**Protocol Number: CVIA 066**

# An Open-label, Randomized, Controlled, Single Centre, Phase IIb Study to Assess the Immunogenicity, Reactogenicity and Safety of Three Live Oral Rotavirus Vaccines, ROTAVAC®, ROTAVAC 5CM and Rotarix® in Healthy Zambian Infants.

Abbreviated Title: Study of BBIL's ROTAVAC® and ROTAVAC 5CM vaccines in Zambia

**Trial Registration:** NCT03602053

**Investigator:** [redacted] Centre for Infectious Disease Research in Zambia, Zambia

**Sponsor:** Centre for Infectious Disease Research in Zambia, Zambia

**In Collaboration with:** PATH, USA

**Pharmaceutical Support:** Bharat Biotech International Ltd, India

**Funder:** PATH, USA

**Protocol Version Number:** 01
**Version Date:** 11 July, 2018

**Confidentiality Statement**

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Abbreviated Title: Study of BBIL's ROTAVAC® and ROTAVAC 5CM vaccines in Zambia

**Trial Registration:** Submission in process

**Investigator:** [redacted] Centre for Infectious Disease Research in Zambia, Zambia

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**Confidentiality Statement**

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## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| AE | Adverse Events |
| AEFI | Adverse Events Following Immunization |
| AGE | Acute Gastroenteritis |
| AIIMS | All India Institute of Medical Sciences |
| ART | Anti-Retroviral Treatment |
| ANC | Acid Neutralizing Capacity |
| BBIL | Bharat Biotech International Limited |
| BMGF | Bill & Melinda Gates Foundation |
| CCHMC | Cincinnati Children's Hospital Medical Center |
| CCID | Cell Culture Infectious Dose |
| CDC | Centers for Disease Control |
| CDSCO | Central Drugs Standard Control Organization |
| CFR | Code of Federal Regulations |
| CI | Confidence interval |
| CIDRZ | Centre for Infectious Disease Research in Zambia |
| CIOMS | Council for International Organizations of Medical Sciences |
| CM | Centimeter |
| CMC | Christian Medical College |
| CRF | Case Report Form |
| CRO | Contract Research Organization |
| DMC | Data Management Centre |
| DBT | Department of Biotechnology |
| DMEM | Dulbecco's Modified Eagle Medium |
| DMP | Data Management Plan |
| DTwP | Diphtheria, Tetanus, Pertussis (whole cell) |
| EC | Ethics Committee |
| EDC | Electronic Data Capture |
| ELISA | Enzyme-linked immunosorbent assay |
| EPI | Expanded Program on Immunization |
| FA | Full Analysis |
| FDA | Food and Drug Administration |
| FFU | Focus Forming Units |
| GCP | Good Clinical Practices |
| GMC | Geometric Mean Concentration |
| GMFR | Geometric Mean Fold Rise |
| GMT | Geometric Mean Titer |
| GRAS | Generally Recognized as Safe |
| GSK | GlaxoSmithKline |
| HepB | Hepatitis B |
| Hib | *Haemophilus influenzae type b* |
| IEC | Independent Ethics Committee |
| IATA | International Air Transport Association |
| Ig | Immunoglobulin |
| IMP | Investigational Medicinal Products |

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|---|---|
| IRB | Institutional Review Board |
| ICH | International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use |
| IMP | Investigational Medicinal Product |
| KG | Kilogram |
| LLOQ | Lower Limit of Quantification |
| MCH | Maternal and Child Health |
| MedDRA | Medical Dictionary for Regulatory Activities |
| mg | milligram |
| mL | milliliter |
| MOH | Ministry of Health |
| MTA | Material Transfer Agreement |
| NIAID | National Institute of Allergy and Infectious Diseases |
| NHRA | National Health Research Authority |
| NLT | Not Less Than |
| NSP | Non Structural Protein |
| OPD | Out Patient Department |
| ORS | Oral Rehydration Solution |
| ORT | Oral Rehydration Therapy |
| ORV | Oral Rotavirus Vaccine |
| PIDC | Post-Immunization Diary Card |
| PP | Per protocol |
| PSRT | Protocol Safety Review Team |
| RCD | Reverse Cumulative Distribution |
| RVGE | Rotavirus gastroenteritis |
| SAE | Serious Adverse Event |
| SAGE | Strategic Advisory Group of Experts on Immunization |
| SAP | Statistical Analysis Plan |
| SAS | Society for Applied Studies |
| SCID | Severe Combined Immunodeficiency |
| SCR | Seroconversion Rate |
| SD | Standard Deviation |
| SDMC | Statistical Data Management Center |
| SIIPL | Serum Institute of India Private Limited |
| SOC | System Organ Class |
| SOP | Standard Operating Procedure (s) |
| SPC | Summary of Product characteristics |
| SST | Serum Separator Tubes |
| ST | Serum Separator tubes |
| ULOQ | Upper Limit of Quantification |
| UNZABREC | University of Zambia Biomedical Research Ethics Committee |
| VP | Viral Protein |
| VVM | Vaccine Vial Monitor |
| WHO | World Health Organization |
| WHO DD | WHO Drug Dictionary |
| ZAMRA | Zambia Medicines Regulatory Authority |

## STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- International Conference on Harmonisation (ICH) Guidance for GCP (E6)
- World Medical Association (WMA) Declaration of Helsinki – Ethical Principles for Research Involving Human Subjects (Oct 2013 or subsequent amendments)
- Applicable Rules of Zambia

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training, Responsible Conduct of Research (RCR) training and ICH-GCP training.

## PROTOCOL SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and ICH-GCP guidelines as outlined in the 'Statement of Compliance.'

Principal Investigator:

Signed: _____ Date: 11th July 2018.

Name: [redacted]

Title: Chief Scientific Officer

Sponsor Representative:

Signed: _____ Date: 11th July 2018

Name: [redacted]

Title: Head Research Operations

PATH Representative:

Signed: [redacted] Date: 11 Jul 2018

Name: [redacted]

Title: Senior Medical Officer

## KEY ROLES AND CONTACT INFORMATION

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## PROTOCOL SUMMARY

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|---|---|
| **Title** | **An Open-label, Randomized, Controlled, Single Centre, Phase IIb Study to Assess the Immunogenicity, Reactogenicity and Safety of Three Live Oral Rotavirus Vaccines, ROTAVAC®, ROTAVAC 5CM and Rotarix® in Healthy Zambian Infants.** |
| **Short Title** | Study of BBIL's ROTAVAC® vaccines in Zambia |
| **Protocol Number** | CVIA 066 |
| **Trial Phase** | Phase IIb |
| **Investigational Products** | **ROTAVAC®:** Bharat Biotech International Ltd's licensed and WHO-prequalified rotavirus vaccine, ROTAVAC® is a live, attenuated G9P[11] monovalent vaccine at a dose of 0.5mL NLT log $10^{5.0}$ focus forming units (FFU) per dose, to be administered orally. The vaccine is recommended to be stored at -20°C.<br><br>**ROTAVAC® 5CM:** Bharat Biotech International Ltd's new Rotavirus vaccine, ROTAVAC 5CM is a live, attenuated G9P[11] monovalent vaccine at a dose of 0.5mL NLT log $10^{5.0}$ focus forming units (FFU) per dose, to be administered orally. The vaccine is recommended to be stored at 2-8°C.<br><br>**Rotarix®:** GSK Biologicals' licensed rotavirus vaccine, Rotarix® is a live attenuated RIX4414 strain of human rotavirus of the G1P[8] type containing not less than $10^{6.0}$ CCID50 (cell culture infectious dose 50%) of the RIX 4414 strain of human rotavirus. 1.5 ml of the liquid vaccine administered orally. The vaccine is recommended to be stored at 2-8°C. |
| **Study Hypotheses** | **Immunogenicity**<br><br>ROTAVAC® and ROTAVAC 5CM administered as a 3-dose series induce comparable immune response in healthy African infants.<br><br>**Safety**<br><br>ROTAVAC® and ROTAVAC 5CM administered as a 3-dose series are both safe and well-tolerated in healthy African infants. |
| **Study Objectives** | **Primary Objective:**<br><br>**Immunogenicity**<br><br>To evaluate and compare the immunogenicity of ROTAVAC® and ROTAVAC® 5CM, 28 days after the last dose of the vaccine, when administered to infants in a three-dose schedule at 6, 10 and 14 weeks of age. |

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| | **Secondary Objectives:**<br>**Safety**<br>To assess the reactogenicity 7 days after each vaccination and safety 4 weeks after the last vaccination of the ROTAVAC® and ROTAVAC® 5CM, when administered to infants in a three-dose schedule at 6, 10 and 14 weeks of age and Rotarix®, when administered to infants in a two-dose schedule at 6 and 10 weeks of age.<br>**Immunogenicity**<br>To evaluate the immunogenicity of Rotarix® 28 days after the last dose of the vaccine, when administered to infants in a two-dose schedule at 6 and 10 weeks of age.<br>**Exploratory Objective**:<br>To evaluate the immunogenicity of the three vaccines by ELISA using 89-12 (G1P8 virus) as a substrate in a subset of the samples collected. |
| **Study Endpoints** | **Primary Endpoint:**<br>**Immunogenicity:**<br>Geometric mean concentrations (GMCs) of serum anti-rotavirus IgA antibodies, 28 days after the last dose of a study vaccine, as measured by enzyme-linked immunosorbent assay (ELISA) using WC3 as the viral lysate.<br>**Secondary Endpoints:**<br>**Safety**<br>The secondary safety endpoints will include:<br>• Immediate adverse events, within 30 minutes' post-vaccination.<br>• Solicited post-vaccination reactogenicity (fever, diarrhea, vomiting, decreased appetite, irritability, decreased activity level) during the 7 day period (Day 0-6) after each vaccination.<br>• Unsolicited AEs from first vaccination through 4 weeks after the last vaccination.<br>• SAEs, including intussusception from first vaccination through 4 weeks after the last vaccination of each study participant.<br>**Immunogenicity**<br>The secondary immunogenicity endpoints (anti-rotavirus IgA using WC3 as the Lysate) will be as follows:<br>• Seroconversion rate 28 days after last dose of study vaccine (Day 84 for ROTAVAC® and ROTAVAC® 5CM; and Day 56 for Rotarix®). Seroconversion is defined as a post-vaccination serum anti-rotavirus IgA antibody concentration of at least 20 U/mL if a baseline |

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| | concentration is < 20 U/mL or a post-vaccination serum anti-rotavirus IgA antibody concentration of ≥ 2-fold baseline level if a baseline concentration is ≥ 20 U/mL.<br>• Seropositivity rate at baseline and 28 days after last dose of study vaccine (Day 84 for ROTAVAC® and ROTAVAC 5CM and Day 56 for Rotarix®). Seropositivity is defined as serum anti-rotavirus IgA antibody concentration ≥ 20 U/mL.<br>• Seroresponse rate 28 days after last dose of study vaccine (Day 84 for ROTAVAC® and ROTAVAC 5CM and Day 56 for Rotarix®). Seroresponse will be assessed as a four-fold, three-fold and two- fold rise in antibody concentration from baseline.<br>• Geometric Mean Fold Rise (GMFR) that is a ratio of GMCs at 28 days after last dose of study vaccine (Day 84 for ROTAVAC® and ROTAVAC 5CM and Day 56 for Rotarix®) with reference to baseline.<br>**Exploratory Endpoints:**<br>The exploratory endpoints based on anti-rotavirus IgA antibodies measured by ELISA using 89-12 as the viral lysate in a subset of samples will be as follows:<br>• GMCs of serum anti-rotavirus IgA antibodies 28 days after the last dose of study vaccine.<br>• Seroconversion rate 28 days after last dose of study vaccine. Seroconversion is defined as a post-vaccination serum anti-rotavirus IgA antibody concentration of at least 20 U/mL if a baseline concentration is < 20 U/mL or a post-vaccination serum anti-rotavirus IgA antibody concentration of ≥ 2-fold baseline level if a baseline concentration is ≥20 U/mL.<br>• Seropositivity rate at baseline and 28 days after last dose of study vaccine. Seropositivity is defined as serum anti-rotavirus IgA antibody concentration ≥ 20 U/mL.<br>• GMFR that is a ratio of GMCs at 28 days after last dose of study vaccine with reference to baseline. |
| **Study Design** | • This study is designed as a Phase IIb, single center, randomized, controlled, open label study with 3 groups of infants (n=150 per group) receiving either three doses of ROTAVAC® three doses of ROTAVAC 5CM or two doses of Rotarix®. 450 participants will be randomized (1:1:1) to receive ROTAVAC®, ROTAVAC 5CM or Rotarix®. The three doses of ROTAVAC® and ROTAVAC 5CM will be administered at 6, 10 and 14 weeks of age whereas two doses of Rotarix® will be administered at 6 and 10 weeks of age. All vaccines will be administered concomitantly with EPI vaccines including Diphtheria, Tetanus, Pertussis, *Haemophilus influenzae* |

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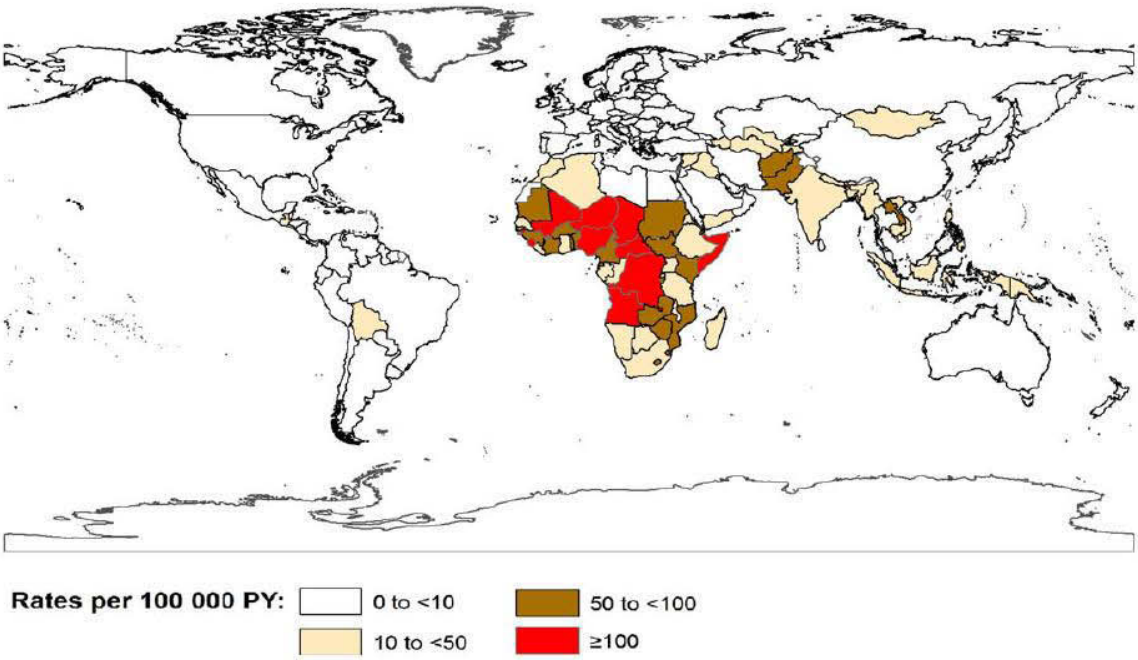
| | *type b* and Hepatitis B vaccine (DTwP-Hib-HepB), Pneumococcal conjugate vaccine and OPV at 6, 10 and 14 weeks and IPV at week 14 (when switched to in Zambia). The participants will be monitored for 30 minutes following vaccine administration for immediate adverse events.<br>• A blood sample will be obtained from all the participating infants before first vaccination and four weeks after the last vaccine dose. This would mean that the blood sample will be collected at approximately 14 weeks of age for infants in the Rotarix® arm and 18 weeks for infants in the ROTAVAC® groups.<br>• Enhanced passive/Active surveillance for vaccine reactogenicity (solicited reactions) over the 7-day period after each vaccination will be conducted on all infants. In addition, surveillance for unsolicited AEs, SAEs including intussusception will be carried out over the period between first vaccination and four weeks after the last vaccination on all infants.<br>• The study will compare the immunogenicity of the two formulations of ROTAVAC® i.e. ROTAVAC® vs. ROTAVAC 5CM and will descriptively analyze the immune response to Rotarix®. |
| **Study population** | 450 infants (150 per arm), 6-8 weeks of age will be enrolled in the study. All enrolments will be done in a single centre in Zambia. |
| **Number of participating sites** | One. At the CIDRZ Research Unit at George Health Centre in Lusaka |
| **Study Duration** | Approximately 12 months |
| **Participant Duration** | 3 - 4.5 months per participant including follow up period of 28 (+7) days after the last vaccination. |

# 1 BACKGROUND AND RATIONALE

## 1.1 Burden of 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. The largest number of rotavirus deaths occurred in sub-Saharan Africa, where the number is estimated at 121 000 (range, 111 000–131 000) deaths in 2013. Rotavirus deaths decreased at a slower rate in sub-Saharan Africa than in the other regions, resulting in an increasing proportion of all rotavirus deaths occurring in this region from 47.3% in 2000 to 56.3% in 2013 [1]. More than 90% of rotavirus deaths occurred in countries eligible for Gavi support. [2]

Figure 1. Mortality rate due to rotavirus disease among children <5 years of age, by country, 2013.




Diarrhea is the third leading cause of child death in Zambia. Up to one-third of diarrhea cases resulting in hospitalization and/or death are caused by vaccine-preventable rotavirus.[3] In line with the world averages, in 2013, Zambia reported a total of 4563 diarrhea related deaths of which 38.2% diarrheas were positive for rotavirus. This corresponds to approximately 3.4% of all-cause mortality in children less than 5 years of age.[4]

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.[5] Studies conducted in Zambia have reported seropositivity rate in approximately 25% of the infants prior to vaccination.[6]

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. The most effective antiseptics against rotavirus are alcohols, which have been found to reduce the number of viruses on the hands by greater than 99%. However, tap water alone, or tap water with regular soap reduces the titer by only 72-84%. 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). 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 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)[7] 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.

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%). [8-12] In countries in Africa which had introduced rotavirus vaccination, the African rotavirus surveillance network has reported a significant drop in the proportion of hospitalized rotavirus diarrhea among children less than five years of age. The surveillance reported a direct correlation of this effect with the time when rotavirus vaccine was introduced. In the countries that introduced rotavirus vaccine by 2013 (Rwanda, Tanzania, Zambia and Ethiopia), average rotavirus vaccine coverage reached up to 90% in 2015 and rotavirus positivity decreased from 35% in 2010 to 19% in year 2015. Understandably, the coverage was lower in year 2015 in countries which implemented vaccination after year 2013 with only 51% in year 2015 but in these countries too, the average rotavirus positivity decreased from 41% in 2013 to 25% in 2015 hence proving the advantages of implementing rotavirus vaccination in programmatic settings. Rotarix® was introduced in the routine public health immunization program in Lusaka, Zambia, in January 2012 and was rolled out countrywide in November 2013. A study in Lusaka Zambia reported that following rotavirus vaccine introduction, seasonal peaks of rotavirus and all-cause AGE were smaller. The study observed a significant reduction in AGE-associated in-hospital morbidity and mortality following

rotavirus vaccine introduction. The greatest reduction was seen in infants <1 year who accounted for 84.4% of rotavirus hospitalizations prior to vaccine introduction.[13] Another case control study in Zambia reported a Vaccine Effectiveness of around 26% against rotavirus diarrhea of all severity and around 56% against hospitalized children concluding that rotavirus vaccines are more effective against disease of increased severity of illness compared with milder disease.[3]

Three rotavirus vaccines, Rotarix® (GSK), RotaTeq® (Merck) and ROTAVAC® (BBIL) are currently WHO prequalified with Rotarix® and RotaTeq® being widely available. One other vaccine, ROTASIIL® (SIIPL), has been licensed in India and may be evaluated for WHO prequalification. 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. To address this, it is critical that a mature market for rotavirus vaccines, including manufacturers from developing countries, develops as quickly as possible. At US$1 per dose, initially, ROTAVAC® is substantially less expensive than other available rotavirus vaccines.

## 1.2 Pathogen

Rotavirus was identified in 1973 from the small intestine's epithelial cells of children who are affected by diarrhea.[14] Humans are mainly infected by species A, B, and C among the available eight species (A, B, C, D, E, F, G, and H), but more than 90% of diarrheal infections are caused by species A.

The rotavirus genome, shelled with three proteins (a core, inner membrane, and outer capsid), contains an 11-segmented double-stranded genome. Both structural proteins (VP1–VP4, VP6, VP7) and non-structural protein (NSP1–NSP4) are encoded by rotaviral RNA gene segment, but only gene segment 11 codes for NSP5 and NSP6. 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 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. It is suspected that clinical protection probably involves mucosal (intestinal), systemic antibody response, and cell-mediated immunity, however it is difficult to realize the mechanism of protection, as well as duration of protection against rotavirus infection.[15,16]

The exact mechanism of rotavirus infection within the public health community is relatively unknown[17],[18] Probably, rotavirus diarrhea is multi-factorial, with malabsorption and secretion components, and may have other components suggested to be related to villus ischemia and intestinal motility. The rotavirus mostly prefer to infect during cool and dry seasons with infection showing peaks in winter to cause diarrhea.[19,20]

The molecular mechanisms underlying the rapid induction of heterotypic protective immunity to rotavirus, which provides the basis for the efficacy of licensed monovalent rotavirus vaccines, have

remained unknown. Epidemiologic and clinical studies demonstrate that heterotypic protective immunity is generated after a single rotavirus infection[21] or immunization.[22,23]

## 1.3 Description of Study Vaccine

Several studies have documented that the first rotavirus infection in infants protects against severe diarrhea during reinfection [21,24]. In the mid-1980s, neonates born at the All India Institute of Medical Sciences (New Delhi) were commonly infected with a rotavirus strain before hospital discharge that was later encoded 116E. These infants remained asymptomatic and experienced 46% fewer episodes of rotavirus diarrhea than did a cohort of babies born at the same institute who were not neonatally infected. A greater reduction in the severity of diarrhea was observed in the neonatally infected than the non-infected neonates. Because infection with strain 116E did not cause diarrhea in newborns and was not found in older patients with diarrhea, it appeared to be naturally attenuated. Furthermore, the strain grew well in the infant gut, induced rotavirus-specific immunoglobulin (Ig) A or IgM antibody responses, and offered clinical protection [25,26]. While rotaviruses do not commonly infect infants in the first few months of life, this strain was unique in that it grew in the presence of transplacental antibodies from the mother[27] Human neonatal strains similar to Rotavirus 116E given alone are likely to protect children against a variety of rotavirus strains of different serotypes.[28]

Bharat Biotech International Limited (Hyderabad, India) developed the oral rotavirus vaccine (ORV) 116E to address the need for a safe, effective, and affordable vaccine for India and the developing world as a part of the Indo-US Vaccine Action Program and with technical support from the Department of Biotechnology (India), the US Centers for Disease Control and Prevention (CDC), the National Institutes of Health, Stanford University, and PATH.[29] The vaccine was successfully developed and named as ROTAVAC®. Further, clinical trials in India proved the efficacy of ROTAVAC® in preventing severe rotavirus gastroenteritis. ROTAVAC® was licensed by CDSCO (National Regulatory Agency) in 2014. ROTAVAC® was initially approved as a 3-dose regimen to be given with routine childhood vaccines at 6, 10, and 14 weeks of age along with 2.5 mL citrate-bicarbonate buffer to facilitate passage through the acidic contents of the upper gastrointestinal tract.

Several studies have been carried out to examine the role of buffer on the performance of rotavirus vaccines [30-32]. Some of these studies noted that rotaviruses are moderately acid labile, and the acidic environment affects the viability of the virus [33,34] although natural transmission of rotavirus occurs via the faecal-oral route and occurs in the presence of un-neutralized gastric acid. It is also postulated that the human infant stomach with higher pH levels (approximately 3.2) compared to adults (approximately 1.0) may be more permissive for the survival of rotavirus. In addition, in humans, homologous rotaviruses (human origin rotavirus in a human) are much more (>1000 fold) infectious than heterologous rotavirus (non-human origin rotavirus in a human) and only very small quantities of homologous rotavirus (10 infectious doses or less) are generally able to cause infection, immune response and illness.[35,36] In general, the results have been found to be mixed in nature and all commercial vaccines use buffering components either as part of the formulation (RotaTeq® and Rotarix®, in some markets) or reconstituted at the time of administration (Rotasiil® in India and Rotarix® in some markets) which pose several logistic and programmatic challenges in terms of either having to separately transport and store the buffering diluent, and administer relatively large volumes (1.5 to 2.16 mL) of reconstituted or ready-to-use vaccine orally to infants.[37-39] From a vaccine administration point of view for infants, low dose volumes, typically

around 0.5 mL, are preferred. Immunization programs prefer vaccines that are "ready-to-use" and do not require reconstitution or administration of separate components, such as a diluent or a buffer, to minimize errors in administration.

Considering the human origin of 116E strain, which belongs to the G9 and P[11] genotypes and the age at immunization, it was hypothesized that the strain may replicate and the vaccine may confer immunity without a buffer administration as it was initially recovered from an asymptomatic neonate. Further, the high pH stability of the 116E strain also suggests that the challenge of premixing the vaccine with the buffer before administration could be avoided, and hence bypass any programmatic challenge when being administered along the other vaccines in The Expanded Program on Immunization (EPI).

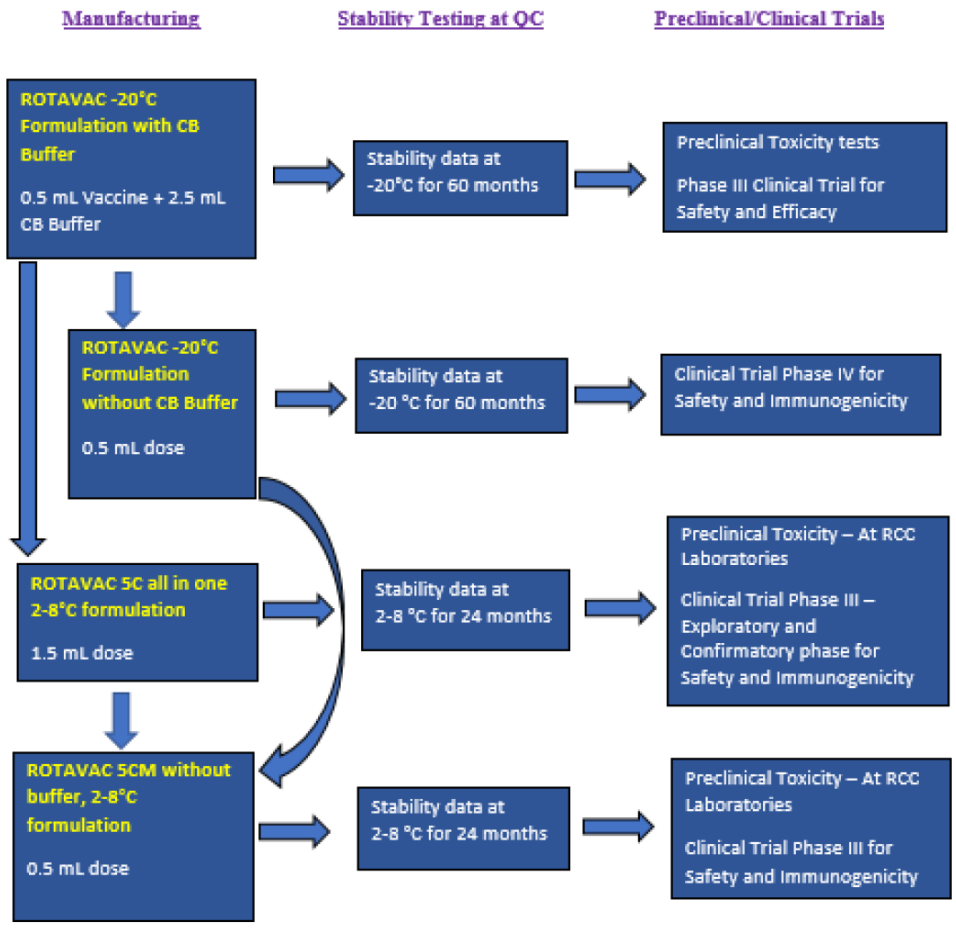
In view of this, and supported by the results of a clinical study demonstrating similar immune response achieved by ROTAVAC® when administered with and without antacid buffer, ROTAVAC® without buffer was also approved and the vaccine was licensed on 30 Apr 2015 and is now available for commercial use. ROTAVAC® has a dosing volume of 0.5 ml/dose with the inherent acid neutralizing capacity of the vaccine eliminating the need to administer an alkali buffer separately prior to the vaccine. Hence the cold chain footprint of these vaccines is markedly reduced with the cold chain volume of ROTAVAC® being between 3.2-4.2 $cm^3$/dose whereas the same is between 46.25 - 75.26 for RotaTeq® and 17.12 - 115.3 cm3/dose per vial for Rotarix®. The vaccine was still recommended to be stored at -20 °C. The Government of India has rolled out rotavirus vaccination as part of the EPI in a phased manner and ROTAVAC® is being used for this purpose in few of the states in India. Till date, the company has supplied 35 million doses to the EPI program in India. It has also initiated supply of WHO Pre-qualified the vaccine to UNICEF and other immunization programs worldwide.[40]

BBIL further undertook the development of a liquid formulation of the vaccine, ROTAVAC 5C that is stable at 2-8 °C for over 24 months thereby making it amenable for easier storage and transport. This formulation contains the same rotavirus strain (116E) as in ROTAVAC®. These formulations also have sufficient Acid Neutralizing Capacity (ANC) to neutralize the gastric juices in stomach, thus, supporting survival of the vaccine virus. The formulation was changed by addition of excipients and stabilizers belonging to the Generally regarded as Safe (GRAS) category.

Further to this and based on the ROTAVAC® clinical trial results without buffer, formulation of ROTAVAC 5C 1.5 mL was modified to 0.5mL volume and the internal buffer components were removed, which later was named as ROTAVAC® 5CM, a liquid formulation that is stable at 2-8 °C storage temperature. ROTAVAC 5CM is without buffer and has additional excipients and stabilizers (same as in ROTAVAC 5C, with established safety and immunogenicity) that belong to GRAS category and additionally any new safety concerns are not anticipated. ROTAVAC 5CM vaccine formulation also has excellent stability at 25°C and 37°C. The advantages with such liquid formulations include stability at 2-8°C which makes the vaccine amenable for easier storage and transport, and the acid neutralizing capacity which eliminates the need to administer an alkali buffer separately prior to the vaccine.

International Patents have been granted in several countries including India, USA, UK and South Africa for these ROTAVAC® vaccine formulations.

The diagram below describes the development of ROTAVAC® formulations.




### 1.3.1 Immune Responses to Rotavirus Vaccines

Challenge of adult volunteers with wild type rotavirus strains and trials of live rotavirus vaccines have shown a correlation between pre-existing rotavirus antibodies, particularly IgA, and the ability of the viruses to infect; correspondingly, serum responses after live virus challenge resulting in infection include a significant increase in antibody levels, IgA in particular. Testing antibodies in serum of infants under 4 months of age, before they receive a live vaccine, has demonstrated the presence of significant amounts of anti-rotavirus specific IgG of maternal origin, the half-life of which is such that by the fourth month such antibodies are barely detectable. On the other hand, IgA antibodies are known not to cross the placenta and therefore are not present in pre-immune serum unless natural infection has occurred. Accordingly, the induction of an IgA seroresponse is the best available tool to assess the immunogenicity of any rotavirus vaccines.

An ELISA-based anti-rotavirus IgA, developed and validated by Ward, et al., at the Cincinnati Children's Hospital Medical Center (CCHMC) and later adapted to use in multiple laboratories, has become the standard test to evaluate immune responses to rotavirus vaccine.[41] When evaluated in developed countries, all previously licensed vaccines (RotaShield®, Rotarix®, and RotaTeq®) were shown to induce a ≥3 or ≥4 fold response (depending on the study analysis) in 80% or more

of the participants with corresponding geometric mean concentrations (GMCs) ≥100. However, when tested in resource poor countries, and in parallel to their diminished efficacy in these countries, both the seroresponse rates as well as the GMCs are greatly diminished with seroresponse rates in some cases below 40% and GMCs as low as 30. Note, however, that in spite of this parallelism, no correlation has been found between IgA responses and efficacy levels. Conversely, in some studies immunogenicity rates have been higher than the efficacy rates. For instance, the seroresponse rates for Rotarix® in Vietnam study have been approximately 97% with efficacy rates of approximately 64%. A study conducted in Zambia reported a baseline seropositivity rate of around 25% in the enrolled infants and the overall seroconversion frequency to Rotarix® for IgA in the infants was 60.2%. The post vaccination GMCs observed in the study were between 63 and 112 and were dependent on the season. It was observed that seropositive infants at baseline were less likely to seroconvert compared to their seronegative counterparts (P = 0.04); and there was no evidence of an association between maternal HIV status and seroconversion (P = 0.25).[6]

### 1.3.2 Potential Safety Risks of Rotavirus Vaccines

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 ROTAVAC® 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; these occurrences have been self-contained and of easy management.[37,38]
Shortly after the introduction of RotaShield® in the US, the vaccine was associated with a very small increase in the incidence of intussusception after vaccination, particularly following the first vaccine dose in older infants. This led to withholding any subsequent use of this vaccine and put a significant burden on future rotavirus vaccine developers to demonstrate with a reasonable degree of certainty that their candidates were not associated with this outcome. Both Rotarix® and RotaTeq® underwent large safety studies in tens of thousands of children in which the occurrence of intussusception was closely followed. The high level of efficacy observed in those studies, along with the vaccine safety profile and lack of association with intussusception were sufficient to achieve licensure in many countries. Additional post-marketing studies initially suggested that intussusception might still be associated with the licensed vaccines, but at much lower rates than could have been detected in the clinical trials. A recently completed study in the US, where post-vaccination surveillance was conducted in 3-34 week old infants after the administration of over 750,000 doses of the RotaTeq® vaccine, showed a lack of association between vaccination and the occurrence of intussusception in the periods of 1 to 7 or 1 to 30 days post-vaccination.[22]

The WHO presently supports seven Rotarix® vaccine-using countries (including Zambia), and another four countries that are using the RotaTeq® vaccine, to systematically collect surveillance data on intussusception, and other Adverse events following immunization (AEFI). Tools for systematic collection of data are readily available for enhanced surveillance.[42]

## 1.4 Summary of Nonclinical Studies of Study Vaccine

Toxicology studies for oral rotavirus 116E vaccine were conducted in Swiss Albino Mice, Sprague Dawley Rat, Fischer F344 Rats and rabbits. The preclinical toxicity studies of Rotavirus 116E Formulations were conducted in RCC Laboratories, a GLP certified testing facility and CPCSEA and AAALAC Accredited animal facility. The characterization from these studies identified that the vaccine did not damage any organ structurally or functionally in animals. The Summary details of the studies performed for Oral Rotavirus 116E vaccine formulations is tabulated below:

| Study | Batch number/Dose | Composition |
|---|---|---|
| A 28-Day Repeated Dose Toxicity Study of Two Formulations of Oral Rotavirus 116E Vaccine, Live Attenuated in Swiss Albino Mice.<br><br>Dosing: Day 0, 3, 6, 9, 12, 15 and day 28 | T/F-11002<br>$10^{6.0}$ FFU/0.5mL | Sucrose 40%, Trehalose0.5%, HA 0.35%, LAH 1%, 3mM Ca, Citrate – 0.052 M, Potassium Phosphate – 0.14 M, |
| | T/F-11012<br>$10^{6.0}$ FFU/1.0mL | Sucrose 30%, Lactose 5%, HA 0.35%, LAH 0.5%, 3mM Ca, Citrate – 0.03 M, Bicarbonate – 0.3 M |
| Repeated Dose 28 Days Oral Toxicity Study with Oral Rotavirus 116Ee Vaccine, II Generation (Four Formulations) In Sprague Dawley Rat<br>Dosing: Day 0, day 7, day 14 and day 21<br><br>Repeated Dose 28 Days Oral Toxicity Study with Oral Rotavirus 116E Vaccine, II Generation (Four Formulations) in Rabbits<br>Dosing: Day 0, day 7, day 14 and day 21 | 61EZ13001<br>$10^{6.10}$ FFU/1.5 mL | Sucrose- 40%, Trehalose- 0.5%, LAH- 0.5%, HA- 0.35%, 1.1 M Potassium Phosphate |
| | 61EZ13002<br>$10^{6.15}$ FFU/1.5 mL | Sucrose- 50%, Trehalose- 0.5%, LAH- 0.5 %, HSA – 0.35%, 0.053 M Trisodium Citrate, 0.144 M Potassium Phosphate |
| | 61EZ13003<br>$10^{6.18}$ FFU/1.5 mL | Sucrose-50%, Trehalose-0.5%, LAH-1.0%, HSA-0.35%, 0.15M Trisodium Citrate, 0.3M Potassium Phosphate |
| | 61EZ13004<br>$10^{5.93}$ FFU/1.5 mL | Sucrose- 55%, Trehalose- 0.8%, LAH- 1.0%, 0.15 M Trisodium Citrate, 0.3 M Potassium Phosphate |
| | 61DA13001(Reference) | $10^{5.38}$ FFU/0.5 mL |
| 28 Day Repeated Oral Dose (Proof of Concept) Study with Oral Rotavirus 116E Vaccine, Ii Generation (Two Formulations) in Fischer F344 Rats<br>Dosing: Day 0, day 14 and day 28 | 61EZ13001<br>$10^{6.10}$ FFU/1.5 mL | Sucrose- 40%, Trehalose- 0.5%, LAH- 0.5%, HA- 0.35%, 1.1 M Potassium Phosphate |
| | 61EZ13004<br>$10^{5.93}$ FFU/1.5 mL | Sucrose- 55%, Trehalose- 0.8%, LAH- 1.0%, 0.15 M Trisodium Citrate, 0.3 M Potassium Phosphate |
| | 61DA13001(Reference) | $10^{5.38}$ FFU/0.5 mL |

In the above studies all animals in the groups were observed and examined for clinical signs, ophthalmoscopy, body weights and food consumption, clinical pathology, organ weights and histopathology of the preserved organs and tissues. The studies revealed no adverse findings in

any of the Oral Rotavirus 116E Vaccine treated animals, and all the formulations were comparable with reference test item.

In conclusion, under the conditions of these studies, 28-day repeated oral dose with Oral Rotavirus 116E formulations revealed no adverse findings in any treated animals, when compared with the placebo. The studies were initiated in 0.5 mL formulations and later on in continuation of developing formulation with buffer wherein the dose volume is 1.5 mL. The proposed ROTAVAC 5CM has similar excipients as in 61EZ13001 and T/F-11002 and differ only in the concentration. Buffer composition is similar to 61EZ13004 wherein the buffer system is citrate phosphate. Dose volume and concentration of buffer system and excipients did not affect the results of the studies. Hence it is proved that the excipients and buffer system used for ROTAVAC 5CM were tested in animal model for toxicity and it is safe. Please refer to ROTAVAC 5CM IB version 1 dated 27 December 2017 for additional details.

## 1.5 Summary of Clinical Studies of Study Vaccines

### 1.5.1 ROTAVAC® (-20°C)

The early clinical development of AGMK Vaccine 116E in adults, children and infants were a collaborative effort between the Society for Applied Studies (SAS), AIIMS, NIAID and PATH. The results of these studies, which were conducted among adults, children ages 2 to 12 years, and infants ages 6 to 9 weeks, provided the basis for BBIL to further develop the vaccine. The following are the studies that were conducted with ROTAVAC®.

**Table 1. List of clinical trials of ROTAVAC®**

| Clinical trial Phase | N | Formulation | Location | Endpoint | Status |
|---|---|---|---|---|---|
| Phase 1: adults | 30 | 116E or I321 ($10^{5.0}$FFU) or Placebo | India | Safety | Completed |
| Phase 1: children | 30 | | India | Safety | Completed |
| Phase 1: infants | 90 | | India | Safety and immunogenicity | Completed |
| Phase 1b/2a: infants | 360 | 116E, $10^{4.0}$ ffu or $10^{5.0}$ ffu or Placebo | India | Safety and immunogenicity | Completed |
| Phase 3: infants | 6800 | 116E ($10^{5.0}$ ffu) with buffer or Placebo | India | Efficacy | Completed |
| Phase 3: infants | 1356 | 116E ($10^{5.0}$ ffu) with buffer or Placebo | India | Lot-to-lot consistency and interference with childhood vaccines | Completed |
| Phase 4: infants | 900 | ROTAVAC® without buffer | India | Immunogenicity and safety | Completed |

| | | | | | |
|---|---|---|---|---|---|
| | | ROTAVAC® with buffer administered 5 minutes before ROTAVAC® with buffer administered simultaneously | | | |
| Phase 4: infants* | 464 | ROTAVAC® or Rotarix® | India | Immunogenicity and safety | Completed |
| Phase 3 Exploratory: infants* | 675 | ROTAVAC® or Rotavac 5C (1.5 ml) or Rotavac 5C (2.0 ml) | India | Reactogenicity and Safety | Completed |
| Phase 3 Confirmatory: infants* | 1,302 | ROTAVAC® or Rotavac 5C (2.0 ml), Lot 1 & 2. | India | Immunogenicity and safety | Completed |
| Phase 3: infants* | 360 | ROTAVAC® or ROTAVAC 5CM | India | Safety and immunogenicity | Completed |

*In these studies, ROTAVAC® without buffer was used

**Phase 1 Study in Healthy Adults in India**

A phase 1 randomized, double-blind, placebo-controlled, safety and immunogenicity study was conducted in healthy adults. A total of 30 volunteers aged 18-45 years received a single oral dose of either the 116E or I321 ($10^{5.0}$FFU) or Placebo according to a 1:1:1 (vaccine: vaccine: placebo) randomization. Vaccine or placebo was administered orally after consuming 2g sodium bicarbonate dissolved in 120 ml sterile water to buffer stomach acidity. The study evaluated safety and immunogenicity of the study vaccines and shedding of the vaccine virus. No vaccine-related SAE was reported. Intussusception was not reported by any participant. None of the participants shed rotavirus in stools as ascertained through antigen detection Enyzme Linked Immunosorbent Assay (ELISA). None of the participants seroconverted (i.e. had a 4-fold rise in rotavirus specific IgA). Both vaccines replicated poorly in adults and appeared to be safe.

**Phase 1 Study in Healthy Children in India**

A phase 1 study with similar study design as the adult study above was conducted in healthy children aged 2-12 years. This study also did not report any vaccine-related SAE or case of intussusception. Symptoms such as fever, diarrhea, vomiting were rare and not differentially distributed. Mild abdominal pain was more common in the study vaccine groups, but only one child required medication. Both vaccines replicated poorly in children and appeared to be safe. None of the participants shed rotavirus in stools as examined by antigen detection ELISA. One participant seroconverted i.e. had a 4-fold rise in rotavirus specific IgA in the 116E group; the baseline concentration in this child was low.

**Phase 1 Study in Healthy Infants in India**

This study was a phase 1 randomized, double blind, safety and immunogenicity study of live, attenuated neonatal rotavirus vaccine candidate strains 116E or I321 in healthy non-malnourished infants aged 8-12 weeks. The study enrolled 90 infants with 30 infants each receiving 116E or I321 vaccines ($10^{5.0}$ fluorescence focus units, ffu in 1 ml) or placebo. UIP vaccines were not administered concomitantly. The study evaluated safety and immunogenicity of the study vaccines

and shedding of the vaccine virus. Expected adverse events such as fever, diarrhea associated with vomiting and diarrhea not associated with vomiting were similar in both groups. Virus was shed on day 3 or 7, by two out of 30 infants in the placebo group, one out of 29 in I321group and 12 out of 30 in 116E group infants. In the per protocol analysis, the proportion of infants showing ≥4 fold rise in IgA antibody during 28 days post administration of vaccine/placebo was five out of 30 (16.7%) in the placebo group, nine out of 28 (32.1%) in I321 group and 16 out of 30 (53.3%) in 116E group. The study concluded that 116E strain is attenuated, clinically safe and highly immunogenic with a single dose.[43]

**Phase 1b/2a Study in Healthy Infants in India**

A frozen (-70ºC) Rotavirus 116E candidate vaccine produced by BBIL supported by Department of Biotechnology (DBT) and PATH was tested in a dose ranging study for safety and immunogenicity in infants 8-20 weeks of age. The randomized, double blind placebo-controlled trial tested two doses of the ORV 116E, $10^{4.0}$ ffu and $10^{5.0}$ ffu. Infants received a dose of either vaccine at 8, 12, and 16 weeks, separately from routine vaccines. There were no vaccine-related serious adverse events, and no significant differences in clinical adverse events or laboratory toxicity were observed between vaccine and placebo recipients. The seroconversion rates with the $10^{5.0}$ ffu dose were 64.5% vs 23.3% with a single dose and 89.7% and 28.1% after three doses in the vaccine and placebo groups, respectively. These were significantly higher than the lower dose. These results led to selection of three doses of the vaccine candidate in a dose of $1X10^5$ FFU/dose for the phase III efficacy study. [44]

**Phase 3 Study in Infants in India**

The frozen (-20ºC) Rotavirus 116E candidate vaccine produced by BBIL supported by Department of Biotechnology (DBT) and PATH has been tested in an efficacy study in infants. The phase 3 pivotal efficacy study was a double-blind placebo controlled, multicenter trial enrolling 6799 infants. The infants were randomized to receive three doses of vaccine (116E; $10^{5.0}$ffu) or placebo at 6, 10, and 14 weeks of age. The primary outcome was severe rotavirus gastroenteritis with ≥11 points on the Vesikari scale. Based on the incidence in the two arms, vaccine efficacy against severe rotavirus gastroenteritis in children in the first year of life was calculated as 56.3% (95% CI 36.7 to 69.9) whereas it was only marginally less at 48.9% in the second year of life. Overall efficacy up to 2 years of age was 55.1% ($p < 0.0001$). The study estimated that the number of infants needed to be immunized to prevent one episode of severe RVGE in the first 2 years of life was 40 and for RVGE of any severity, it was 21. Seroconversion rates were analysed using 2-, 3- and 4-fold response. The IgA immune response rates were moderate; 4-fold (39.9%), 2-fold (56.3%) and 3-fold (46.9%). Viral shedding was highest on day 3 or 7 after dose 1 (9.2%). The. The protection offered by the vaccine during the first 2 years of life was against the array of commonly circulating genotypes including G1P[8], G2P[4], G12P[6], G12P[8] and G9P[4], although 116E vaccine has an unusual G9P[11] genotype that is rarely associated with clinical disease in India or other countries. This suggests that the vaccine could offer significant protection in varying geographical settings over time. SAEs, deaths and cases of intussusception were similar between vaccine and placebo groups. [23]

**Phase 3 Lot Consistency and Non-Interference with Childhood Vaccines Study in India**

This is a placebo-controlled study designed to assess non-interference of ORV 116E to the childhood vaccines (namely pentavalent vaccine and oral polio vaccine), when co-administered at

6-7, 10-14 and 14-18 weeks of age) and clinical consistency in the immune responses to the three production lots of ORV 116E. This study demonstrated that three doses of ROTAVAC® can be safely co-administered with three doses of pentavalent vaccine and oral polio vaccine without diminishing an infant's serum antibody responses to each component of these vaccines. The three ROTAVAC® lots tested were proven to be not different as statistical clinical equivalence across them was established. ROTAVAC® is generally well tolerated with respect to solicited adverse events of special interest (fever, vomiting, diarrhea, cough, listlessness or less active, runny nose, irritability, and rash). There was no case of intussusception associated with the administration of ROTAVAC®. [45]

Based on promising results of the phase 3, safety and efficacy clinical trial of the oral rotavirus human 116E strain (ORV 116E), ROTAVAC® (with buffer), manufactured by Bharat Biotech International Limited, Hyderabad, India, it was licensed in India in 2014.

**Phase 4 With and Without Buffer Study in Infants in India**

This was a single blind study to evaluate the immunogenicity and safety of ROTAVAC®. A total of 900 participants were enrolled in the study in three different treatment groups with 1:1:1 ratio. ROTAVAC® was administered in a 3-dose series with and without buffer to healthy infants. Immunogenicity was tested in terms of GMCs of serum anti rotavirus IgA and seroconversion in all three treatment groups. Seroconversion was defined as achievement of post-vaccination titer of >20 U/mL in infants with pre-vaccination titer of <20 U/mL or achievement of a 2-fold rise in their post-vaccination titer if pre-vaccination titers were > 20 U/mL. Immune response in three treatment groups is comparable with no statistically significant difference detected. Seroconversion and GMCs achieved in group II (ROTAVAC® administered without buffer) was similar to that in the other two treatment groups where ROTAVAC® is administered with buffer. Post vaccination anti-rotavirus IgA GMCs in the group to which ROTAVAC® was administered without buffer was 20.7 U/mL in comparison to 19.6 U/mL and 19.2 U/mL in the two groups which received antacid buffer 5 minutes prior to vaccine and simultaneously mixed with vaccine, respectively. Similarly, the three-fold seroconversion observed in the groups that received ROTAVAC® without antacid buffer and with buffer were between 24.5% and 29.2%. ROTAVAC® vaccine was well tolerated in all three treatment groups that received the vaccine with or without the antacid buffer. The reactogenicity and safety with respect to solicited and unsolicited adverse events were comparable across the three groups with no statistically significant difference.[46]

**Phase 4 Comparator Study**

Immunogenicity and safety of ROTAVAC® was compared with a WHO prequalified rotavirus vaccine Rotarix® in a separate phase IV, multicenter, open-labelled, randomized clinical trial in healthy infants in India. Four hundred and sixty-four infants aged 6-8 weeks were equally randomized to receive 3-doses of ROTAVAC® (group I) or 2-dose Rotarix® (group II). Infants in group 1 were vaccinated on day 0, day 28 and day 56 with 3 doses of ROTAVAC® and infants in group II were vaccinated on day 0 and day 28 with 2 doses of Rotarix®. The two groups were compared for differences in serological responses and safety. Seroconversion was defined as achievement of post-vaccination titer of >20 U/mL in infants with pre-vaccination titer of <20 U/mL or achievement of a 2-fold rise in their post-vaccination titer if pre-vaccination titers were

> 20 U/mL. Seroconversion was reported in 34.1% participants and 30.6% participants in group I and II respectively. Anti-rotavirus geometric mean concentration (GMC) was 20.4 and 24.8 for group I and II respectively. The proportion of infants who achieved fourfold rise in antibodies from baseline was 25% and 21.9% in group I and II respectively. Adverse Events were captured and analyzed for the entire study population. A total of 100 and 106 participants in the treatment ROTAVAC® and ROTARIX® groups reported at least one adverse event. The distribution of solicited, unsolicited, and serious adverse events was similar across groups. The study demonstrated immunological non-inferiority with the comparator Rotarix® with a clinically acceptable safety profile (Data on file; CTRI/2015/12/006428)).

The study results clearly establish that buffer administration is not required to ensure the immunogenicity of the vaccine. Not having buffer also addresses the programmatic issues, such as incorrect reconstitution of a vaccine, temporary unavailability of the buffer, and reduction of the cold chain footprint, etc. With due importance to all these findings, ROTAVAC® without buffer was licensed in India in the year 2015. This formulation was included in the WHO list of prequalified vaccines on 5th January 2018. The formulation still required storage and transportation at -20°C.

### 1.5.2 ROTAVAC 5C

The currently licensed frozen ROTAVAC® vaccine needed to be stored and transported at -20°C. BBIL has been engaged since 2009 in the development of a liquid formulation that is stable at 2-8°C storage temperature over 24 months and also has sufficient Acid Neutralizing Capacity (ANC) to neutralize the gastric juices in the stomach, thus supporting survival of the vaccine virus. This changes the formulation by addition of excipients and stabilizers belonging to the GRAS (Generally Recognized as Safe) category. The advantages with this formulation are twofold: (1) ROTAVAC 5C is a stable liquid vaccine that offers many advantages as discussed later (2) the acid neutralizing capacity eliminates the need to administer an alkali buffer separately prior to the vaccine. ROTAVAC 5C contains the same rotavirus strain (116E) as in ROTAVAC®. International Patents have been granted in several countries including India, USA, UK and South Africa for these rotavirus vaccine formulations.

**Phase 3 Exploratory Study**

In this phase 3 exploratory study, 6-8 week-old healthy infants (n=675) were randomized a 1:1:1 ratio to receive ROTAVAC 5C 1.5 mL (F1 arm, n=225) or ROTAVAC 5C 2 mL (F2 arm, n=225) or ROTAVAC® 0.5 mL (n=225). There were no statistically significant differences in the pre and post vaccination IgA concentrations between the ROTAVAC 5C (F1) and (F2) and ROTAVAC® arms (mean baseline concentration 20.7, 22.3 and 24.2 U/mL respectively (p=0.84 comparing all arms); and post vaccination concentration 63.5, 59.1 and 76.0 U/mL, respectively (p=0.12 comparing all arms). Seroconversion occurred by day 84 in 28.8% (95% CI: 22.7, 34.9%) of infants in the ROTAVAC 5C (F1) arm, 37.6% (95% CI: 31.1%, 44.2%) of the ROTAVAC 5C (F2) arm, and 41.3% (95% CI: 34.7%, 47.8%) of the ROTAVAC® arm. Significantly greater proportions of healthy infants seroconverted in the ROTAVAC® arm compared to the ROTAVAC 5C (F1) arm (p=0.0085). There was no significant difference in seroconversion rates between the ROTAVAC® and ROTAVAC 5C (F2) formulation (p=0.489). Adverse event rates were found to be 22.2% in the ROTAVAC 5C (F1) arm, 14.7% in the ROTAVAC 5C (F2) arm, and 20.6% in the ROTAVAC® arm. The majority of these adverse events were reported within seven days of

the study vaccine administration. There was no statistically significant difference in the proportion of healthy infants experiencing adverse events between those receiving either ROTAVAC 5C formulation (F1 or F2) and those receiving ROTAVAC® (p=0.109). The safety profile of both ROTAVAC 5C formulations (F1 and F2) was found to be similar to the ROTAVAC® formulation. Fewer healthy infants experienced gastrointestinal disturbances with the ROTAVAC 5C 2 mL (F2) formulation compared to the ROTAVAC 1.5 mL (F1) formulation. There were no AEs leading to discontinuation, no AEs leading to death, and no cases of intussusception. Thus, it is concluded that the ROTAVAC 5C 2 mL (F2) formulation is non-inferior to the ROTAVAC® -20°C 0.5 mL formulation. (Data on File; CTRI/2015/02/005577)

**Phase 3 Confirmatory Study**

In this phase 3 confirmatory study, 6-8 week-old healthy infants (n=1302) were randomized to receive ROTAVAC 5C 2 mL Lot 1 (n=327), ROTAVAC 5C 2 mL Lot 2 (n=324), ROTAVAC 5C 2 mL Lot 3 (n=320), or ROTAVAC® -20°C 0.5 mL (n=331). There were no statistically significant differences in the pre- and post-vaccination IgA concentrations between the ROTAVAC 5C and ROTAVAC® arms (mean baseline concentration 24.0, 23.6, 21.5 and 28.5 for ROTAVAC 5C Lot 1, 2 and 3; and ROTAVAC®, respectively (p=0.7275). Seroconversion (4-fold or greater increase in IgA concentration over baseline), occurred by day 84 in 31.5% (95% CI 26.4 to 37.2%) in the ROTAVAC 5C Lot 1 arm, 32.1% (95% CI 26.9 to 37.9%) in the ROTAVAC 5C Lot 2 arm, 34.7% (95% CI 29.3 to 40.6%) in the ROTAVAC 5C Lot 3 arm, and 31.2% (95% CI 26.0 to 36.8%) in the ROTAVAC® arm. The differences in seroconversion between arms was not statistically significant (p=0.8156). There were 861 healthy infants who experienced 2,984 adverse events. Of these, 30 infants experienced 33 vaccine-related adverse events. Vaccine-related adverse events occurred in 1.6% of healthy infants in the ROTAVAC 5C Lot 1 arm, 3.2% of healthy infants in the ROTAVAC 5C Lot 2 arm, 2.9% of healthy infants in the ROTAVAC 5C Lot 3 arm and 1.9% of healthy infants in the ROTAVAC® - arm (p=0.486). The majority of adverse events occurred within 7 days of vaccine administration. There were no adverse events leading to discontinuation, no deaths and no cases of intussusception reported during this study. Non-inferiority against the ROTAVAC® - formulation was established using two-sided 95% confidence intervals for the GMC ratio between ROTAVAC 5C (all lots combined) to ROTAVAC®, requiring a lower limit greater than 0.50. (Data on File; CTRI/2015/02/005577).

### 1.5.3 ROTAVAC 5CM

Further to this and based on the ROTAVAC clinical trial results without buffer, formulation of ROTAVAC 5C 1.5 mL (Formulation 1/EZ) was modified to 0.5mL volume and the internal buffer components were removed, which later was named as ROTAVAC 5CM, a liquid formulation that is stable at 2-8°C (5±3°C) storage temperature. ROTAVAC 5CM is without buffer and has additional excipients and stabilizers (same as in Formulation 1/EZ, with established safety and immunogenicity) that belong to GRAS (Generally Recognized as Safe) Category and new safety concerns are not anticipated. The ROTAVAC 5CM vaccine has also been filed for international patents.

**Phase 3 Study**

A phase 3, multicenter, randomized, open labeled clinical study was conducted to evaluate the immunogenicity, reactogenicity and safety of ROTAVAC 5CM (stored at 2-8 °C), in comparison

with ROTAVAC® stored at -20ºC) in healthy infants aged 6-8 weeks. The study enrolled 360 participants which were randomized in the two groups in a ratio of 3: 1 with 270 participants in the ROTAVAC 5CM arm and 90 in the ROTAVAC® arm. Both formulations were administered in a dose of 0.5 ml at 6, 10 and 14 weeks of age along with routine childhood vaccinations. Blood samples for immunogenicity analysis were collected before first vaccination and 4 weeks after last vaccination. The post-vaccination GMCs reported in the ROTAVAC 5CM and ROTAVAC® groups were 18.70 and 19.55, respectively. The ratio of GMC (ROTAVAC 5CM/ ROTAVAC®) was estimated as 0.81 and the lower bound of two-sided 95 % CI was found to fall in the 2-fold margin bounds of 0.5 to 2. Hence the two vaccines were assessed to be equivalent. Also, the two vaccines were found to be similar when assed in terms of seroconversion (34.24% vs. 28.92%), four-fold rise (22.18% vs. 21.25%), three-fold rise (27.42% vs. 30.00%), two-fold rise (38.31%vs. 31.25%) for ROTAVAC 5CM and ROTAVAC® arms, respectively. The adverse events were, Pain at the site of injection (34.22% and 38.64%), Redness at the site of injection (34.60% and 32.95%), Swelling at the site of injection (32.70% and 37.50%), crying (14.83% and 14.77%) and fever (65.40% and 67.05%) in ROTAVAC 5CM and ROTAVAC® groups respectively. Many of these may be ascribed to routine childhood immunization administered concomitantly. No case of intussusception was reported among the study population who were administrated with either of two vaccines. Overall, this study demonstrated that immunogenicity and safety profiles of both vaccines ROTAVAC 5CM and ROTAVAC® was similar. (Data on file (Data on File; CTRI/2016/11/007481).

### 1.6 Potential Risks and/or Benefits of Study Vaccine

ROTAVAC 5CM is the next generation formulation of ROTAVAC® and has the same virus strain as ROTAVAC® with addition of some stabilizers and excipients of GRAS category. ROTAVAC® and ROTAVAC 5CM have been extensively studied in pre-clinical and clinical studies. ROTAVAC® has been administered to more than 7,000 children in clinical trials in India (including one study demonstrating efficacy of the vaccine and another study demonstrating non-inferiority of immune response vs. Rotarix). In these studies, ROTAVAC® was found to be efficacious (efficacy of 55.1% up to 2 years of age) and immunogenic. The pivotal study had active surveillance for adverse events and the vaccine was found to be safe. ROTAVAC 5CM has been shown to be equally immunogenic as ROTAVAC. Based on these data ROTAVAC® has been licensed in India and was recently included in the WHO list of pre-qualified vaccines. It has already been administered to millions of children in India under the national immunization plan without any major concern. The potential risk for the vaccine include commonly reported adverse events like 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. In the Phase 3 efficacy study, 11 intussusception cases as defined by Brighton grade 1 have been reported (8 of 4532 participants in vaccine group and 3 of the 2237 in placebo group). None occurred within 30 days of a vaccine dose and all were reported only after the third dose. The intussusception events following the third dose occurred between 112 and 587 days post-vaccination in the vaccine group and between 36 and 605 days in the placebo group. Ten of the cases were rotavirus negative when tested for presence of rotavirus antigen in stools by ELISA. None of the four rotavirus positive cases had the vaccine virus strain.

Since the earlier studies were performed in India (another developing country) it can be expected that the vaccine will be similarly beneficial in Zambia, and will have similar safety profile.

## 1.7 Overall Development Strategy

ROTAVAC® is indicated for active immunization of infants from the age of 6 weeks for the prevention of gastroenteritis due to rotavirus when administered as a 3-dose series. ROTAVAC® has been licensed in India in 2014 and has been included on the WHO list of prequalified vaccines since early 2018. These milestones were achieved based on the data generated in India. This trial will generate immunogenicity and safety data on ROTAVAC® and ROTAVAC 5CM outside of India to support WHO SAGE policy recommendations and country-level decision-making.

## 1.8 Study Rationale

This study has been designed to compare ROTAVAC 5CM with the newly WHO prequalified vaccine ROTAVAC® among infants in Africa. Four hundred and fifty infants, 6-8 weeks of age will be enrolled and randomized to receive either three doses of ROTAVAC® or ROTAVAC 5CM, four weeks apart, at 6, 10 and 14 weeks of age or two doses of Rotarix® (current standard of care in Zambia), four weeks apart at 6 and 10 weeks of age. The main purpose of this study is to assess the immunogenicity of a full course of the two formulations of ROTAVAC®, i.e., ROTAVAC® and ROTAVAC 5CM and to assess the safety and reactogenicity of the vaccine among African infants. Rotarix® has been included as the third arm since it is being used for routine immunization of children in Zambia and holds the vast majority of the GAVI-eligible country market share. This arm will act as an internal control to validate immune response of ROTAVAC® and ROTAVAC 5CM which are being tested for the first time in African continent. The study will also assess the safety and reactogenicity of a full immunization course and of each dose of the study vaccines, with the aim of describing its safety in Zambia.

Because Rotarix® is a two-dose human rotavirus vaccine and ROTAVAC® and ROTAVAC 5CM are three-dose human rotavirus vaccines, maximizing comparability of immunogenicity data is a critical driver of the design and tests to be used. In order to ensure maximal comparability, blood sampling time points in a three-dose arm (i.e. ROTAVAC® and ROTAVAC 5CM) are at 18 weeks of age, and for Rotarix® at 14 weeks of age i.e. 4 weeks after last dose. ROTAVAC® has already been studied in a similar study design in studies conducted in Indian population.

This trial will generate immunogenicity and safety data on ROTAVAC® and ROTAVAC 5CM outside of India to support WHO SAGE policy recommendations and country-level decision-making. At the conclusion of the study, PATH, public health leaders and site investigator(s), together with Bill & Melinda Gates Foundation (BMGF) officers will support BBIL in communication of results to global and national policymakers and to the global public health community. Presentation of data to Zambian Ministry of Health, WHO and in peer reviewed open access publications will be key audiences targeted for communication of results.

Primary immunogenicity analysis of all samples will be based on a validated ELISA which uses strain WC3 as a substrate. The same test was also used for clinical trials supporting licensure of the ROTAVAC® (without buffer) in India and for WHO pre-qualification of the vaccine. A subset of the samples (50 pairs/arm) collected will also be tested by a validated ELISA which uses strain 89-12 (G1P8 virus) as a substrate. Strain 89-12 was the isolated strain that was used to develop the Rotarix® Vaccine. Since no correlation between the two assays has been established and it is

expected that tests employing heterologous strains as substrate may give lower concentrations, no within-group or between assay comparisons will be done.

# 2 HYPOTHESES, OBJECTIVES AND ENDPOINTS

## 2.1 Study Hypotheses

**Immunogenicity**

- ROTAVAC® and ROTAVAC 5CM administered as a 3-dose series induce comparable immune response in healthy African infants.

**Safety**

- ROTAVAC® and ROTAVAC 5CM administered as a 3-dose series are both safe and well-tolerated in healthy African infants.

## 2.2 Study Objectives

### 2.2.1 Primary Objective

**Immunogenicity**

- To evaluate and compare the immunogenicity of ROTAVAC® and ROTAVAC 5CM 28 days after the last dose of the vaccine, when administered to infants in a three-dose schedule at 6, 10 and 14 weeks of age.

### 2.2.2 Secondary Objectives

**Safety**

- To assess the reactogenicity 7 days after each vaccination and safety 4 weeks after the last vaccination of the ROTAVAC® and ROTAVAC 5CM, when administered to infants in a three-dose schedule at 6, 10 and 14 weeks of age and Rotarix®, when administered to infants in a two-dose schedule at 6 and 10 weeks of age.

**Immunogenicity**

- To evaluate the immunogenicity of Rotarix® 28 days after the last dose of the vaccine, when administered to infants in a two-dose schedule at 6 and 10 weeks of age.

### 2.2.3 Exploratory Objective

- To evaluate the immunogenicity of the three vaccines by ELISA using 89-12 (G1P8 virus) as a substrate in a subset of the samples collected.

## 2.3 Study Endpoints

### 2.3.1 Primary Endpoint

**Immunogenicity:**

Geometric mean concentrations (GMCs) of serum anti-rotavirus IgA antibodies 28 days after the last dose of a study vaccine, as measured by enzyme-linked immunosorbent assay (ELISA) using WC3 as the viral lysate.

### 2.3.2 Secondary Endpoints

**Safety**

The secondary safety endpoints will include:

- Immediate adverse events, within 30 minutes post-vaccination.
- Solicited post-vaccination reactogenicity (fever, diarrhea, vomiting, decreased appetite, irritability, decreased activity level) during the 7 day period (Day 0-6) after each vaccination.
- Unsolicited AEs from first vaccination through 4 weeks after the last vaccination.
- SAEs, including intussusception from first vaccination through 4 weeks after the last vaccination of each study participant.

**Immunogenicity**

The secondary immunogenicity endpoint (anti-rotavirus IgA using WC3 as the Lysate) will be as follows:

- Seroconversion rate 28 days after last dose of study vaccine (Day 84 for ROTAVAC® and ROTAVAC 5CM and Day 56 for Rotarix®). Seroconversion will be defined as a post-vaccination serum anti-rotavirus antibody IgA concentration of at least 20 U/mL if a baseline concentration is < 20 U/mL or a post-vaccination serum anti-rotavirus IgA concentration of ≥ 2-fold baseline level if a baseline concentration is ≥ 20 U/mL.
- Seropositivity rate at baseline and 28 days after last dose of study vaccine (Day 84 for ROTAVAC® and ROTAVAC 5CM and Day 56 for Rotarix®). Seropositivity is defined as serum anti-rotavirus IgA antibody concentration ≥ 20 U/mL.
- Seroresponse rate 28 days after last dose of study vaccine (Day 84 for ROTAVAC® and ROTAVAC 5CM and Day 56 for Rotarix®). Seroresponse will be assessed as a four-fold, three-fold and two- fold rise in antibody concentration from baseline.
- Geometric Mean Fold Rise (GMFR) that is a ratio of GMCs at 28 days after last dose of study vaccine (Day 84 for ROTAVAC® and ROTAVAC 5CM and Day 56 for Rotarix®) with reference to baseline.

### 2.3.3 Exploratory Endpoints:

The exploratory endpoints based on anti-rotavirus IgA antibodies measured by ELISA using 89-12 as the viral lysate in a subset of samples will be as follows:

- GMCs of serum anti-rotavirus IgA antibodies 28 days after the last dose of study vaccine.
- Seroconversion rate 28 days after last dose of study vaccine. Seroconversion is defined as a post-vaccination serum anti-rotavirus IgA antibody concentration of at least 20 U/mL if a baseline concentration is < 20 U/mL or a post-vaccination serum anti-rotavirus IgA antibody concentration of ≥ 2-fold baseline level if a baseline concentration is ≥20 U/mL.

- Seropositivity rate at baseline and 28 days after last dose of study vaccine. Seropositivity is defined as serum anti-rotavirus IgA antibody concentration ≥ 20 U/mL.
- GMFR that is a ratio of GMCs at 28 days after last dose of study vaccine with reference to baseline.

## 3 STUDY DESIGN

This study is designed as a Phase IIb, single-center, randomized, active-controlled, open-label study enrolling a total of 450 healthy infants 6-8 weeks (42-56 days) of age. Prospective participants, whose parent or legal guardian sign an informed consent form and pass the test of understanding, 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.

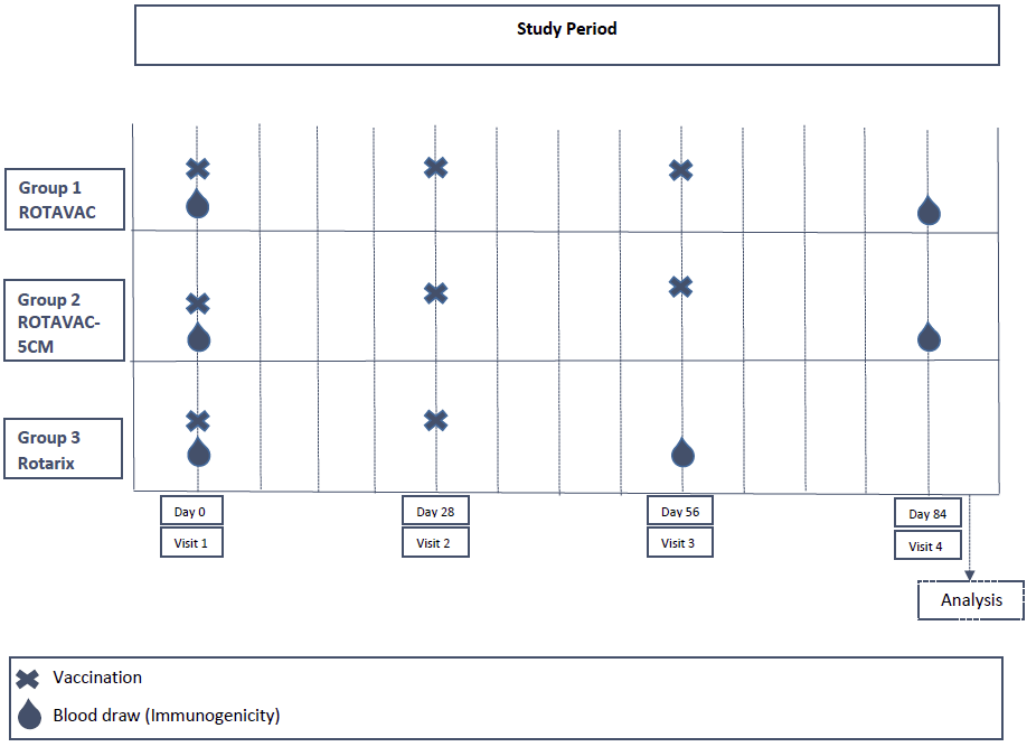
If the participant is found to be eligible, the infants will be allocated to one of the three groups in a ratio of 1:1:1 (n=150 per group) to receive either three doses of ROTAVAC®, three doses of ROTAVAC 5CM or two doses of Rotarix® 4 weeks apart (minimum interval of 4 weeks and maximum of 5 weeks). Allocation of treatment to individual participants will be based on a randomization schedule developed in coordination with the data management organization for unbiased randomization of participants to the treatments. Three doses of ROTAVAC® and ROTAVAC 5CM will be administered at 6, 10 and 14 weeks of age whereas two doses of Rotarix® will be administered at 6 and 10 weeks of age (see table below). All vaccines will be administered concomitantly with EPI vaccines including Diphtheria, Tetanus, Pertussis, *Haemophilus influenzae type B* and Hepatitis B vaccine (DTwP-Hib-HepB), Pneumococcal conjugate vaccine and OPV at 6, 10 and 14 weeks and IPV at week 14 (when switched to in Zambia). Should any changes to the EPI schedule in the Zambia occur before or during the study the vaccines given alongside the study vaccines will be allowed to reflect the current program without a protocol amendment unless this is considered to interfere with the assessment of the study endpoints in any way. The participants will be monitored for 30 minutes following vaccine administration for immediate adverse events like anaphylaxis, vomiting etc.

| **Group** | No. of participants | Visit 1 (6-8 weeks) | Visit 2* V1+28 (+7) days | Visit 3* V2+28 (+7) days |
|---|---|---|---|---|
| A | 150 | ROTAVAC® | ROTAVAC® | ROTAVAC® |
| B | 150 | ROTAVAC 5CM | ROTAVAC 5CM | ROTAVAC 5CM |
| C | 150 | Rotarix® | Rotarix® | NA |

To evaluate the Rotavirus vaccine immunogenicity, a blood sample will be obtained from all the participating infants before first vaccination and four weeks (+1 week) after the last vaccine dose. This would mean that the blood sample will be collected at approximately 14 weeks of age for infants in the Rotarix® arm and 18 weeks for infants in the ROTAVAC Groups. Serum Anti-rotavirus IgA antibodies will be analyzed at Wellcome Trust Research Laboratory, Christian Medical College, Vellore, India using a validated ELISA which uses WC3 virus as a substrate. A subset of samples (50 sample pairs per arm) will also be analyzed by another ELISA using the Rotarix specific strain 89-12 as a lysate at Cincinnati Children's Hospital Medical Center (CCHMC), Cincinnati, Ohio, USA. The study will compare the immunogenicity of the two

formulations of ROTAVAC® i.e. ROTAVAC® vs. ROTAVAC 5CM in terms of GMCs and will describe without comparing the immune response to Rotarix®.

**Study Schema**




Enhanced passive/Active surveillance for vaccine reactogenicity (solicited reactions) over the 7-day period after each vaccination will be conducted on all infants. In addition, surveillance for unsolicited AEs, SAEs including intussusception will be carried out over the period between first vaccination and four weeks after the last vaccination on all infants. Participants will be evaluated for:

- Immediate adverse events within 30 minutes post-vaccination.
- 7-days post-vaccination reactogenicity, i.e., the occurrence of solicited reactions (fever, diarrhea, vomiting, decreased appetite, irritability, decreased activity level) after each vaccination.
- Unsolicited AEs from first vaccination through four weeks after the last vaccination.
- Incidence of serious adverse events (SAEs) including intussusception, from first vaccination through four weeks after the last vaccination.

For evaluation of post-vaccination reactogenicity, the parent will be given a digital thermometer and a Post-Immunization Diary Card (PIDC) covering Days 0-6 (Day 0 = day of vaccination). They will be instructed to record their child's axillary temperature as well as other solicited reactions on the PIDC for 7 days post-vaccination. Study staff will make a home visit on Days 2 (+1 day) and Day 7 (+1 day) to determine health status and to support completion of the PIDC as well as capture information on the PIDC recorded to-date. Completed PIDC will be collected by

the field officer on the Day 7 (+1 day) following vaccination. The data in the PIDC collected by the parents will be reviewed by the study physician/designee and entered in the CRF.

All SAEs will be reported following established regulatory requirements.

Safety will be monitored by on-site clinical staff and routinely by a Protocol Safety Review Team (PSRT), an internal group of physicians which includes the Investigator team and PATH Medical Officers. The PSRT will have provision of expedited meeting that could be triggered by reporting of predefined AEs. The PSRT may also seek independent expert medical opinion as dictated by the occurrence of certain events.

It is expected that the enrolment will be completed over approximately 6 months and total study duration for each participant will be 3-4.5 months.

# 4 STUDY POPULATION

## 4.1 Description of Study Site & Population

The study will be conducted at George Clinic in Lusaka where CIDRZ has a research facility. This Research Unit is located on the premises of a busy public clinic that offers all basic outpatient services including general OPD, MCH, ART, pharmacy and allied primary care services in a peri-urban setting. The center has a catchment population of 145,230. This study site is at a typical peri-urban clinic setting in Lusaka, and participants will be recruited as they randomly attend the health facility.

After necessary approvals are in place, the CIDRZ Community Advisory Board will facilitate meetings in the community with key gate keepers and interest groups in order to inform them about the study. This is the mechanism that is also used to address any community issues that may arise during the course of the study. With a catchment population of over 140,000 and more than 600 antenatal attendances each month, George clinic has sufficient number of healthy infants to be screened with parental consent in order to enroll 450 infants in the study. Participants will be 6-8 week (42-56 days; both inclusive) old healthy infants. Parents attending clinics for the vaccination of their infants will be approached for their child's enrollment in the study. A signed written form consent will be obtained before any trial-related procedures are performed.

Final eligibility determination will depend on the results of the medical history and clinical examination, fulfillment of eligibility criteria, and appropriate understanding of the study.
Investigators should always use good clinical judgment in considering a participant's overall fitness for inclusion in the trial. Some participants may not be appropriate for the study even if they meet all inclusion/ exclusion criteria. For instance, medical, occupational or other conditions present in the parents may make safety evaluations difficult or make the infants poor candidates for retention. All infants targeted for enrollment will need to have parents who can comprehend the purpose of the study and provide written informed consent. In addition, the families should be resident in the area without plans to leave the area during the course of the study.

## 4.2 Inclusion Criteria

Fulfillment of all of the following criteria is required to accept an infant in the study:

1. Healthy infant as established by medical history and clinical examination before entering the study.

2. Age: 6-8 weeks (42-56 days, both days inclusive) confirmed by Immunization Record.
3. Infants received age-appropriate EPI vaccines till enrolment.
4. Ability and willingness to provide informed consent as per local consenting procedures.
5. Parent can be contacted on phone and confirms intention to remain in the study area with the participant during the study period.

### 4.3 Exclusion Criteria

Any of the following will exclude an infant from the study:

1. Presence of diarrhea or vomiting in the previous 72 hours or on the day of enrolment (temporary exclusion).
2. Presence of fever on the day of enrolment (temporary exclusion).
3. Acute disease at the time of enrolment (temporary exclusion).
4. Concurrent participation in another clinical trial throughout the entire timeframe of this study.
5. Presence of severe malnutrition (weight-for-height z-score < -3SD median).
6. 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 child's health or is likely to result in non-conformance to the protocol.
7. History of congenital abdominal disorders, intussusception, abdominal surgery
8. Known or suspected impairment of immunological function based on medical history and physical examination.
9. Prior receipt or intent to receive rotavirus and other age specified EPI vaccines outside of the study center and during study participation.
10. A known sensitivity or allergy to any component of the study vaccine.
11. Clinically detectable significant congenital or genetic defect.
12. History of persistent diarrhea (defined as diarrhea more than 14 days).
13. Participant's parents not able, available or willing to accept active follow-up by the study staff.
14. Has received any immunoglobulin therapy and/or blood products since birth or planned administration during the study period.
15. 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.
16. History of any neurologic disorders or seizures.
17. Any medical condition in the parents/infants that, in the judgment of the investigator, would interfere with or serves as a contraindication to protocol adherence or a participant's parent's/legally acceptable representative's ability to give informed consent.

18. Participant is a direct descendant (child or grandchild) of any person employed by the Sponsor, the CRO, the PI or study site personnel.

### 4.4 Continued Eligibility Confirmation for Subsequent Vaccination

The following events constitute absolute contraindications to rotavirus vaccination and all participants should be evaluated for these before further administration 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, within the time window specified in the protocol, 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.

### 4.5 Reasons for Withdrawal

Participants have the right to decline study treatment or procedures for any reason and at any time during the study. If a participant 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 will be encouraged to complete the remaining applicable safety-related follow-up to complete safety follow-up for atleast 28 days after last dose and immunogenicity blood draw at the end of safety follow-up. If the participant does not wish to remain in the study by declining any follow-ups or procedures, the participant 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 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

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 if the interval between the participant's last visit and the time of withdrawal is within the protocol-defined reporting period
- Conduct physical examination
- Collect blood for immunologic testing if withdrawal occurs before end of study
- Update contact information

# 5 STUDY PRODUCT/S

## 5.1 Study Vaccine

### 5.1.1 Product Description

Two formulations of Rotavirus vaccine manufactured by Bharat Biotech International Ltd will be used in the study. Both the formulations are monovalent vaccines containing suspension of $10^{5.0}$ FFU live rotavirus 116E prepared in Vero cells. Based on the dual classification system using G and P surface proteins, 116E is classified as G9P[11] type. Both the vaccines are ready to use (no reconstitution or dilution is required).

Rotarix® is a live attenuated RIX4414 strain of human rotavirus of the G1P[8] type and will be used as an internal control. The vaccine is ready to use (no reconstitution or dilution is required). Rotarix® is a GSK Biologicals' licensed rotavirus vaccine which has been reviewed by WHO and has been approved and included in the WHO pre-qualified list of vaccines.

Details of the vaccines are provided below:

| | |
|---|---|
| **ROTAVAC® (Frozen formulation)** | This is the licensed and WHO prequalified rotavirus vaccine from Bharat Biotech International Limited. The vaccine is a live, attenuated G9P[11] monovalent vaccine at a dose of 0.5 mL containing NLT log $10^{5.0}$ focus forming units (FFU) per dose. The vaccine should be stored at -20°C. Before administration, liquid frozen vaccine vial will be shifted from -20°C to room temperature for thawing. The liquid vaccine will be administered per oral.<br>Manufactured by Bharat Biotech International Limited, India |

| | |
|---|---|
| **ROTAVAC 5CM (liquid formulation)** | The vaccine is a live, attenuated G9P[11] monovalent vaccine to be administered in a dose of 0.5 mL containing NLT log $10^{5.0}$ focus forming units (FFU) per dose. Since this is a fully liquid vaccine stored at 2-8°C, no thawing is required before administration. The liquid vaccine will be administered per oral.<br>Manufactured by Bharat Biotech International Limited, India |
| **Rotarix®** | This is the licensed and WHO prequalified rotavirus vaccine from GSK Biologicals. Rotarix is a live attenuated RIX4414 strain of human rotavirus of the G1P[8] type containing not less than $10^{6.0}$ CCID50 (cell culture infectious dose 50%) of the RIX 4414 strain of human rotavirus. 1.5 ml of the liquid vaccine will be administered per oral.<br>Manufactured by GlaxoSmithKline Vaccines, Belgium |

### 5.1.2 Manufacturer

ROTAVAC® and ROTAVAC 5CM are manufactured by Bharat Biotech International Ltd.

Rotarix® is manufactured by GSK Biologicals.

### 5.1.3 Presentation and Formulation

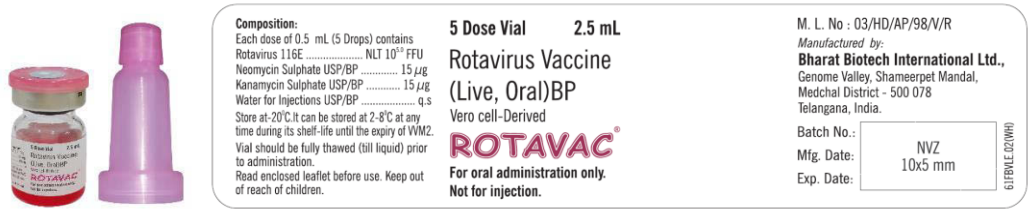
**ROTAVAC®** is a liquid in frozen form. In liquid form, the vaccine is generally pink in colour and may sometimes change to orange (or light yellow) in colour. This change in colour does not impact the quality of vaccine. ROTAVAC® is packed in USP type 1 glass vials and will be supplied as multi dose vials containing 5 (2.5 mL vial) or 10 doses (5 mL vial) of the vaccine per vial.

Each dose of 0.5 mL (5 Drops) contains

| **Ingredient** | **Quantity / 0.5 mL** |
|---|---|
| Rotavirus 116E Bulk, Live Attenuated | NLT $10^{5.0}$ FFU |
| Potassium Phosphate Monobasic USP/BP | 0.258 mg |
| Potassium Phosphate Dibasic USP/BP | 0.625 mg |
| Sucrose USP/BP | 37.31 mg |
| Potassium L-glutamate Monohydrate USP/BP | 1.0 mg |
| Neomycin Sulphate USP/BP | 15 µg |
| Kanamycin Sulphate USP/BP | 15 µg |
| Dulbecco's Modified Eagle's Medium (DMEM) | 4.4 mg |
| Water for Injections USP/BP | q.s |

pH range: 7.2 to 8.0

**ROTAVAC®** vials supplied through BBIL comes with a Vaccine Vial Monitor2 (VVM2) dot to guide its usage. The expiry date of the vaccine is indicated on the label and packaging. A picture of the prototype vial and label has been provided below:




An additional label to comply with the regulatory requirements will be pasted on the carton of the vaccine. A template has been provided below:

**FOR CLINICAL TRIAL USE ONLY**

**Protocol Number: CVIA 066**

Sponsor: Centre for Infectious Disease

Research in Zambia, Lusaka, Zambia, 10101

Site: George Research Clinic, Centre for Infectious Disease Research in Zambia, Lusaka, Zambia, 10101

**ROTAVAC 5CM** is a fully liquid vaccine and is generally pink in colour and may sometimes change to orange (or light yellow) in color. This change in colour does not impact the quality of vaccine. ROTAVAC 5CM is packed in USP type l glass vials and will be supplied as single dose Glass Vials (2mL) containing 0.5 mL of the vaccine.

Each dose of 0.5 mL (5 Drops) contains

| **Ingredient** | **Reference** |
|---|---|
| Rotavirus 116E Bulk, Live Attenuated | NLT $10^{5.0}$ FFU |
| Kanamycin acid sulphate | IP |
| Neomycin sulphate | USP |
| Sucrose | USP |
| Trehalose | In-House |
| Lactalbumin hydrolysate (LAH) | In-House |
| Human Albumin | IP/BP |
| Di Potassium hydrogen orthophosphate/Potassium Phosphate dibasic | USP |
| Potassium di hydrogen orthophosphate/ Potassium Phosphate Monobasic | USP |
| Tri sodium citrate dihydrate | USP |
| Water for Injection | IP/BP/In-House |

pH range: 7.2 to 8.0

A template of the label for has been provided below:

**FOR CLINICAL TRIAL USE ONLY**

**Protocol No.: CVIA 066**

**ROTAVAC 5CM®**

**Instructions for use and route of administration:**

For oral administration only. Not for injection.

**Directions of storage:**

Store at 2-8°C

Batch No: XX

Mfg. Date: Expiry date:

**Manufactured by:** Bharat Biotech International Ltd, Genome Valley, Shameerpet, Hyderabad, Telangana, India-500 078

**Sponsor:** Centre for Infectious Disease Research in Zambia, Lusaka, Zambia, 10101

**Site:** George Research Clinic, Centre for Infectious Disease Research in Zambia, Lusaka, Zambia, 10101

**Rotarix®** is presented as a clear, colourless liquid suspension in a squeezable tube (polyethylene) fitted with a membrane and a tube cap (polypropylene). The vaccine is meant for oral administration and is free of visible particles. Each tube contains 1.5 ml of the vaccine which maybe packed in pack sizes of 1, 10 or 50.

1 dose (1.5 ml) contains:

| **Ingredient** | **Quantity** |
|---|---|
| Human rotavirus RIX4414 strain (live, attenuated)* | not less than $10^{6.0}$ CCID50 |
| Excipients: Sucrose, Di-sodium Adipate, Dulbecco's Modified Eagle Medium (DMEM), sterile water. | |

*Produced on Vero cells

An additional label to comply with the regulatory requirements will be pasted on the carton of the vaccine. A template label has been provided below:

**FOR CLINICAL TRIAL USE ONLY**

**Protocol Number: CVIA 066**

Sponsor: Centre for Infectious Disease Research in Zambia, Lusaka, Zambia, 10101

Site: George Research Clinic, Centre for Infectious Disease Research in Zambia, Lusaka, Zambia, 10101

### 5.1.4 Stability and Storage

The expiry date of the vaccine will be indicated on the label and the packaging. The vaccines should not be mixed with any other medicinal products/active immunizing agents.

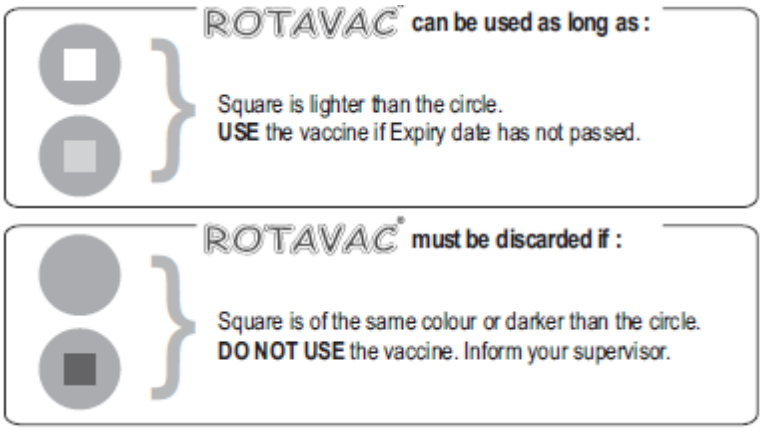
ROTAVAC® should be stored at -20 °C in a deep freezer with a power backup. It is absolutely critical to ensure that the storage conditions specified above are complied with. The vaccine should be thawed before and used immediately after opening. ROTAVAC® should be transported in a vaccine carrier using conditioned gel packs with temperature maintained at 2-8 °C. Any unused vaccine after 8 hours of thawing should be discarded.

ROTAVAC 5CM and Rotarix® should be stored at 2-8 °C in a refrigerator with a power backup. The vaccine should be transported in a vaccine carrier using conditioned gel packs with temperature maintained at 2-8 °C. It is absolutely critical to ensure compliance with the storage conditions specified above.

Freezing is not recommended for storage of Rotarix®. The vaccine should be used immediately after opening. The vaccine should be stored in the original package, in order to protect from light.

### 5.1.5 The Vaccine Vial Monitor2

Vaccine Vial Monitor2 (VVM2) dot is on the seal of the ROTAVAC® vials supplied through BBIL. This is a time temperature sensitive dot that provides an indication of the cumulative heat to which the vial has been exposed. It warns the end user when exposure to heat is likely to have degraded the vaccine beyond an acceptable level.



Interpretation of VVM2: Focus on the central square. Its colour will change progressively. As long as the colour of this square is lighter than the colour of the ring, the vaccine can be used. As soon as the colour of the central square is the same colour as the ring or of a darker colour than the ring, the vial should be discarded.

The Vaccine Vial Monitor (VVM) is also part of the label used for all Rotarix® batches supplied by GlaxoSmithKline Biologicals. The colour dot that appears on the label of the tube is a VVM. This is a time-temperature sensitive dot that provides an indication of the cumulative heat to which the tube has been exposed. It warns the end user when exposure to heat is likely to have degraded the vaccine beyond an acceptable level.

To interpret the VVM status the colour of the central square should be observed and decisions on use are exactly the same as described for Rotavac® above.

### 5.2 Dose Preparation and Administration

The Investigational Medicinal Products (IMP) will be prepared and administered by designated qualified staff only to infants included in this study. The vaccine will be administered as per the randomization list.

The date and time of the IMP administration must be recorded. The Investigator must track vaccines received, used and wasted and will retain all unused or expired products.

**ROTAVAC®, ROTAVAC 5CM and Rotarix® for oral use only and should under no circumstances be injected.**

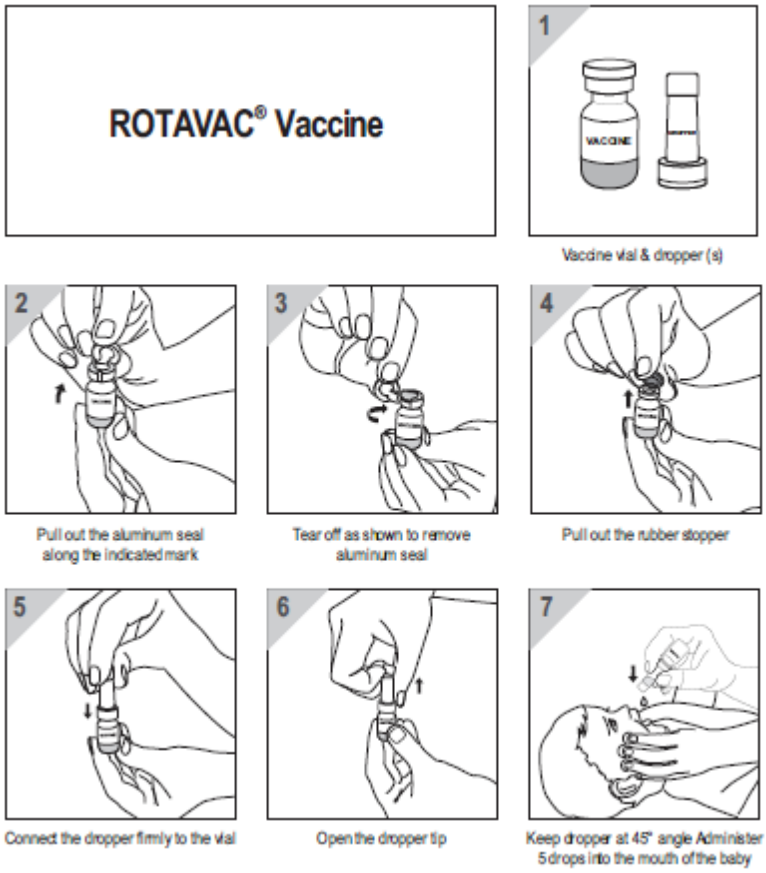
The investigational products must not be mixed with other medicinal products.

**ROTAVAC® and ROTAVAC 5CM instructions for use:**

- The appropriate dosing vial to be chosen.
- Check the expiry date.
- 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.
- Check the vial has not been damaged nor is already open.
- Check the VVM2 on the seal and let the vaccine to thaw to liquid state before administration (applicable for frozen ROTAVAC® formulation only).
- Pull out the aluminum seal along the indicated mark and tear it off.
- Pull out the rubber stopper
- Connect the dropper firmly on the vial.
- Open the dropper tip.
- The vaccine should be administered immediately after opening.
- Seat the child leaning slightly backwards. Keep dropper at 45 degrees and administer 5 drops in the mouth of the baby toward the inner cheek. Note that ROTAVAC **5CM** comes as a 0.5ml single dose vial and all the contents should be administered to one child.

- Observe the child for at least 30 minutes for any immediate reactions.

Please refer to Package Insert of ROTAVAC® for more information on administration of the vaccine.




**Rotarix® instructions for use**

- The appropriate dosing tube to be chosen.
- Check the expiry date.
- 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.
- Check the tube has not been damaged nor is already open.
- Check the liquid is clear and colorless, without any particles in it.
- Check the VVM2 on the label.
- Hold the tube upright and repeatedly flick the top of the tube until it is clear of any liquid
- Keep the tube held upright and hold the side of tube
- There is a small spike inside the top of the cap - in the center. Turn the cap upside down (180°) and press the cap down to pierce the membrane. Then lift off the cap.
- Check the membrane has been pierced and there is a hole at the top of the tube.

- Administer the vaccine immediately.
- Seat the child leaning slightly backwards.
- Squeeze the liquid gently into the side of the child's mouth - towards the inside of their cheek.
- You may need to squeeze the tube a few times to get all of the vaccine out - it is okay if a drop remains in the tip of the tube.
- Observe the child for 30 minutes for any immediate reactions.

Please refer to Package Insert of Rotarix® for more information on administration of the vaccine.

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.

## 5.3 Accountability and disposal

### 5.3.1 Vaccine supply

Bharat Biotech International Limited will supply sufficient quantities of study vaccines (ROTAVAC® and ROTAVAC 5CM) to allow completion of this study only after the IEC and regulatory approval of final protocol. Rotarix® will be procured locally from the commercial market by the Sponsor (CIDRZ). Local regulatory requirements require that an additional label "For Clinical Trial Use ONLY" be added to the vaccine lots intended for research studies. The product commercial packaging or appropriate package will be unaltered and remain visible to maintain the integrity of the products. The batch numbers, expiry dates and physical description of investigational products will also be included in the study report.

The vaccines will be labeled according to the local regulations and requirements of study protocol. All labels will contain the following minimum information**:**

- Imprint "For Clinical Trial Use Only"
- Kit Number (if applicable)/study vaccine name
- Name and Address of Manufacturer,
- Protocol number
- Store between 2-8 oC or -20 oC,
- Batch Number,
- Expiry Date,
- Imprint "For Oral use only"
- Name and address of Site

Upon receipt of the study supplies, an inventory must be performed by the designated study staff at site. It is important that the designated study staff verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study product in a given shipment will be documented in the study files. The Investigator must notify Bharat Biotech International

Limited immediately of any damaged or unusable investigational products that were supplied to the Investigator's site. The IMP must be stored as per the storage instructions, which will be under close temperature monitoring and connected to a source with reliable back up power. Changes in temperature outside the allowed range should be immediately reported and any vaccine lots experiencing such out of range changes will be brought to the attention of Bharat Biotech International Limited for determination of procedures to follow.

In case there is any temperature excursion during shipment or storage, follow the steps given below:

1. Quarantine the shipment in appropriate storage area (2-8°C or -20°C).
2. Inform immediately to Site Monitor and BBIL / designee about excursion.
3. Send completed acknowledgment forms to BBIL / designee.
4. Wait to hear on usage decision from BBIL.
5. In case BBIL confirms use, release investigational products for study use.
6. In case usage decision is 'To be Rejected', transfer the investigational products to rejected area.

The logistic provider's designee will provide temperature data to PATH/BBIL for each shipment. Temperature recorded for storage will be reviewed by the monitor during monitoring visits.

After administration, the study staff will complete accountability label on the carton and store the used products at designated area. A monitor from the CRO will verify the use of investigational products as per the randomization list and also the accountability of investigational products.

The site PI (or designee) must maintain 100% accountability for all investigational products received and dispensed during his or her entire participation in the study. Proper drug accountability includes, but is not limited to:

- Frequently verifying that actual inventory matches documented inventory.
- Verifying that all containers used / broken / unused / lost are documented accurately on the log.
- Verifying that required fields are completed accurately and legibly.

If any dispensing errors or discrepancies are discovered, PATH/BBIL must be notified immediately.

### 5.3.2 Dispensing

After the inclusion and exclusion criteria check, the PI or designated personnel must confirm the eligibility and then randomize the participant. The procedure for randomization will be designed to minimize allocation bias in the study. Designated personnel will prepare and administer the IP as per the instructions respecting randomization. The used IMP tubes/vials must be kept for accountability. For ROTAVAC® multi dose vial, only one dose to be used for one participant. Used vials with remaining vaccines should be kept at room temperature for accountability.

### 5.3.3 Return or destruction

The site will receive instruction from BBIL/PATH /Designee regarding the final disposition of used and unused IMP tubes/vials. The used IMP containers must be kept separately for the accountability. At the completion of the study (after the last visit of the last participant), there will be a final reconciliation of IMP shipped, IMP consumed, and IMP remaining. Any discrepancies noted will be investigated, resolved, and documented. All the unused and used vials/tubes will be destroyed on site after PATH/BBIL approval. Destruction of IMP at site will be documented (including ZAMRA permit to destroy the products) in the study files and certificate will be provided to PATH/BBIL.

## 6 STUDY PROCEDURES

A manual will be developed and maintained at site with detailed descriptions of the procedures involved. All relevant study team members will have documented training on their respective responsibilities to be undertaken in the study.

### 6.1 Recruitment

Participants will be 6-8 week (42-56 days; both days inclusive) old healthy infants at enrolment.

Eligible parent (those above the age of 18 years i.e. legal age for consenting in Zambia) to young infants will be approached during the initial visit to MCH of George Health Centre by the study nurses with information about the study in the local language of choice. Those generally interested will be invited to walk to the research unit within 20 meters of the clinic premises for more detailed information during which motivated mothers will be recruited and taken through the written informed consent process by the study nurse. The standard operating procedures will follow Good Clinical Practice and ethical norms of research including confidentiality, provision of adequate information and allowing sufficient time for answering questions, voluntariness and clear information to confirm that there are no penalties whatsoever for refusal to participate. For illiterate participants, an independent, literate individual will witness and validate the informed consent process. A comprehension test for the participant information will be administered to assess knowledge and understanding of the basic study information, and only those meeting the minimum threshold score 7 out of 10 will proceed to sign the consent. Those scoring less, will have information re-administered and will take the comprehension test again. This will be repeated only once.

Investigators should always use good clinical judgment in considering a participant's overall fitness for inclusion in the trial. Some participants may not be appropriate for the study even if they meet all inclusion / exclusion criteria. For instance, medical, occupational or other conditions present in the parents may make safety evaluations difficult or make the infants poor candidates for retention. All infants targeted for enrollment will need to have parents/ Legally Acceptable Representative who can comprehend the purpose of the study and provide written informed consent. In addition, the families should be resident in the area without plans to leave the area during the course of the study.

Sufficient number of healthy infants will be screened with parental consent to enroll 450 infants in the study. It is expected that the recruitment will be completed over a period of 6 months.

## 6.2 Study visits

Children will be seen at the study clinic on the day of screening/enrollment/first vaccination, visits for second and third vaccinations, if applicable, and follow up visit 4 weeks after the third dose of ROTAVAC/ROTAVAC 5CM and second dose for those in the Rotarix® arm. Below is a detailed description of study activities on each scheduled visit day.

### 6.2.1 Day 0 –Screening/Enrolment Visit

Screening and enrolment will be done on the same day. An information sheet approved by IEC/IRB with details of the study will be provided and discussed with the families. If the family is interested in participating in the study a Participant ID will be assigned and the study physician will counsel the parent and signed consent will be obtained before any trial-related procedures. After obtaining written consent for the study, 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:** This will include age, ethnicity, gender, date of birth, birth weight (kg), present weight (kg), and length (cm). A copy of the Children's Clinic Card will be taken as a source document.

Complete address of the parent will be recorded.

**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, vaccines; current medication history; and family history, including history of immunodeficiency in any household member.

**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 0. The following procedures will be conducted on this day:

1. Reconfirm eligibility: If screening and enrolment/vaccination are conducted separately, a targeted (symptom-based) physical exam with vital signs (axillary temperature, heart rate and respiratory rate), and brief medical history update will be performed prior to vaccination to ensure continued eligibility.
2. Randomization: Once eligibility is ascertained, the child would be assigned a randomization code which will assign him/her to receive either ROTAVAC®, ROTAVAC 5CM or Rotarix® The participants will receive same treatment at all the applicable vaccination visits.
3. Blood collection: Collect baseline blood sample of up to 2 ml for immunogenicity assessment.
4. Vaccination: Vaccination will be done when the child comes back to normal state after blood collection. The participant will receive a dose of either ROTAVAC®, ROTAVAC 5CM or

Rotarix® as assigned by the randomization schedule. Vaccines will ONLY be administered if participant does not meet any contraindication to vaccine administration. During each vaccination visit, a section of study worksheet/eCRF will be completed that records information about vaccines received, date and time. Proper accountability of all vaccines will be maintained.

5. Concomitant Vaccination and Other Treatments: All participants will receive other EPI vaccinations concomitantly as per the national immunization schedule; the research nurses will ensure that all study participants receive the due vaccines on schedule. Use of prescription medications and any treatments / procedures during the study period will be recorded on source documents / eCRF. The name of prescription medication and duration of treatment should be recorded in the eCRF, if available.
6. 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. Clinically significant abnormal findings on targeted physical examination and vital signs will be reported in the CRF as an unsolicited AE.
7. 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. Train the parents to complete the PIDC.
8. Parents will be instructed that if an AE requiring medical attention is identified during the study, parent(s) will contact a study physician/designee and appropriate medical care will be given. Also, at all times between first and last visit, unsolicited AEs and /or SAEs including intussusception will be recorded in eCRF and managed medically as needed.
9. Inform the parent on the safety follow up home visit by the field officer on days 2 & 7, and advise him/her on the next clinic visit after 28 days.

**Safety Follow-Up:** Parents will be shown how to fill in the PIDC for 7 days (day 0-6) after vaccination. Study staff will make a physical home visit on Days 2 (+1 day) and Day 7 (+1 day) 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. Completed PIDC can be collected by the field officer at this visit or the parent can bring it to the site at the next visit, if parents were not available during the home visit. 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, at all times between first and last visit, unsolicited AEs and / or SAEs including intussusception will be recorded in eCRF and managed medically as needed.

### 6.2.2 Second Visit (Day 28)

The date for this visit will be calculated 28 days from the date of the first study vaccination. A maximum window period of +7 days will be allowed for this visit. Parents will be contacted by

phone a day 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 immunizations and ensure that EPI vaccines and investigational vaccines are administered in a timely manner. The following procedures will be carried out at this visit:

1. Check for any adverse events since last visit and record them in the source note and eCRF. Update medical history including follow-up of prior AEs.
2. Collection and review of the diary cards if not already collected by field worker earlier.
3. 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.
4. Conduct physical examination and vital examination.
5. Check for any contraindication to vaccination. Reschedule visit if the infant is experiencing an illness that is temporary contraindication to vaccination.
6. Vaccines will be administered if participant does not meet any contraindication to Investigational Medicinal Product (IMP) administration. The participant will receive a dose of either ROTAVAC®, ROTAVAC 5CM or Rotarix® as assigned by the randomization schedule. During each vaccination visit, a section of study worksheet/eCRF will be completed that records information about vaccines received, date and time.
7. Procedures 5-9 under section 6.2.1 above will be repeated.
8. Remind the parent of the safety follow up visit by the field officer on days 30 & 35, and advise him/her on the next clinic visit after 28 days.

### 6.2.3 Third Visit (Day 56)

The date for this visit will be calculated 28 days from the date of last vaccination. A maximum window period of +7 days will be allowed for each of these visits. Parents will be contacted by phone a day 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 immunizations and ensure that EPI vaccines and IMPs are administered in a timely manner. The following procedures will be carried out at this visit:

1. Check for any adverse events since last visit and record them in the source note and eCRF. Update medical history including follow-up of prior AEs.
2. 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 worksheet/eCRF.
3. Conduct physical examination and vital examination.
4. For participants in the Rotarix® arm
    - Review/update address and contact details.
    - Collection and review the diary cards if not already collected by field worker earlier.
    - Perform physical examination.

- Obtain up to 2 ml of blood for immunological assays.
- Ensure all ongoing SAEs are followed up as per the protocol.
- Review vaccination history and advise the parent on further vaccination of the infant.
- Fill up the study termination/completion form. This will be the final study visit for participants in the Rotarix® arm.

5. For participants in the ROTAVAC®, ROTAVAC 5CM arms
   - Check for any contraindication to vaccination. Reschedule visit if the infant is experiencing an illness that is temporary contraindication to vaccination.
   - Collection and review of the diary cards if not already collected by field worker earlier. Vaccines will be administered if participant does not meet any contraindication to IMP administration. The participant will receive a dose of either ROTAVAC or ROTAVAC 5CM as assigned by the randomization schedule. During each vaccination visit, a section of study worksheet/eCRF will be completed that records information about vaccines received, date and time.
   - Procedures 5-9 under section 6.2.1 above will be repeated.
   - Remind the parent of the safety follow up visit by the field officer on days 58 & 63, and advise him/her on the next clinic visit after 28 days.

### 6.2.4 Fourth Visit (Day 84)

(applicable only for ROTAVAC® and ROTAVAC 5CM arms)

The date for this visit will be calculated 28 days from the date of last vaccination. A maximum window period of +7 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. Review / update address and contact details.
2. Check for any adverse events since last visit and record them in the source note and eCRF. Update medical history including follow-up of prior SAEs. Ensure all ongoing AEs are followed up as per the protocol.
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. Perform physical examination. Review vaccination history and advise the parent on further vaccination of the infant.
6. Collect up to 2 ml of blood for immunological assays.
7. Review vaccination history and advise the parent on future vaccination of the infant.
8. Complete the study termination/completion form

### 6.2.5 Interim Contacts and Visits

Interim contacts and visits (those between regularly scheduled follow up visits) may be performed at participant's parent'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"

### 6.2.6 Management of Serious Adverse Events including Intussusception

The research team will be available on-site at all times during normal working hours and will be able to provide basic services for out-patient care. In case of serious conditions that require admission to the tertiary hospital, a study vehicle stationed on-site will be available to provide ambulance service for timely travel. Medical personnel at the sites will be trained on screening tools to promptly identify and assess any suspected cases of intussusception. The key symptoms include bloody stools, continuous vomiting, abdominal distension and/or abdominal "lumps." Children presenting with any suspected symptoms or signs will be promptly escorted for consultation with a pediatrician or pediatric surgeon or referred for hospitalization. Parents will be instructed by staff to keep close watch of the symptoms noted above and contact study staff if they are detected. The study team will be obliged to ensure rapid consultation with the team led by the pediatric surgeon who leads the intussusception surveillance work at the university teaching hospital. Any access fees related to diagnosis and care for these cases will be taken by the Sponsor. A SAE form will be completed for all cases of intussusception and notified to authorities as well as the Protocol Safety Review Team (PSRT) immediately, and follow-on full report as soon as the case is ascertained.

### 6.2.7 Schedule of events

The schedule of events / assessments performed during the trial is provided below:

| **Visit** | **V1** | **V2** | **V3** | **V4** |
|---|---|---|---|---|
| **Time point** | **D0** | **D28** | **D56** | **D84** |
| **Interval** | **6-8 weeks** | **V1+28 (+7) days** | **V2+28 (+7) days** | **V3+28 (+7) days** |
| Information process and written informed consent | X | | | |
| Collect baseline demographic data | X | | | |
| Collect/review medical history | X | | | |
| Perform physical and vital signs examination | X[a] | X | X | X |
| Check inclusion/ exclusion criteria | X | | | |
| Check withdrawal criteria | | X | X | X |
| Participant enrollment and Randomization | X | | | |

| **Visit** | **V1** | **V2** | **V3** | **V4** |
|---|---|---|---|---|
| **Time point** | **D0** | **D28** | **D56** | **D84** |
| **Interval** | **6-8 weeks** | **V1+28 (+7) days** | **V2+28 (+7) days** | **V3+28 (+7) days** |
| Collect blood sample for immunogenicity testing | X (Pre-dosing) | | X[c] | X[b] |
| Study vaccination along with EPI vaccination | X | X | X[b] | |
| Check contraindications, warnings and precautions to vaccine receipt | | X | X[b] | |
| Observe for immediate adverse events for 30 minutes post vaccination | X | X | X[b] | |
| Perform post-vaccination Vital examination and targeted physical examination if required. | X | X | X[b] | |
| Issue and Instruct participant's Parent/LAR on use of diary card | X | X | X[b] | |
| Record solicited AEs for 7 days post-vaccination | X | X | X[b] | |
| Safety follow up post vaccination by social worker | X[d] | X[d] | X[db] | |
| Record unsolicited AEs , including SAEs | X | X | X | X[b] |
| Record any concomitant medications/vaccinations | X | X | X | X[b] |
| Review interim medical history and record any intercurrent medical conditions | | X | X | X[b] |
| Participant completion of study | | | X[c] | X[b] |

D = day; PRE = pre-vaccination; AE = adverse events, SAE = serious adverse event.

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

[b] Not applicable for participants in Rotarix arm

[c] Applicable only for participants in Rotarix arm

[d] This includes visit by the health worker to the parent on day 2 and 7 after vaccination.

## 6.3 Study Termination

### 6.3.1 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.

### 6.3.2 Suspension and/or Premature Termination of the Trial

CIDRZ (in consultation with PATH) 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 Centre for Infectious Disease Research in Zambia 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. For all participants enrolled in the study, safety follow-up will be conducted as decided by the PSRT 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.

## 6.4 Lost to follow-up

To prevent lost to follow-up, the study team will ensure that valid contact details are obtained prior to enrolment, including a physical visit to the participant's home at the end of visit. Also, the

participant's parent will be reminded by phone at least one day before their scheduled visit. In the event of a missed visit, the participant 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 and have missed 2 consecutive visits 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 also be classified as lost to follow-up. There will be no replacement for participant who are lost to follow-up.

## 6.5 Use of concomitant vaccine(s)/medication during the study

Routine childhood vaccines mandated by EPI should be given concomitantly with the study vaccine as per the national immunization schedule. All infants will receive all EPI vaccines as per routine schedule. The EPI schedule consists of administering Diphtheria, Tetanus, Pertussis, *Haemophilus influenzae type b* and Hepatitis B vaccine (DTwP-Hib-HepB), Pneumococcal conjugate vaccine and OPV at 6, 10 and 14 weeks and IPV at week 14 (when switched to in Zambia). The study participants may receive additional doses of OPV as required during special immunization rounds. BCG vaccine will be offered to infants who did not receive it at birth.

Concomitant and other vaccines used during the study will be reported in the Concomitant vaccination section of the CRF.

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.

## 6.6 Unblinding procedure

The study will be conducted under open-label condition. This means that the patient, clinical site, CRO, sponsor and PATH will be unblinded to the treatment assignments. Therefore, unblinding procedures are not applicable. The study laboratory will be kept blinded for the treatment administered until the end of testing.

## 6.7 Clinical procedures

**Vital Signs**

- Temperature in degrees Celsius (recorded to the nearest 0.1 degree) will be measured by axillary thermometer for infants.
- Respiratory rate will be recorded in breaths per minute.
- Heart rate in beats per minute will be measured manually.
- Height/length will be measured and recorded to the nearest 0.1 cm.
- Weight will be measured in kg and recorded to the nearest 0.01 kg.

**Physical Examination**

Full physical examination will include assessment of vital signs, head, eyes, ears, nose, oropharynx, neck, chest (auscultation), lymph nodes (neck, supraclavicular, axillary, inguinal),

abdomen (auscultation and palpation), genitourinary, musculoskeletal, skin (especially injection sites), and neurological.

**Targeted physical examination**

A focused physical examination based on symptoms reported by the participant and the presence or absence of solicited adverse events collected to assess vaccine reactogenicity.

**Medical History**

A comprehensive medical history will be collected including details of any previous vaccinations and reaction to vaccinations, participation in clinical trials, surgery, previous hospitalization, allergy to food/drugs, current medication and history of any chronic or recurrent medical conditions.

An interval medical history will consist of inquiring regarding changes since the last medical history discussion (healthcare events, signs, symptoms and changes in use of prescription or nonprescription drugs or herbal preparations).

### 6.8 Termination of withdrawn study participant

A participant who can no longer continue with the study for whatever reason, will need to be concluded in the eCRF via the termination page which will mark the close of the case. The possible reasons will include:

1. Voluntary withdrawal from the study by the participant
2. Investigator decides to withdraw the participant from the study for safety or other clinical reasons
3. Participant is confirmed as lost to follow up.

This site principal investigator must review each case and confirm the status before completion of the termination form.

## 7 LABORATORY EVALUATIONS /REQUIREMENTS

### 7.1 Sample collection, distribution and storage

To quantitate antibody concentrations elicited by the study vaccines, a baseline blood sample will be collected from all participants before the first vaccination and four weeks after the last vaccination (after 3rd dose for ROTAVAC®/ROTAVAC 5CM and after 2nd dose for Rotarix®).

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. A maximum of 2 ml of blood, from a peripheral vein, will be collected by an aseptic technique into gel (SST) tubes. A maximum of 2 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. Clotted blood in the tube will be centrifuged to obtain serum. Designated laboratory personnel at the site will aliquot the samples into two aliquots before storage. Serum will be distributed in the two aliquots such that the primary receives at least 0.5 ml of the serum and the remaining is transferred to the second cryotube. All serum samples will be labelled appropriately. The two samples will be stored in separate controlled- freezers at or below

–20°C until the time of shipment to the testing laboratories. 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 each will be sent separately to each of the two testing laboratories (i.e., laboratory at CMC Vellore, India and 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 (see below) and storing of samples till study completion.

The Sponsor will be responsible for obtaining the appropriate Material Transfer Agreement (MTA) from the local National Health Research Authority.

### 7.2 Immunological laboratory assays

Serological assays for quantification of anti-rotavirus IgA antibodies by ELISA will be performed at the following labs.

| Name of Laboratory | Assay specification | Number of participants (Pre and Post-vaccination samples) | | |
|---|---|---|---|---|
| | | ROTAVAC® | ROTAVAC 5CM | Rotarix® |
| The Wellcome Trust Research Laboratory, Christian Medical College, Vellore, India | Estimation of serum anti-rotavirus IgA antibodies by ELISA using WC3 as the viral lysate | All | All | All |
| Cincinnati Children's Hospital Medical Center (CCHMC), Division of Infectious Diseases, Cincinnati, Ohio, USA. | Estimation of serum anti-rotavirus IgA antibodies by ELISA using 89-12 as the viral lysate | 50 | 50 | 50 |

If warranted, the samples may be subjected to additional tests to explain the immune responses seen in the primary analysis.

### 7.3 Assays qualification, standardization, validation

The Wellcome Trust Research Laboratory, Christian Medical College, Vellore, India has a validated assay to measure serum anti-rotavirus IgA antibodies using WC3 as the lysate which has been used in earlier clinical trials of ROTAVAC® vaccines. Cincinnati Children's Hospital Medical Center (CCHMC), Division of Infectious Diseases, Cincinnati, Ohio, USA also has a validated

assay for estimating serum anti-rotavirus IgA antibodies using 89-12 as the viral lysate which is the parent strain of Rotarix® vaccine.

### 7.4 Future use of stored samples

Any remaining serum samples will be retained for a maximum of 5 years at the laboratories in case further investigations are required after completion of the study. No genetic testing will be performed on samples collected in the study. After this period these samples will be destroyed after written communication from PATH.

### 7.5 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.

## 8 SAFETY ASSESSMENT AND REPORTING

### 8.1 Definitions

#### 8.1.1 Adverse Event (AE)

An adverse event is 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 unfavorable 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. 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

**Solicited AEs** are pre-specific local and systemic 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. Participants 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 will not be considered solicited events and should be reported as unsolicited AEs.

**Unsolicited AEs** are any AEs reported spontaneously by the participant, 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.

### 8.1.2 Adverse drug reaction (ICH) / Suspected Adverse Reaction (FDA)

An adverse drug reaction (ADR) is any AE in which the casual relationship to the investigational vaccine is at least a reasonable possibility, i.e., the relationship cannot be ruled out. Having a reasonable suspected causal relationship to the investigational vaccine qualify as ADR. The concept of "reasonable causal relationship" is meant to convey in general that there are facts (evidence) or arguments to suggest a causal relationship.

**Unexpected adverse drug reaction** is an adverse reaction, the nature or severity of which is not consistent with the information in the product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).

### 8.1.3 Serious Adverse Event (SAE)

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 a SAE. In absence of an AE, a hospitalization or prolongation of a hospitalization should not be reported as a 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.

### 8.1.4 Reporting Period and Parameter

Safety events are reported from the time of the first study vaccination through completion of the study at 4 weeks after the final vaccination. Specifically,

- Immediate adverse events will be collected for 30 minutes after each vaccination.
- Solicited AEs to assess systemic reactogenicity will be collected for 7 days after each vaccination. If a solicited AE started during the 7 days (Day 0-6) post vaccination and continues beyond the 7 days it will continue to be reported as a solicited AE.
- Unsolicited AEs and SAEs will be collected from day 0 up to four weeks after last vaccination inclusive. Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. AEs characterized as intermittent require documentation of onset and duration of each episode. No specific safety laboratory tests are planned in the study; however, clinical and laboratory investigations conducted in order to diagnose or treat a condition in a study participant will warrant documentation of all the key laboratory results. Events will be followed for outcome information until resolution or stabilization.

## 8.2 Severity of Adverse Events

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/breast feeding less than usual / no effect on normal activity |
| | 2/Moderate | Eating/breast feeding less than usual / interferes with normal activity |
| | 3/ Severe | Not eating/breast feeding 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 are graded with the worst severity grade during the illness/symptoms.

### 8.3 Causality of Adverse Event

The study investigator/s will determine the causal relationship between the study product and the AE. The causality assessment is made on the basis of the available information at the time of reporting and can be subsequently changed according to follow-up information. Determining of causality is based on clinical judgment and should take into consideration the following factors:

- 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 investigator/s 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.

## 8.4 Follow-up of Adverse Event

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.

PATH 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 PSRT within the time frames outlined in section 8.7.2.

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

## 8.5 General Guidance on Recording Adverse Events

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 a 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 a 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.

## 8.6 Reporting of SAE

### 8.6.1 Investigator Reporting to Sponsor

Within 24 hours of an investigator's awareness of a SAE as defined in the protocol, a SAE form must be completed and submitted to PATH and Sponsor as per reporting requirement. An SOP will be developed to include the contact details of PATH and CIDRZ and will contain specific details of the process including timelines.

The investigator must not wait to collect additional information to fully document the event before submitting the SAE form. When additional information become available follow-up submission must be completed. The initial SAE form should be completed with all information known at the time and should include as much information as possible on the following:

- Name and contact of the investigator submitting the AE/SAE report
- Participant ID number
- Date participant received study vaccine/s, including cohort group if applicable
- Description of the serious adverse event and date of event onset
- Investigator's assessment of severity, causality and expectedness
- Action taken and current status
- If available, any diagnostic test reports or hospital records that may help the PATH to evaluate the SAE

The investigator will be responsible for notifying UNZABREC and WIRB (through PATH) as per the respective EC/IRB's reporting requirement.

The sponsor and PATH must be notified, within 15 calendar days after first knowledge by the investigator, when there is a suggestion of a change in the nature, severity or frequency of expected adverse drug reactions or when new risk factors are identified. The basis on which these assessments are made should be included. An SOP will be developed to include specific details of responsibilities of the personnel, process and timelines.

### 8.6.2 Notification and Review of SAEs

The Medical monitor (PATH) is responsible for evaluating SAEs submitted by the investigator and for notifying the protocol safety review team (PSRT) within 24 hours to convene an expedited PSRT review if any criteria outlined in section 9.2 or 9.3 is met. The PSRT will be responsible for reviewing adequacy of information and query investigator team to supplement information or act as a consultant (if required) on treatment approach. The medical monitor must also indicate whether he/she concurs with the details of the report provided by the site PI. The medical monitor will also be responsible for conducting the expectedness analysis of the SAE and inform the team on the reporting requirement for ZAMRA. Medical monitor from PATH serving as technical consultants will provide technical guidance regarding SAE management including classification and reporting.

### 8.6.3 Sponsor Reporting to Regulatory Agency

The Sponsor will be responsible for safety reporting to the appropriate regulatory authorities within the specified time period of notification.

- All fatal and life-threatening, serious, unexpected adverse drug reactions should be reported within 7 calendar days after first knowledge by the applicant. The initial notification must be followed by as complete a report as possible, within an additional 8 calendar days.
- Serious, unexpected adverse drug reactions that are not fatal or life-threatening must be reported as soon as possible, and not later than 15 calendar days after first knowledge by the applicant.
- Adverse drug reaction that are serious and already known (described in Investigator's Brochure or the Summary of Product characteristics (SPC)/Prescribing Information) there will be no fixed time limit set. These cases will be reported as soon as the necessary information is available.
- Any information, that may in any way influence the benefit-risk assessment of a medicine or that would be sufficient to consider changes in the administration of the medicine or in the overall conduct of a clinical trial, must be reported to the PSRT. The applicant must submit this information to the PSRT within three calendar days of first knowledge by the applicant.

## 9 SAFETY OVERSIGHT

For this study, a diligent mechanism for review of safety of the participants will be created. It will ensure safety oversight by the site PI and Protocol Safety Review Team (PSRT) with the provision of expedited review by the team in case high grade AEs are reported. The study also has provisions for referring to infectious disease specialists in case a consultation is recommended by the PSRT.

### 9.1 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.

### 9.2 Routine Reviews by Protocol Safety Review Team (PSRT)

An internal team, the PSRT, will be established to examine safety at periodic intervals. The Protocol Safety Review Team (PSRT), a group of physicians which includes the principal investigator, other physicians from the site and the medical officer(s) from PATH will routinely monitor safety throughout the duration of the trial. The PSRT will be chaired by a 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 PSRT. 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 PSRT safety review will be conducted by teleconference occurring approximately fortnightly to monthly (depending on rate of enrolment) 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 assigned medical monitor/officer will be responsible for informing the other members and convening the meeting. The PSRT may seek independent expert medical opinion as dictated by the occurrence of certain events.

In addition to safety review, the PSRT 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 PI or medical monitor will also notify the PSRT for ad hoc safety reviews whenever it is aware of SAE or adverse events that meet pre-specified study pause criteria as per Section 9.3

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

### 9.3 Study Pause Rule

This study has no formal pause rules. However, if during the expedited safety reviews, the PSRT identifies safety concerns that warrant a safety pause, the PSRT will notify the Sponsor, PATH and BBIL. Examples of safety concerns which may be considered (but may not necessarily trigger automatic pause) include:

- Two or more participants experience the same related serious adverse event over a period of two weeks.
- Two or more participants experiencing a grade 4 solicited systemic adverse event over a period of two weeks
- Two or more participants with the same severe (grade 3) solicited systemic adverse event or laboratory abnormality, within seven days.
- Any case of intussusception with Diagnostic Certainty Level 1 as per Brighton Collaboration Intussusception Working Group* within 30 days of vaccination.

*Brighton collaboration definition of **Diagnostic Certainty Level 1of** intussusception
• Surgical criteria: The demonstration of invagination of the intestine at surgery
and/or
• Radiologic criteria: The demonstration of invagination of the intestine by either air or liquid contrast enema or the demonstration of an intra-abdominal mass by ultrasound with target sign or doughnut sign on transverses section and a pseudo-kidney or sandwich sign on longitudinal section that is proven to be reduced by hydrostatic enema on post reduction ultrasound
and/or • Autopsy criteria: the demonstration of invagination of the intestine at autopsy

Based on the criteria if a decision is made to pause the study, all enrollment and further vaccinations will be paused, pending consultation with independent infectious disease expert, EC and ZAMRA.

PATH/CIDRZ retains the right to temporarily suspend or prematurely discontinue this study at any time related to safety. If the study is stopped or suspended prematurely, the sponsor (CIDRZ) will inform the EC and ZAMRA about the decision and the reasons for termination or suspension. If such action is taken, all efforts must be made to ensure the safety of the participants enrolled in the study. In case of premature study or study clinic closure, the monitor will conduct all activities as indicated in the close out monitoring visit.

### 9.4 Study Pause Procedure

At scheduled study review, the PSRT may also identify adverse events that could potentially qualify as pause criteria.

If the PI (or designee), the medical monitor or any other member of the PSRT identifies and propose the study be paused on a discretionary basis, all vaccinations and enrollment will be suspended. The PSRT reviews will be summarized with consensus recommendations to the study Sponsor as to whether there are safety concerns and whether the study should continue without change, be modified, or be stopped.

If at any time, a decision is made to discontinue administration of study product in all participants, expeditious notification will be provided by the Sponsor to the ZAMRA and the EC within 48 hours.

If the Sponsor re-starts the study after PSRT review and recommendation, enrollment and vaccination may resume.

## 10 DATA HANDLING AND RECORDKEEPING

The Principal Investigator is responsible for assuring that the data collected are complete, legible, attributable, accurate, and recorded in a timely manner. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the eCRF should be consistent with source documents, and a quality control plan will be put in place to ensure the same. All source documents including laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the site PI or designee.

The Data Management CRO (DMC) is responsible for data management activities, including setting up the data base, allocation of access to various teams with appropriate access authority levels, quality review, analysis, and reporting of the study data according to SOPs.

The study site will maintain appropriate medical and research records for this trial, in compliance with ICH-GCP, regulatory, sponsor and institutional requirements for the protection of confidentiality of participants.

### 10.1 Definitions

#### 10.1.1 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),

### 10.1.2 Source Documents

For this study source documents 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.
- AE evaluations.
- Concomitant medications.
- Post Immunization Diary Card (PIDC).
- Certified copies of hospital records.
- Study visit worksheets.

## 10.2 Data Capture Methods (Case Report Form Development and Completion)

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 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 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 study 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 PATH's training requirements and must only access the electronic data capture tool using the unique user account provided by the Sponsor. 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.

### 10.3 Data Management

The DMC responsible for data management will develop a Data Management Plan (DMP) and corresponding database compliant with International Conference on Harmonization (ICH) requirements for approval by PATH before implementation. The Plan will describe roles of stakeholders and specific procedures to ensure appropriate handling of data at all steps of the data management process to assure a valid and high-quality database at the end of the study, ready for analysis.

Entered data will be verified by an independent monitoring CRO who will query the site for potential discrepancies. The site Investigator, or designee, will be responsible for resolution of queries and appropriate documented changes in the database. DMC will perform all activities as per their standard operating procedures (SOPs). All study specific processes will be described in the Data Management Plan (DMP). Coding of medical history and adverse events will be performed using MedDRA and coding of medications will be performed using WHO Drug Dictionary. eCRFs and any supporting documentation should be available for retrieval or review at any given time.

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 DMC 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.

### 10.4 Retention of Study Records

The site PIs are responsible for retaining study records until 5 years after the investigation is discontinued or completed and the UNZABREC, NHRA and ZAMRA is notified. No records will be destroyed without the written consent of PATH. PATH will inform the Investigator in writing of the need for record retention and will notify the Investigator in writing when the trial related records are no longer needed.

These records are also to be maintained in compliance with local EC and local authority medical records retention requirements, whichever is longest. Storage of all trial-related documents will be such that confidentiality will be strictly maintained to the extent required by local law.

## 11 STATISTICAL CONSIDERATIONS

### 11.1 Overview and General Considerations

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

Serum anti-rotavirus IgA antibody concentrations 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.

## 11.2 Randomization Procedures

Healthy infants will be randomized to receive one of the three study vaccines in a 1:1:1 ratio. For vaccine allocation, a randomization blocking scheme will be used in order to ensure that balance between vaccine groups will be maintained. Infants will be randomized sequentially in the order that they are enrolled. The randomization scheme that contains a participant identification number and the corresponding randomization assignment will be generated using computer software prior to the initiation of the study and provided to the designated site personnel.

## 11.3 Sample Size and Power

The immunogenicity of the study vaccines will be primarily assessed by geometric mean concentrations (GMCs) of serum anti-rotavirus IgA antibodies at 28 days after the last dose of the study vaccine, along with its two-sided 95% confidence interval (CI), by exponentiating the corresponding $log_{10}$-transformed mean and its two-sided 95% CI limits.

Table 2 below presents the precision of the 95% CI of the GMC in log10-transformed scale per group under different assumptions of group sizes and assumed standard deviation (SD) of log10-transformed anti-rotavirus IgA concentrations.

**Table 2. The half width of a two-sided 95% CI of the GMC in log10-transformed scale based on various group sizes and assumed standard deviation (SD) of log10-transformed anti-rotavirus IgA concentrations**

| Assumed SD of log10-transformed anti-rotavirus IgA concentrations | Number of evaluable participants per group | | |
|---|---|---|---|
| | 120 | **135** | 150 |
| 0.50 | 0.090 | 0.085 | 0.081 |
| 0.55 | 0.099 | 0.094 | 0.089 |
| **0.60** | 0.108 | **0.102** | 0.097 |
| 0.65 | 0.117 | 0.111 | 0.105 |
| 0.70 | 0.127 | 0.119 | 0.113 |

With a sample size of 150 for each group and an assumed SD of $log_{10}$-transformed anti-rotavirus IgA concentrations of 0.60, the half width of a two-sided 95% CI for anti-rotavirus IgA GMC will be 0.102 in $log_{10}$-transformed scale for each group, assuming a dropout rate of 10%.

Power to show that the ratio of anti-rotavirus IgA GMCs in ROTAVAC 5CM group to that in ROTAVAC® group is at least 0.5 was calculated using two-sample t-test according to various

group sizes, different assumed SDs of $log_{10}$-transferred anti-rotavirus IgA concentrations and equal true underlying geometric mean and is provided in Table 3.

**Table 3. Power to show comparability of immune response in terms of GMCs for rotavirus-specific serum IgA antibody concentrations between ROTAVAC 5CM and ROTAVAC® groups, based on various group sizes, different assumed SD of log10 anti-rotavirus IgA concentrations, and equal true geometric mean**

| Assumed SD of log10 anti-rotavirus IgA concentrations | Number of evaluable participants *per* group | Power to demonstrate that the lower limit of 95% CI of the ratio of GMC between groups is > 1/2 |
|---|---|---|
| 0.50 | 120 vs. 120 | >99% |
| | 135 vs. 135 | >99% |
| | 150 vs. 150 | >99% |
| 0.55 | 120 vs. 120 | 99% |
| | 135 vs. 135 | 99% |
| | 150 vs. 150 | >99% |
| **0.60** | 120 vs. 120 | 97% |
| | **135 vs. 135** | **98%** |
| | 150 vs. 150 | 99% |
| 0.65 | 120 vs. 120 | 95% |
| | 135 vs. 135 | 97% |
| | 150 vs. 150 | 98% |
| 0.70 | 120 vs. 120 | 91% |
| | 135 vs. 135 | 94% |
| | 150 vs. 150 | 96% |

Assuming the true standard deviation of $log_{10}$-transformed anti-rotavirus IgA concentration is below than or equal to 0.60, with a sample size of 150 and a dropout rate of 10% per group (135 evaluable participants per group), the study has power of 98% to detect at least 0.5 of GMC ratio between ROTAVAC®5CM and ROTAVAC® groups.

With 150 infants vaccinated per group, this study design allows a greater than 90% chance of observing at least one AE if true incidence is 1.53%. Conversely, if no AEs are observed in 150 vaccine recipients, the study will be able to rule out AEs occurring at a rate of approximately 2.5% or above based on the upper bound of the two-sided 95% CI.

## 11.4 Analysis Populations

### 11.4.1 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.

### 11.4.2 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.

### 11.4.3 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 before the database is locked.

### 11.4.4 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.

## 11.5 Analytical Methodology

### 11.5.1 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 ANOVA 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 up to 1 month prior to enrolment will be summarized by anatomical therapeutic chemical (ATC) classification and preferred drug name coded by WHO Drug Dictionary (WHO DD) for each vaccine group.

### 11.5.2 Analysis of primary objective

The GMCs of serum anti-rotavirus IgA antibodies at 28 days after the last dose of ROTAVAC® or ROTAVAC 5CM will be calculated along with its two-sided 95% CI, by exponentiating the corresponding log10-transformed mean and its two-sided 95% CI limits.

To compare the immunogenicity of ROTAVAC® and ROTAVAC 5CM at 28 days following the last dose of the study vaccine, the following hypothesis will be tested,

$H_0$: $GMC_{ROTAVAC\ 5CM} / GMC_{ROTAVAC®} \leq ½$

$H_1$: $GMC_{ROTAVAC\ 5CM} / GMC_{ROTAVAC®} > ½$

The test will be done at 28 days following the last dose of ROTAVAC 5CM and ROTAVAC® 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 ROTAVAC 5CM and ROTAVAC® 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 ROTAVAC 5CM and ROTAVAC® groups is larger than 1/2, ROTAVAC 5CM is considered to be non-inferior to ROTAVAC®.

The comparison of the GMCs between the two study vaccines will be also 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.

The percentage of participants with seroconversion, seropositivity, and seroresponse will be computed for the ROTAVAC 5CM and ROTAVAC® 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.

The Geometric Mean Fold Rise (GMFR; GMC post vaccination / GMC at baseline) will be provided with its two-sided 95% CIs, by exponentiating the difference in means of $log_{10}$-transformed anti-rotavirus IgA concentrations between post vaccination and baseline and its two-sided 95% CI that will be obtained by paired t-test method.

In addition, a reverse cumulative distribution (RCD) curve will be created by vaccine group and visit.

### 11.5.3 Analysis of secondary objectives

#### 11.5.3.1 Analysis of secondary safety objective

The percentage of participants with safety endpoints along with its exact two sided 95% CI based on Clopper-Pearson method will be provided for each vaccine group.

**Reactogenicity:**

The number and percentage of participants experiencing immediate adverse events within 30 minutes post each vaccination and solicited post-vaccination reactogenicity (fever, diarrhea, vomiting, decreased appetite, irritability, decreased activity level) during the 7-day period after each vaccine will be tabulated by vaccine group and severity. For the percentage, an exact two-sided 95% CI will be provided using Clopper-Pearson method.

**Unsolicited AEs:**

The number and percentage of participants experiencing immediate adverse events within 30 minutes, unsolicited AEs 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 twill be tabulated by ATC classification and preferred drug name of WHO DD for each vaccine group.

#### 11.5.3.2 Analysis of secondary immunogenicity objective

The immunogenicity analysis for Rotarix® will be descriptive. The GMC and GMFR will be computed for the Rotarix® group along with its two-sided 95% CI using the same methods outlined in Section 11.5.2. The percentage of participants with seroconversion, seropositivity, and seroresponse will be provided for the Rotarix® group along with its exact two-sided 95% CI using Clopper-Pearson method.

A reverse cumulative distribution (RCD) curve will be created for Rotarix® group by visit.

### 11.5.4 Analysis of exploratory objective

The exploratory analysis conducted on immune response measured by ELISA using 89-12 (G1P8 virus) as a substrate in a subset of the samples collected (50 per group) will be descriptive. GMCs of serum anti-rotavirus IgA antibodies using 89-12 virus as a substrate will be calculated for each group along with its two-sided 95% CI, by exponentiating the corresponding log10-transformed mean and its two-sided 95% CI limits.

The percentage of participants with seroconversion and seropositivity will be provided for each group along with its exact two-sided 95% CI using Clopper-Pearson method.

GMFR will be provided with its two-sided 95% CIs, by exponentiating the difference in means of $log_{10}$-transformed anti-rotavirus IgA concentrations using 89-12 virus as a substrate between post vaccination and baseline and its two-sided 95% CI that will be obtained by paired t-test method.

### 11.5.5 Multiplicity

No multiplicity adjustment will be carried out as ROTAVAC 5CM and ROTAVAC® will be primarily compared based on a single primary endpoint.

### 11.5.6 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 treatment group. All withdrawn participants 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 as applicable.

## 11.6 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.

# 12 QUALITY ASSURANCE AND QUALITY CONTROL

Guidance on internal and external processes to assure effective protocol implementation, quality of the research conducted and compliance with sponsor/PATH's and applicable regulatory requirements.

## 12.1 General Considerations

The study will be conducted in full compliance with the protocol and ICH GCP to provide public assurance that the rights, safety, and well-being of trial participants are protected, and that the clinical trial data are credible. To ensure quality and standardization, the site will develop Standard Operation Procedures (SOPs) for key protocol procedures and conduct the study guided by the study Manual of Procedures or other written guidelines. The site will also develop routine operational checks to verify that critical protocol requirements and procedures are executed correctly and completely at the time the work is being performed. Prior to the initiation of the study, PATH and / or the CRO delegated by PATH will conduct protocol training, including applicable SOPs, for study staff.

The investigational site will provide direct access to all trial-related site, source data/documents, and reports for the purpose of monitoring and auditing by the PATH/designee, and inspection by local IRB/EC and regulatory authorities.

## 12.2 External Monitoring

PATH/designee is responsible for ensuring that the study is conducted in accordance with ICH GCP and regulatory requirements. For this purpose, monitors under contract from PATH will provide external monitoring for this study. A site initiation visit will be conducted prior to beginning the study, and monitoring will be conducted at initiation, during, and at closeout of the study. During the course of the study monitors will visit the clinical site at intervals to verify compliance to the protocol; completeness, accuracy, and consistency of the data and study product accountability; adherence to ICH GCP and applicable regulations. As needed and when appropriate, the monitors will also provide clarifications, additional training to help the site resolve issues identified during the monitoring visit. As appropriate and informed by risk assessment, remote centralized monitoring activities may be considered in place of or to supplement onsite monitoring. These may include analysis of data quality (e.g. missing or inconsistent data, outlier data), identify data trend not easily detected by onsite monitoring and performance metrics (e.g., screening or withdrawal rates, eligibility violations, timeliness and accuracy of data submission).

The extent and frequencies of the monitoring visits will be described in a separate Study Monitoring Plan developed prior to study initiation. The investigator will be notified in advance of the scheduled monitoring visit. The monitor should have access to all trial related locations, participant medical records, study product accountability and other study-related records needed to conduct monitoring activities. The PATH/designee will share the findings of the monitoring visit, including any corrective actions, with the site investigator. The site PI and the monitor must agree to cooperate to ensure that any problems detected in the course of these monitoring visits are resolved in a predefined timeframe.

## 12.3 Independent Auditing

PATH or its designee may audit the study to ensure that study procedures and data collected comply with the protocol and applicable SOPs at the clinical site and that data are correct and complete. The site PI will permit auditors (employees of the PATH or employee of a company designated by the PATH) to verify source data validation of the regularly monitored clinical study. The auditors will compare the entries in the eCRFs with the source data and evaluate the study site

for its adherence to the clinical study protocol and GCP guidelines and applicable regulatory requirements.

### 12.4 Regulatory Agency Inspection

The site PI must be aware that regulatory authorities, including EC/IRB may wish to inspect the site to verify the validity and integrity of the study data; and protection of human research participants. The site PI will notify the Sponsor, and PATH within 24 hours following contact by a regulatory authority. The site PI must make the relevant records available for inspection and will be available to respond to reasonable requests and audit queries made by authorized representatives of regulatory agencies. The site PI will provide the Sponsor and PATH with copies of all correspondence that may affect the review of the current study or his qualification as an investigator in clinical studies. During the audits or inspections, the participant's confidentiality will be maintained at all times to the extent permitted by the law. Site visit logs will be maintained at the study site to document all visits. PATH will provide any needed assistance in responding to regulatory audits or correspondence.

## 13 ETHICAL CONSIDERATIONS (AND INFORMED CONSENT)

### 13.1 Ethical Standards

This study will be conducted in accordance with the ethical principles set forth in the World Medical Association Declaration of Helsinki / The Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines for Biomedical Research Involving Human Participant and in conformity with ICH GCP and "Guidelines on Regulating the Conduct of Clinical Trial in Human Participants" as set forth by Zambia Medicines Regulatory Authority (ZAMRA).

NB. The Declaration of Helsinki provides detail on what must be included in a protocol: funding, sponsorship, affiliations and potential conflicts of interest, incentives to participate, compensation for harm and post-trial access to drugs and care.

International Ethical Guidelines for Biomedical Research Involving Human Trial participants CIOMS 2002 also provides guidance on ethical requirements.

### 13.2 Ethical Review

This study will be conducted under the auspices of the University of Zambia Biomedical Research Ethics Committee. In addition, the study will also be reviewed by Western Institutional Review Board and an approval would be required before initiation of enrolment.

These committees will review and approve the protocol, informed consent form, and any recruitment materials (advertising or informational material); including any modifications to these documents prior to, or during the study. All changes to the protocol or informed consent form must be reviewed and approved by the IEC, WIRB and PATH prior to implementation, except where necessary to eliminate apparent immediate hazard to study participants. The investigator is also responsible for obtaining continuing review throughout the duration of the study in accordance with existing regulations. The site will be responsible for submitting to and obtaining initial and subsequent annual/biannual review approval from the UNZABREC, NHRA and ZAMRA as applicable.

## 13.3 Informed Consent Process

Eligible parent (those above the age of 18 years i.e. legal age for consenting in Zambia)/LAR of potential participants will typically be approached during routine child immunization clinics by study staff with general information about the study. Those interested, will be invited to the Research Unit within 20 meters of the clinic and will be offered further information about the study. Motivated parents/LAR will then be provided one on one study information in the local language of their choice. They will be taken over the approved participant information sheet going through details of what the study is about, what is required to participate, procedures, benefits of participation and risks involved, number of clinic visits required etc.

The participant's parent/LAR will be given a copy of the ICF and allowed ample time to read the consent form, 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 participant/parent has the right to decline to participate or subsequently withdraw from the study at any time without prejudice.

A simple comprehension test will be administered at the end of the session. Participant's parent/LAR scoring at least 7 out of 10 basic questions will be deemed sufficiently informed, and those scoring less will be taken through the participant information again (repeat will be done only once) until they are able to pass the comprehension test. After this, the parent or appropriate legally acceptable representative will be required to sign two originals of the ICF; one for the study file, and another for themselves to keep.

Those who are illiterate will require an independent witness of their choice to sign attesting to the process; while the parent/LAR will be required to thumb print the form.

## 13.4 Participant Confidentiality

The investigators, PATH and all staff from organizations involved with the implementation of the trial must ensure that the participant's confidentiality is maintained. Personal identifiers will not be included in any study report. All study records will be kept confidential to the extent provided by national and local laws. 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. Direct access 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.

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.

## 13.5 Reimbursement

Participants will be compensated for their time and effort in this study, and be reimbursed for travel to study visits. There is no direct payment for participating in this study. However, a reimbursement [redacted] has been proposed for approval by the ethics committee. This amount is expected to be reasonably sufficient to compensate for time, inconvenience and travel cost, and yet not too much to unduly induce them into participating. This information will be provided in the participant information sheet.

Participants/parents of study participants will not be charged for study medication, research clinic visits, research-related examinations, or research-related laboratory tests.

## 13.6 Risk and Benefits

### Benefits to Study Participants

Potential benefits to enrolled participants in the current trial could be protection from rotavirus gastroenteritis, the identification of undetected medical conditions during medical examination, education about rotavirus and diarrhea and its prevention and treatment, education on personal hygiene and access to medical care for the participant for any illnesses occurring during the trial period.

### Potential Risks

**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 its SOP 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.

In the case of expected and unexpected reactions after the use of vaccines, study participants will receive appropriate medical care and treatment. The sponsor (CIDRZ) will ensure coverage for the cost of medical treatment and resolve these cases according to current standard of care in Zambia.

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 an appropriate clinic outside of the institution.
- 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.

**Risks to Privacy:** Anyone participating in research using their real name and medical information can face a loss of privacy. These risks are mitigated by using unique IDs in place of a participant's name, restricting access to study information, and not naming or identifying a participant in any publication.

**Foregoing approved rotavirus Vaccination:** Rotarix® is being provided free of cost by the national immunization plan whereas ROTAVAC® and ROTAVAC 5CM have not yet been studied or approved for use in Zambia. However, ROTAVAC® has been administered to more than 12,000 children in clinical trials in India (including one study proving efficacy of the vaccine and another study proving non-inferiority of immune response vs. Rotarix®) and has already been administered to millions of children in India under national immunization plan. ROTAVAC 5CM has the same virus strain as ROTAVAC® with addition of some stabilizers and excipients of GRAS category and the immune response has been shown to be similar to WHO approved ROTAVAC® vaccine. Two third of the participants will receive either ROTAVAC® or ROTAVAC 5CM vaccine which has not been studied in Zambian population and the immune response cannot be predicted. If the immune response in the population is not as per the expectation, then there is a risk that the infant may not be protected against rotavirus infections.

### 13.7 Compensation for Research Related Injury

The Sponsor (CIDRZ) will provide appropriate treatment for injuries directly attributable to participation. Clinical trial insurance in favor of participants will be undertaken to cover injury and other trial related eventualities including compensation for disability and death. Information about this will be provided in the participant information as well as how to access it.

## 14 PROTOCOL MODIFICATIONS AND AMENDMENTS

The protocol will not be amended without prior written approval from PATH. In case the protocol is amended, the Investigator will submit and, where necessary, obtain approval from the Ethics Committee for all protocol amendments and changes to the ICF document. Submission and approval of the amended protocol to ZAMRA will be the responsibility of the sponsor.

## 14.1 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 UNZABREC and WIRB (through PATH) as an amendment to the protocol by the investigator.

## 14.2 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 NHRA, ZAMRA, UNZABREC and WIRB. The Sponsor, PATH and Investigator will agree to implement / adhere to such modifications only after written approval from ZAMRA and UNZABREC.

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 immediately. The IEC / IRB should be informed immediately.

## 14.3 Protocol Deviations and Violations

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 enrolment 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 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.

## 15 FINANCING AND INSURANCE

The trial is supported by a grant from Bill and Melinda gates Foundation to PATH under Opportunity ID: OPP1168597. ROTAVAC® and ROTAVAC 5CM vaccine are provided by Bharat Biotech International Ltd., India.

The sponsor will secure insurance that complies with local regulatory requirements to cover for research related injury. The vaccine manufacturer will self-insure against product liability.

## 16 PUBLICATION POLICY

It is understood by the investigators that the information generated in this study will be used by PATH in connection with the development/use of the product and therefore may be disclosed to government regulatory agencies in various countries. PATH (and manufacturer) 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.

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# APPENDIX 1 - 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 2 – APPLICABLE DEFINITIONS FOR GRADING OF AES

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.

## Addendum to Protocol: CVIA 066

# An Open-label, Randomized, Controlled, Single Centre, Phase IIb Study to Assess the Immunogenicity, Reactogenicity and Safety of Three Live Oral Rotavirus Vaccines, ROTAVAC®, ROTAVAC 5CM and Rotarix® in Healthy Zambian Infants.

## Addendum Number: 01; Dated 25, October, 2018

The purpose of this addendum is to emphasize the changes that are to be made in the study protocol, version number 1.0; Version date: date 11 July, 2018.

Namely, after the above mentioned version of the protocol was submitted and approved by University of Zambian Biomedical Ethics Committee for the CIDRZ protocol to be conducted at George Health Centre and Zambia Medicines Regulatory Authority (ZAMRA), 4 minor changes are to be implemented in the protocol.

The following outlines these changes:

| Place within the document | Section | Present text | Corrected/updated text |
|---|---|---|---|
| Title page | NA | Trial Registration: Submission in process | Trial Registration: ClinicalTrial.gov study reference no NCT03602053 |
| Page 12<br>(New addition) | Administrative structure | NA | [redacted]<br>Clinical Research Officer<br>PATH - 15th Floor, Dr Gopal Das Bhawan,<br>28, Barakhamba Road, Connaught Place,<br>New Delhi - 110001 India<br>Phone: [redacted]<br>Email: [redacted] |
| Page 10, 44-46 | Participating Institutions<br>5.1.3 Presentation and Formulation | George Research Clinic | |

| | | | |
|---|---|---|---|
| 39 | 4.1 Description of Study Site & Population | George clinic | George Health Centre |

Given the fact that the changes are administrative in nature, the clinical study protocol number remain the same. Please review these changes in conjunction with the study protocol version number 1.0; Version date: date 11 July, 2018.

Principal Investigator: [redacted] Sign: _____ Date: 25th October 2018

Sponsor Representative: [redacted] Sign: _____ Date: 26th October 2018

PATH Representative: [redacted] Sign [redacted] Date: 26 OCT 2018

# Addendum to Protocol: CVIA 066

## An Open-label, Randomized, Controlled, Single Centre, Phase IIb Study to Assess the Immunogenicity, Reactogenicity and Safety of Three Live Oral Rotavirus Vaccines, ROTAVAC®, ROTAVAC 5CM and Rotarix® in Healthy Zambian Infants.

**Protocol Number: CVIA 066**
Version number 1.0; Version date: 11 July, 2018

**Addendum Number: 02; Dated: 11 October 2019**

The purpose of this addendum is to inform the change in name of investigational product from Rotavac 5CM to Rotavac 5D® pursuant to change in name of the product after registration in India. It is therefore stated that henceforth, all documents including the protocol, wherein the investigational product is named as "Rotavac 5CM" should be read as "Rotavac 5D®" except the title of the study which will remain unchanged. It is also to be noted that all documents prepared henceforth including the clinical study report will use the name of the investigational product as "Rotavac 5D®". The change is being implemented to avoid any confusion resulting from difference in name of the product being referred to in the study and the trade name of the vaccine under which the vaccine is being marketed.

Given the fact that the changes are administrative in nature, the clinical study protocol number remain the same. Please acknowledge this change in conjunction with the study protocol version number 1.0; Version date: 11 July, 2018 and Addendum Number: 01; Dated: 25 October, 2018.

Principal Investigator: [redacted] Sign: _____ Date: 13-October 2019

Sponsor Representative: [redacted] Sign: _____ Date: 13-October 2019

PATH Representative: [redacted] Sign: [redacted] Date: 14 OCT 2019