVIN} / GMC_{ROTAVIN\text{-}M1} > ½$

The test will be done at 28 days following the second dose of ROTAVIN and ROTAVIN-M1 and will be conducted with one-sided with a type I error rate of 0.025. The ratio of the post-vaccination anti-rotavirus IgA GMCs between the ROTAVIN and ROTAVIN-M1 groups will be provided with its two-sided 95% CI. The log_{10}-transformed anti-rotavirus IgA concentrations will be used to construct a two-sided 95% CI for the mean difference between the two study groups using t-distribution. The mean difference and corresponding 95% CI limits will be exponentiated to obtain the GMC ratio and the corresponding 95% CI. If the lower limit of the 95% CI of the ratio of GMCs between the ROTAVIN and ROTAVIN-M1 groups is larger than 1/2, the liquid formulation of ROTAVIN is considered to be non-inferior to the licensed frozen formulation of the vaccine (ROTAVIN-M1). Serum anti-rotavirus IgA antibody concentration below the lowest limit of quantitation (LLOQ) (i.e. below the starting dilution of assay recorded as "<LLOQ") will be set to half that limit (i.e. LLOQ/2). In case of that the concentration is above the upper limit of quantitation (ULOQ), an actual value reported will be used.

The comparison of the GMCs between the two study vaccines will also be performed using analysis of covariance (ANCOVA) method with log_{10}-transformed anti-rotavirus IgA concentrations as the dependent variable, the vaccine group as the explanatory variable, and log_{10}-transformed baseline concentrations as a covariate. This adjusted analysis will be considered as supportive.

Analysis of primary safety objective

The number and percentage of participants experiencing solicited post-vaccination reactogenicity (fever, diarrhea, vomiting, decreased appetite, irritability, decreased activity level) during the 7-day period after each vaccination will be tabulated by vaccine group and severity. For the percentage, an exact two-sided 95% CI will be provided using Clopper-Pearson method.

The percentage of participants with at least one solicited post-vaccination reactogenicity between ROTAVIN and ROTAVIN-M1 groups after each vaccination will be compared using a two-sided Fisher's Exact test with a significance level of 0.05.

12.4.3 12.4.3 Analysis of secondary objectives

Analysis of secondary immunogenicity objectives

The percentage of participants with seroconversion and seropositivity 28 days after the second vaccination will be computed for the ROTAVIN and ROTAVIN-M1 groups along with exact two-sided 95% CIs based on Clopper-Pearson method. The difference in the percentage between the two groups will be provided along with its two-sided 95% CI obtained by Miettinen and Nurminen method.

In addition, a reverse cumulative distribution (RCD) curve for anti-rotavirus IgA concentrations will be created by vaccine group and visit.

Analysis of secondary safety objectives

The number and percentage of participants experiencing immediate adverse events within 30 minutes after each vaccination, unsolicited AEs four weeks after each vaccination and SAEs including intussusception reported through 4 weeks after the last vaccination will be provided by vaccine group, severity, and causality. For the percentage, an exact two-sided 95% CI will be provided using Clopper-Pearson method.

The original verbatim terms used by investigators to identify adverse events on case report forms (CRFs) will be coded according to MedDRA dictionary. AEs including SAEs will be summarized and classified by SOC and preferred term of the MedDRA dictionary. They will be displayed by vaccine group as both frequencies and percentages.

The analysis of unsolicited adverse events comprises the following categories:

- Any unsolicited AEs;
- Related unsolicited AEs;
- SAEs;
- Related SAEs;
- Unsolicited AEs leading to withdrawal from the study;
- Unsolicited AEs leading to withdrawal from study vaccination but remaining in the study;
- Unsolicited AEs leading to hospitalization;

- Any AEs leading to death.

All reported AEs that start after vaccination will be tabulated. If a given disease is already reported as ongoing at the first visit on the medical history pages, it will be counted and tabulated as a vaccine emergent adverse event only if it worsens after the immunization with the study vaccine. When an adverse event occurs more than once for a participant, the maximal severity and strongest relationship to the vaccine group will be counted.

SAEs and discontinuation due to AE(s) will be described in detail by vaccine group.

Data listings of all adverse events will be provided by participant.

The medications taken during study period will be coded using WHO DD. The concomitant medications will be tabulated by ATC classification and preferred drug name of WHO DD for each vaccine group.

12.4.4 Multiplicity

No multiplicity adjustment will be carried out for primary objectives according to intersection-union property. ROTAVIN and ROTAVIN-M1 will be primarily compared based on a single primary immunogenicity endpoint and a single safety endpoint, respectively.

12.4.5 Handling of Dropouts and Missing Data

Missing immunogenicity data will not be imputed and will be analyzed as if they were missing randomly.

Over the whole study period, the number and percentage of participants who withdraw from the study will be provided by vaccine group. All withdrawn participant's post-randomization will be further described regarding their time to dropout and their reasons for withdrawal. For participants who withdraw from the study, their data collected before withdrawal will be analyzed under full analysis (FA) population and safety population when applicable.

12.5 Analysis Sequence

No interim analysis is planned for this study. A final analysis on all safety and immunogenicity data will be performed after the study ends and database is cleaned and locked.

13 DATA MANAGEMENT

13.1 Case Report Form (CRF)

The eCRF for the EDC system will be developed by the Data Management CRO who will also provide training in the use of the system. Write access to the system will be limited to authorized Investigators / sub-Investigators / study staff and the system will automatically keep an audit trail of all entries and corrections in the eCRF. Read access to the participant data will be restricted to authorized staff working within the project team. The clinical data in source documents will be continuously entered directly into a 21 CFR Part 11-compliant Electronic Data Capture (EDC) system by trained and qualified study staff. The data system includes internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data for each participant will be entered directly into the eCRF from the source documents.

It is the site PIs' responsibility to ensure the accuracy, completeness, and timelines of the data reported in the participant's eCRF and any supporting documentation. All source documents

should be completed in a neat, legible manner to ensure accurate interpretation of data. Source documentation supporting the eCRF data should document the dates and details of study procedures, AEs and participant status. The study database will identify study participants only by a patient identification number and will not contain any identifying information such as name, address or personal contact information, or any other regional / state / national identification number. The site PIs/institutions will maintain all information in the eCRFs and all source documents that support the data collected from each participant in a secure area and treated as confidential material. The confidentiality of records that could identify participants must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s). For electronic CRFs, review and approval / signature is completed electronically through the electronic data capture tool. Each individual having write access to electronic CRFs must meet the CRO's training requirements and must only access the electronic data capture tool using the unique user account provided by the CRO. User accounts are not to be shared or reassigned to other individuals.

The site PI will retain all essential documents and a CD-ROM copy of the eCRF data after the study is closed and the Clinical Study Report is completed or at such time that the site no longer has access to the electronic data system.

13.2 Source Documents and Source Document Access

Source Data: All information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies. (ICH E6 section 1.51).

Prior to the start of the trial, the sponsor will determine which documents or data fields completed by the investigative team will be considered source documents and documented on a Source Documentation Table.

Source documents for this study would include but are not limited to:

- Documentation of the study eligibility evaluation.
- Signed Informed Consent Documents.
- Visit documentation that includes dates of study visits and dates of study vaccinations.
- Reported laboratory results (AEs).
- AE evaluations.
- Concomitant medications.
- Post Immunization Diary Card (PIDC).
- specimen collection logs certified copies of hospital records.
- Study visit worksheets.

For some data fields, the eCRF may be the source document. Data fields on the eCRF for which there are separate primary source documents will be carefully completed using the named source document.

Only authorized study staff and representatives of POLYVAC and study monitors authorized by POLYVAC, PATH, overseeing ethical review committees, and regulatory agencies may have direct access to source documents containing study data. Participant identification will be revealed to authorized representatives of these organizations only when necessary.

13.3 Database Management and Analysis Software

A detailed data management plan and statistical analysis plan for preparation of the final study report will be created and made final prior to database lock and unblinding.

The Electronic Data Capture System (EDC) will detect and flag univariate data discrepancies or inconsistencies to alert the Investigator / designee and provide a satisfactory resolution within the EDC. The Data Manager shall review all the discrepancies to ensure corrective and preventive actions. In addition, the Medical Reviewers will also review data for medical inconsistencies and generate manual queries if required or queries can be routed through data management following medical review. After completion of data coding and resolution of all the queries in the database, the database will be declared as complete and accurate and will be locked for final statistical analysis.

Analysis and reporting of the created study database will be done by the trial statistician at the Data Management CRO using SAS® software Version 9.4 or later.

Medical history and AEs will be coded using MedDRA dictionary Version 15.1 or higher. All medication histories will be coded using latest available version of WHO drug dictionary (WHO DD). The frequency count and percentage of participants will be summarized according to the coded terms of system organ class and preferred term. Participant-wise data listings will be provided.

13.4 Entering, Cleaning, and Management of the Database

Procedure of entering, cleansing, and management of the database will be developed and implemented by Contract Research Organization (CRO). CRO will perform data management, data analysis, and report writing. A Data Management Plan will be drafted by CRO and submitted to POLYVAC and PATH for approval before implementation.

13.5 Source Data Verification

For source data verification (SDV), the monitor (on behalf of the study Sponsor) must have direct access to source documents that support the data recorded, e.g., medical records, original laboratory records and ICFs. If source data are electronic, these data must be printed, signed and dated by the site PI and stored in the participant's study file. Essential documents, including ICFs, must be filed and kept in the study files.

13.6 Database Locking Procedures

A final database lock for the primary safety analysis will occur after all participants have completed all follow-up visits, review of the severity of any AEs has been performed and finalized, all data queries have been resolved to the satisfaction of the sponsor and monitoring is complete.

Immunology data will be maintained in a separate immunology database. These data will also be locked.

13.7 Potential Deviations and Method of Limiting Deviations

To further limit the potential deviations, during the study process, the most critical study activities will be based on standard operation procedures (SOPs), particularly the activities related to the study record database, copying data from source documents, data entry, and the cleaning process. All relevant team members will be provided trainings on these processes before study start.

Retraining may also be planned in case of repeated deviations from the study specific SOPs or protocol.

During the data entry process, questionable, inappropriate data will be detected by the data management system developed by the CRO with data management responsibilities, and feedback will be given to clarify the questionable data via a data clarification form (DCF). All fixes on CRF must be signed by study staff and the fix date must be recorded.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 General Considerations

All key personnel (those responsible for the design and conduct of this study) will have completed Human Subjects Protection Training, Responsible Conduct of Research (RCR) training and ICH-GCP training prior to interaction with any participants or to have access to their confidential study data.

The study will be conducted in accordance with the procedures specified in the protocol and staff will be guided by a study Manual of Procedures (MOP) or other written guidelines. Study data collection forms will be designed to guide staff on study conduct. Forms will also include areas for documenting that activities did, in fact, occur (even if these activities did not require recording of data) and that they are recorded in the appropriate sequence. All study staff must attend mandatory protocol implementation training prior to participant enrollment.

Individual SOPs will be developed and documented for key study procedures and refined/revised as necessary. These SOPs will be included in the study MOP at the site or in the laboratory.

Study data will be recorded on worksheets or source documents and then entered into the database throughout the study. After data has been entered, it will be checked systematically by data management staff according to a pre-specified data validation plan. Queries will be generated for site staff to clarify or correct throughout the study. Additionally, an audit trail will be kept of all changes to the data. All listings of the database will be reviewed and discussed for assessment of consistency and medical plausibility. After resolution of all issues, the database will be locked.

14.2 Trainings

Trainings for the research team (including the project manager, branch project manager, study coordinator, researchers, physicians, nurses, and technicians) participating in the clinical trial include basics of research and ethics, information on how to conduct the trial, the SOPs to be used in the trial, and the procedures for drug management and use.

There will be two formal trainings before study initiation for all NIHE staff and doctors and nurses from nearby hospitals who will participate in the study. The first training course on good clinical practice (GCP) and ethics is expected to last three days. The second training will be related to the research protocol, SOPs, and implementing the study.

15 ETHICS/PROTECTION OF HUMAN SUBJECTS

15.1 Ethical Standard

The study will be conducted in full conformity with the Vietnam GCP guidelines of MOH, the Declaration of Helsinki, and all of the Vietnam MOH requirements and current laws in order to ensure the best protection for study participants.

15.2 Study Risks and Benefits

Study Risks

Potential Physical Risks: The main risks of this study are risks of rotavirus vaccines to cause some side effects including development of fever, vomiting, diarrhea, cough, runny nose, irritability and rash. In babies, natural rotavirus infection can cause diarrhea, vomiting and fever. It is possible that enrolled participants may have a few looser than usual stools, cry or have a slightly higher than usual temperature for a day or two after vaccination with the attenuated rotavirus vaccine. These events are similar to those reported in other rotavirus vaccine clinical trials. These will be explained to parents in the consent form.

Serious or allergic reactions also may be possible. This risk is addressed by trying to screen out any children with known allergic reactions to vaccines in the past and who may have an allergy to one of the components of the vaccine. Should an allergic reaction occur with vaccination, the study clinic will follow the guidance/regimen issued by MOH of Vietnam for handling medical emergencies and have supportive medicines in place, in addition to trained staff.

Post-marketing studies suggest that intussusception may be associated with the licensed rotavirus vaccines at a low rate with an attributable risk for intussusception of the rotavirus vaccine between 1-7 per 100,000 vaccinated infants. The huge benefit in terms of number of lives saved as a result of excellent efficacy of these vaccines against rotavirus diarrhea outweigh the small potential risk of intussusception and therefore the vaccine continues to be recommended for immunization schedules of all countries.

Collection of blood specimens may cause some discomfort to participants. Venipuncture is sometimes associated with discomfort, pain, bleeding, bruising, redness, swelling, local hardness, and/or infection at the puncture site. This risk will be mitigated by ensuring that only study staff members adequately trained in safe drawing of blood, conduct this procedure.

To minimize risk to the participants and early identification and treatment of side effects the following assessments are planned in the protocol.

- Conduct of study procedures like administration of vaccines, safety assessment and blood draws by staff trained in these procedures and in this age group.
- Monitoring of participants closely for 30 minutes after vaccination and providing emergency care for any immediate reactions. If medical issues arise that cannot be managed by the clinic performing the study, the study doctor will refer the participant to district or provincial hospital which would have specialists and more advanced treatment modalities.
- Telephonic call and visit of heath care worker to infant’s home twice within 7 days of each vaccine dose.
- Regular follow up of all infants with severe and serious adverse events.

If any study product related adverse event occurs during the study period, POLYVAC will ensure coverage for the full cost of treatment according to the laws of Vietnam for research participants. The study site will establish an agreement with nearby medical centers to provide treatment at no cost to the participant for reactions that are not life-threatening, but nonetheless warrant medical observation or care.

Potential Risks to Participant Privacy: Personal identifiers, including name, birth date, sex, and location/address of residence will be collected and recorded on some study data collection forms. As a result, a potential risk of "loss of confidentiality" exists. To avoid this risk, participants will be assigned a unique study participant number that will be used to identify the participant and link, using a master linking document, an individual to his/her study data and/or biological specimens. Whenever feasible, use of identifiers will be avoided and the unique study participant numbers will be used instead. Case report forms (CRF) to be sent to the CRO selected for data management and will contain only unique study participant numbers to identify the participant. Paper-based records will be kept in a secure location and only be accessible to authorized personnel involved in the study. Computer-based files will only be made available to personnel involved in the study through the use of access privileges and passwords. Individual participants will not be identified in any study related reports. Study staff will treat all study data, including participant identifiers and laboratory testing results, as confidential and not to be shared with anyone unauthorized to view such data.

Biological specimens will be identified by study participant number and no personal identifiers will be utilized on any biological specimen. Biological specimens will be stored in the laboratories of PPMC during the study period and with POLYVAC subsequently. The specimens will continue to have no personal identifiers even if they are stored at another laboratory. Likewise, laboratory reports will utilize only study participant numbers.

Potential Benefits

Potential participants will have aspects of their health status screened by qualified clinicians. This screening will be free of cost to the participant's family and may provide important health information to the screening candidates.

The study will provide opportunity to the selected participant to be tested for anti-rotavirus antibodies, a test which is not commonly available. Results will be reviewed with them and shared with their personal physician, if so desired by the participant's parent/LAR.

The information sheet and the physician will educate the family about rotavirus diarrhea and its prevention and treatment.

Protection from rotavirus diarrhea is a possible benefit, although because the vaccine is experimental it cannot be guaranteed. Participants may not benefit personally through study participation. By participating in this study, participants will contribute information on the safety and immunogenicity of this new vaccine against rotavirus in Vietnam. Development of new rotavirus vaccine, such as this new formulation of ROTAVIN-M1, that is locally produced, more stable and potentially less expensive, would be a significant contribution to public health in Vietnam and regionally.

There are no other known potential benefits to the participant for participation in this trial. Given the history of good safety profiles of rotavirus vaccines, the risk-benefit ratio is considered favorable, especially considering that all participants will be under the observation of qualified medical personnel.

15.3 Financing and Insurance

PATH will fund this trial. The trial is supported by a grant from The Department for International Development (DFID) to PATH. The study vaccines will be provided by POLYVAC. Any financial engagement with the clinical study site/NIHE and POLYVAC will be regulated by a separate agreement. Financing of the study by PATH may be disclosed to study participants in the ICFs.

POLYVAC will maintain the product liability insurance to cover treatment for study related injuries. POLYVAC will also maintain employer liability insurance or shall self-insure, as necessary, to meet its liability obligations under this protocol as well as sufficient levels of all legally mandated insurance, including at a minimum: professional liability coverage for the investigator, trial team, and all other employees, contractors, and agents providing services to this trial. As applicable, POLYVAC will maintain insurance to cover any general liability and product liability to meet its obligations under Vietnam law.

15.4 Assurance of Emergency Medical Care And Care For Other Adverse Events

Study participants will be observed by qualified clinicians after each vaccination, and emergency care will be immediately available to participants who need it. If additional urgent care or resources are needed, depending on each case, the participant will be provided emergency care and transported to a hospital. The study site will inform the nearby medical centers of the study to assure admittance of those with severe or life threatening side effects requiring urgent or supportive care. The personnel at Provincial Preventive Medicine Center will be part of the study team and any participant requiring specialist treatment will be referred to the nearest district and provincial hospital. After obtaining approval from People committee, the Provincial Preventive medicine center will inform in writing officials at hospitals near each study site to assure admittance of those with severe or life threatening side effects requiring urgent or supportive care. The study will provide this care to the participant at no cost to the participant.

Most adverse events that are expected and of less severity can be handled at the study site by the clinical staff as they occur at no cost to the participant. These would be events such as bruising or swelling from blood draws, etc.

15.5 Institutional Review Boards and Independent Ethics Committee

There are several Institutional Review Boards and Independent Ethics Committees reviewing this study because of the various collaborations. No human subject research activities will be conducted without the review and approval from all relevant ethics committees of the entities involved in the study.

NIHE maintains an independent institutional review board. This study will be reviewed and approved by NIHE IRB prior to submission of Vietnam MOH IEC. The investigator is responsible for completing and submitting the clinical trial application documents to NIHE IRB.

The protocol and all amendments will have initial and continuing review and approval by an independent ethics committee (IEC) responsible for clinical trials in Vietnam. This IEC is the Vietnam MOH Ethics Committee, which is the ultimate authority for decisions related to this trial. The MOH will acknowledge the review by participating institutions' IRBs designed to ensure that their staff meet their responsibilities in conducting human subjects research. In Vietnam, the investigator is responsible for completing and submitting the clinical trial application documents to the MOH for review. All amendments will be approved by Vietnam MOH IEC before implementation, as appropriate.

In addition, the study will be reviewed by Western Institutional Review Board (WIRB) a delegate of PATH for reviewing all interventional research studies where PATH is engaged. The investigator is responsible for completing and submitting the clinical trial application documents to WIRB. PATH will support the investigator to do the submissions.

The PI or designate shall maintain copies of all application documents and forward copies of all IRB and IEC documents and approvals prior to the start of the study. The approval letters must identify all documents approved and list the study site, study investigator, protocol title, version number, and date. Date and number of the ICF as well as the date of IRB or IEC approval must also be included in approval letters. The PI will sign all approved versions of the protocol.

The investigator is responsible for notifying the IEC and all IRBs of problems related to risks for participants, according to the requirements of the IEC and each IRB.

The investigator may not change or deviate from the protocol without prior written IRB/ IEC approval of appropriate amendments, except when necessary to eliminate immediate hazards to the participants or when the changes involve only logistical or administrative aspects of the study (e.g., change of telephone number, etc.).

POLYVAC will report to the MOH and the IEC/IRBs any new information related to the study vaccine which possibly affects the safety of participants or their risk/benefit ratio for participating in this trial. Reports on the implementation of projects are to be periodically submitted to the Ethics Committee of the MOH.

The investigator will be responsible for reporting to the MOH and the IEC/IRBs when the clinical study has been completed. This must occur within 90 days after the end of experimental phase of the study (i.e., involvement of human participants in any study procedure). If the clinical study is terminated earlier than planned, the notice must be submitted within 15 days and reasons must be clearly explained.

15.6 Media Planning for the Community

Before conducting the study, a media communication plan will be developed between PATH, POLYVAC, and NIHE in case there is media interest in the study. This plan will address issues such as who are appropriate contacts for each of the trial partners in case of contact by the media; identification of appropriate spokesperson for each entity; and an Internal Q&A on the vaccine candidate, trial, partner(s), project, and other issues as necessary.

15.7 Informed Consent Process

A clinical trial involving human subjects cannot be conducted until the following factors have been fully met:

1. IEC/IRBs concludes that risk/benefit ratio is favorable.
2. Investigator provides complete information on the clinical trial to the participant's parent/LAR and has answered the participant parent/LAR questions to his/her satisfaction.
3. Participant's parent/LAR signs the written informed consent to participate in research.
4. There is a commitment to respect the individual participant's freedom and confidential information as well as the participant's physical and mental security.
5. A provision is made for health care for all participants in the case of adverse events related to the trial or study vaccine.

6. Participant's parent/LAR are provided full contact information of the investigator should he/she request more information or need to report any health concerns he/she may have during the trial.

Written informed consent of the participant's parent/LAR must be obtained before performing any trial procedures. ICFs will embody the elements of consent as described in the Declaration of Helsinki and the ICH Harmonized Tripartite Guidelines for Good Clinical Practice.

Parent/LAR of potential participants will typically be approached during routine child immunization clinics by study staff with general information about the study. This dissemination of study information may be completed in groups. Motivated parents/LAR will then be provided a copy of the consent form in local language and allowed ample time to read the consent form. Then, they will be provided one on one study information going through details including the following issues prior to obtaining their informed consent to participate in the study:

- The purpose of research, time duration that participant need to participate, procedures involved in the study, scientific evidence that justifies conducting this experimental trial in humans, and the potential risks and known benefits of participating in the research.
- Randomization and what chances the participant have of receiving each of the study vaccines
- That study staff will not know which study product (liquid formulation of ROTAVIN or ROTAVIN-M1) the participant will receive and that the study staff and participant parent/LAR will have no way to choose which product is received by the participant.
- That the child's participation is voluntary and refusal to participate will not result in any fine, loss of rights, or access to medical care that the participant is normally entitled receive.
- In the event of unforeseen circumstances or needs, the investigator may decide to withdraw the participant from continued participation in the research, even without the consent of the parent/LAR.
- That the participant's parent/LAR will be provided with the results of any new important findings related to the trial or the study vaccine which may influence the participant's parent/LARs decision whether or not to continue participation in the research.
- The number of other participants participating in the research.
- Who to notify as the point of contact with the investigator and IRBs in case the parent/LAR would like to know more information regarding the trial and his/her rights as research participants parent/LAR.
- That the investigator is responsible for collecting signed and dated written informed consent forms from the participant's parent/LAR regarding participation in the study before participation is allowed; that he/she will be given a signed and dated copy of the form to keep; and that the investigator must keep an original signed and dated copy of individual's signed and dated written informed consent form in the investigator's research files.

They will be encouraged to ask questions about the study, have the questions answered and then be given time to decide if s/he would like to have her/his child participate in the study. It will be emphasized that participation is voluntary, and that the parent/LAR has the right to decline to participate or subsequently withdraw from the study at any time without prejudice. Parent/LAR will be made aware that authorized representatives of health agencies and POLYVAC and its designee, PATH will have access to their confidential medical information for the purposes of monitoring trial conduct or performing audits.

If the parent/LAR agree to participate in the study they will be asked to sign the consent form. Original ICFs must be kept on file by the investigator for possible inspection by regulatory authorities or POLYVAC. The parent/LAR must receive a copy (or second original) of the signed and dated ICF(s), and any subsequent updates or amendments to the ICF. The study monitor shall check the documentation of the individual ICFs during each monitoring visit.

Parents will be informed that they will be compensated for their time and effort for participation in this trial, travel, and meals during study visits. For each study visit, each participant's parent/LAR will be paid a set amount of 300,000 VND that is the equivalent of about 13.5 US dollars.

15.8 Inclusion of Women, Minorities and Children

Enrollment in this study is open to healthy children of any gender and race or ethnicity whose parent is able to read the study consent and complete the participant diaries. No child may be denied enrollment based on gender or race or ethnicity. The investigator may enroll different races and ethnicities in proportion to their presence in the local population; however, no special recruitment methods will be used to ensure certain levels of participation by any specific minorities residing in the source population. Enrollment will be closed when 825 participants have been screened and determined eligible for entry into the trial.

The trial is open to children 60-91 days of age (both days inclusive) only.

15.9 Participant Confidentiality

15.9.1 Confidentiality of Data

By signing the protocol, the Principal Investigator agrees that the study protocol, documentation, data, and all other information generated regarding the vaccines will be held in strict confidence. The investigator may divulge such information within regulatory restrictions and ethical considerations only to ethical review committees or similar expert boards or committees, and their affiliated institutions and employees, only under an appropriate understanding of confidentiality with such board or committee, and their affiliated institutions and employees. No information concerning the study or the data may be released to any unauthorized third party without prior written approval of POLYVAC. Any regulatory agency deemed appropriate, may consult study documents in order to verify CRF data. Investigators will ensure that all employees involved in the study respect the same rules.

Medical information about individual participants obtained during the course of this study is confidential and may not be disclosed to third parties, except authorized monitors, auditors, or inspectors, or as a requirement by law. Confidentiality will be ensured by the use of study participant numbers for the identification of each participant; these study participant numbers will also be used for participant data in the participant files at the site and for the CRFs.

15.9.2 Confidentiality of Participant Records

Participant confidentiality is strictly held in trust by the participating investigators, their staff, PATH and POLYVAC. This confidentiality is extended to cover testing of biological specimens in addition to the clinical information relating to participating participants.

Study participants should not be identified by name on any data collection form or on any other documentation sent to POLYVAC and will not be reported by name in any report or publication resulting from data collected in this study.

Documents and data pertaining to the study will be kept in a locked room or in locked files under the responsibility of the Principal Investigator. POLYVAC/PATH or their designee will conduct periodic monitoring visits to ensure that the data is stored securely. Only study clinicians and study staff will be granted access to the study data and records. Study data will be kept for 15 years after completion of the study, in compliance with Vietnamese law. All study records will be kept confidential to the extent provided by national and local laws. All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with access limited to study staff. Data collection, process, and administrative forms, laboratory specimens, and other reports will be identified by a coded number only to maintain participant confidentiality. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by code number. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link Participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. Participants' study information will not be released without their written permission, except as necessary for monitoring.

The investigators will keep individual data confidential to the extent permitted by law. Information will not be released to anyone other than the participant unless required to do so by law or directed by the participant (e.g., to release information to his or her health care provider). Medical records containing identifying information may be made available for review when the study is monitored by the sponsor/designee or an authorized regulatory agency. This may include examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study.

When appropriate and to the extent possible, study procedures will be conducted in private to protect participant privacy and confidentiality.

15.10 Sharing of Study Result

15.10.1 Sharing of Study Results with the Participant

All results of rotavirus immunological testing available should be shared with each participant's parent or made available for review by the parent. For the rotavirus immunologic results, the participant will be told that the results are not confirmed to provide protection against rotavirus disease because the anti-rotavirus IgA antibody concentration do not correlate with protection; however, if he or she would like the results, the Principal Investigator will provide them.

15.10.2 Sharing of Study Results with the Community

The results of this study will be published in peer-reviewed international and national scientific journals to share information with the international community.

15.10.3 Incidental Health Findings

The investigator may release participant clinical results data to the participant's primary care physician only if the parent agrees in writing to this action. The clinical staff will share and discuss

any incidental health findings with the participant's parent/LAR and help the participant seek proper medical follow-up.

15.11 Study Discontinuation

Study discontinuation is not expected to occur. However, POLYVAC retains the right to temporarily suspend or prematurely discontinue this study at any time related to safety, administrative, or other reasons including but not limited to the following:

- Risk to participant's safety.
- AEs occur with such severity and frequency that the proposed schedule can no longer be adhered to.
- The scientific question is no longer relevant or the objectives will not be met (i.e. slow accrual).
- Failure to comply with GCP or terms of Clinical Trial Agreement.
- Risks that cannot be adequately quantified.
- Ethical concerns raised by the local community or local medical care / health care authorities.
- Failure to remedy deficiencies identified through site monitoring, substandard data or failure to meet identified Sponsor performance standards.
- The manufacturer decides to discontinue the development of the formulation.
- It becomes apparent that participant enrollment is unsatisfactory with respect to quality and / or quantity.
- Data recording is inaccurate and / or incomplete on a chronic basis.

Documentation explaining premature termination of the study must be forwarded to the Site, Regulatory Authority, and Ethics Committee in accordance with local guidelines. If the study is stopped or suspended prematurely, a summary report will be submitted by POLYVAC to inform the regulatory authorities and the ethics committee/ Institutional Review Board overseeing the study about the decision and the reasons for termination or suspension. The summary report will provide a brief description of the study, the number of participants exposed to the vaccine, dose and duration of exposure, details of adverse drug reactions if any, and the reason for discontinuation of the study. If such action is taken, all efforts must be made to ensure the safety of the participants enrolled in the study. If the study is discontinued for safety reasons, participants parent/LAR will be informed of the reasons for discontinuation and of the implications/potential consequences for the participant. For all participants enrolled in the study, safety follow-up will be conducted as decided by the SRC or as advised by the EC/IRB or the MOH. In case of premature study or study clinic closure, the monitor will conduct all activities as indicated in the close out visit.

15.12 Future Use Of Stored Specimens

Biological specimens will be stored until the vaccine is approved (or for a period of 5 years after the trial) at POLYVAC. Stored samples will be used only if a retesting is required and for assay development related to Rotavirus. No genetic testing will be performed on samples collected in the study. Parent/LAR of participants do not have to agree to storage and future use of specimens to be in the vaccine study. In addition, they can change their mind and notify the study site, which will contact POLYVAC for destruction of the specimens for that participant. After this period,

these samples will be destroyed after written communication from POLYVAC/PATH. Remaining samples at CCHMC will be destroyed within 1 year of CSR submission.

15.13 Study Record Retention

Study data will be kept for 15 years after completion of the study. No records will be destroyed without the written consent of POLYVAC. Storage of all trial-related documents will be such that confidentiality will be strictly maintained to the extent required by local law.

16 PROTOCOL MODIFICATIONS AND AMENDMENTS

The protocol will not be amended without prior written approval from POLYVAC/PATH. In case the protocol or ICF is amended, the Investigator will submit and, where necessary, obtain approval from the all IRBs and IEC overseeing the study, before implementation of the same. Submission and approval of the amended protocol to Vietnam MOH and IRBs will be the responsibility of the PI.

16.1 Administrative Modifications

Administrative or technical modifications (like change in study team or telephone numbers), which do not have an impact on the participant's health or influence study outcome will be communicated in writing and filed to NIHE IRB, MOH and WIRB (through PATH) as an amendment/addendum to the protocol by the investigator.

16.2 Clinical Modifications

Modifications affecting or interfering with the participant's health interests and involving changes in the design of the study or its scientific significance or quality or safety will require protocol amendments and new approvals by NIHE IRB, Vietnam MOH and WIRB (through PATH). The Sponsor, PATH and Investigator will agree to implement / adhere to such modifications only after written approval from the concerned authorities.

These requirements for approval should in no way prevent any immediate action from being taken by the Investigator in the interests of preserving the safety of all participants included in the trial. If an immediate change to the protocol is felt to be necessary by the Investigator and is implemented by him for safety reasons, the Sponsor and PATH should be notified and the IEC / IRB should be informed immediately.

17 PROTOCOL DEVIATIONS

Any changes from protocol-specified procedures and study-related SOPs occurring during the conduct of the trial will be documented and reported as protocol violations or deviations.

A Protocol Violation is any departure from the approved protocol, trial documents or any other information relating to the conduct of the study which may affect the safety of trial participants or the study outcomes. Examples include wrong randomization or enrollment of participants that do not meet inclusion / exclusion criteria.

A Protocol Deviation is any departure from the approved protocol, trial documents or any other information relating to the conduct of the trial that does not result in harm to the trial participants and does not significantly affect the study outcomes. Examples of deviations include a protocol

visit date outside the study visit window or an isolated incident of a missed or incomplete study procedure or study evaluation. Serious or repeated protocol violations or deviations will require assessment of the root cause and implementation of corrective and preventive action plans. They may constitute grounds to interrupt the trial at a study site.

The noncompliance may be either on the part of the participant, the investigator, or the study clinic staff. As a result of any deviations, corrective actions are to be developed by the site and implemented promptly. Trial procedures shall not be changed without the consent of POLYVAC/PATH. Deviations of the protocol will be examined on an individual basis, taking into account recorded information for the reason(s) that the deviation occurred.

It is the responsibility of the site to use continuous vigilance to identify and report deviations to CRO in a timely manner after identification. If required, reports of protocol deviations must be sent to the research ethics committees overseeing the research. The PI and his/her staff are responsible for knowing and adhering to their research ethics committee's/IRB's requirements.

To limit the potential for protocol deviations, the trial site will receive training (or retraining, as necessary) on protocol implementation and will operate according to written procedures.

18 HUMAN RESOURCES

18.1 Human Resource for the Study

The study research team will be qualified by experience, education, and training to conduct their responsibilities on this study.

The study site will make arrangements with local health centers for adequately care for participants who experience side effects/illnesses that need care beyond what the study site can provide.

The research team may use commune health centers to prepare a list for potential participants, keep contact with study participant parents/LARs, conduct non-vaccination follow up visits, and conduct home visits.

18.2 Training Plan

All key personnel (those responsible for the design and conduct of this study) will have completed Human Subjects Protection and/or GCP Training and/or Responsible Conduct of Research (RCR), as appropriate to their role, prior to interaction with any participants or to having access to their confidential study data. In addition, staff will be trained on any written procedures that pertain to their role in the study.

19 CLINICAL STUDY REPORT AND PUBLICATION POLICY

19.1 Clinical Study Report

A Clinical Study Report (CSR) composed of text and results tables reflecting all safety and immunogenicity data will be generated by POLYVAC/ PATH or their designates. The CSR will be compliant with ICH E3 guidelines.

All data, documents, recordings, and information transferred to any contractor or obtained or prepared by any contractor, his consultants or persons associated by contractual relationships with any contractor during the trials, belong to POLYVAC and PATH.

All confidential information communicated to the Principal Investigator by POLYVAC/ PATH shall be kept strictly confidential by him/her or any other person connected with the study and shall not be disclosed, either orally or in written form, by him/her or such person to any third party without prior written consent of the organization of which the information is the exclusive property.

19.2 Publication Policy

It is understood by the investigators that the information generated in this study will be used by POLYVAC and PATH in connection with the development/use of the product and therefore may be disclosed to government regulatory agencies in various countries. POLYVAC and PATH also recognizes the importance of communicating study findings and therefore encourages their publication in reputable scientific journals and presentation at seminars or conferences, while protecting the integrity of the ongoing trial. Any publication, lecture, manuscripts of the findings of this study by any individual involved with the study will be governed by the procedure outlined in the Clinical Trial Agreement. Within any presentation or publication, confidentiality of individual participants will be maintained, with identification by participant code number and initials, if applicable.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry. It will be the responsibility of PATH to register this trial in an acceptable registry.

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APPENDIX A-DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, Sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and / or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the Sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and

potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and / or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, Sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, Sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

APPENDIX B-TABLE OF GRADING SEVERITY FOR CERTAIN EVENTS

The severity of all unsolicited AEs will be assessed by the investigator based on the guidance provided in Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, corrected version 2.1, July 2017, of the US National Institute of Health. The applicable symptoms and their adapted definitions has been provided below:

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Diarrhea	Liquid stools (more unformed than usual) but usual number of stools	Liquid stools with increased number of stools OR Mild dehydration	Liquid stools with moderate dehydration	Life-threatening consequences (e.g., liquid stools resulting in severe dehydration, hypotensive shock)
Vomiting	Transient or intermittent AND No or minimal interference with oral intake	Frequent episodes with no or mild dehydration	Persistent vomiting with aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Seizures	Seizure lasting < 5 minutes with < 24 hours postictal state	Seizure lasting 5 to < 20 minutes with < 24 hours postictal state	Seizure lasting ≥ 20 minutes OR > 24 hours postictal state	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
Acute Allergic Reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with intervention indicated OR Mild angioedema with no intervention indicated	Generalized urticaria OR Angioedema with intervention indicated OR Symptoms of mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR Laryngeal edema
Fever (axillary temperatures)	≥ 37.5 – ≤ 38.0°C	> 38.0 – ≤ 39.0°C	> 39.0 – < 40.0°C	≥ 40.0°C

Injection Site Tenderness	Tenderness causing no or minimal limitation of use of limb	Tenderness causing greater than minimal limitation of use of limb	Tenderness causing inability to perform usual social & functional activities	Tenderness causing inability to perform basic self-care function OR Hospitalization indicated
Injection Site Erythema or Redness *Report only one*	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Injection Site Induration or Swelling *Report only one*	Same as for Injection Site Erythema or Redness	Same as for Injection Site Erythema or Redness	Same as for Injection Site Erythema or Redness	Same as for Injection Site Erythema or Redness

Grade 5/Death- All AEs leading to death are Grade 5 events.