STATISTICAL ANALYSIS PLAN
for
PATH Protocol VAC 041

Study Title:

A Phase I/II double-blind, randomized, placebo-controlled, descending-age, dose-escalation study to examine the safety, tolerability and immunogenicity of the trivalent P2-VP8 subunit rotavirus vaccine in healthy South African adults, toddlers and infants

Version 1.0

DATE: 24 AUG 2017

Prepared and distributed by:
The Emmes Corporation
Rockville, Maryland USA

Summary

Protocol Number Code:	VAC 041
Development Phase:	Phase I/II
Investigational Product:	Trivalent P2-VP8 subunit rotavirus vaccine produced in *E. coli* is adsorbed onto aluminum hydroxide (0.5625 mg/dose)
Form/Route:	Vaccine/Intramuscular
Dosing:	15 µg, 30 µg and 90 µg (consisting of equal amounts of VP8 antigen derived from a P[4], a P[6] and a P[8] strain of rotavirus), all to be administered in 0.5 mL volumes
Sponsor:	PATH Vaccine Solutions (PVS) 2201 Westlake Avenue, Suite 200, Seattle, WA 98121 USA
Biostatistician:	Len Dally, MSc The Emmes Corporation 401 North Washington Street, Suite 700, Rockville, MD 20850

STUDY HYPOTHESES - PRIMARY

Safety: The trivalent P2-VP8 subunit rotavirus vaccine is safe and well-tolerated in healthy South African adults, toddlers and infants.

Immunogenicity: The trivalent P2-VP8 subunit rotavirus vaccine is immunogenic in healthy South African infants and will induce neutralizing immune responses to at least 2 of the 3 strains from which vaccine antigens are derived in 60% or more of participants in at least one of the study groups.

STUDY OBJECTIVES

Primary Objectives:

Safety

- To evaluate the safety and tolerability of the trivalent P2-VP8 subunit rotavirus vaccine at escalating dose levels in healthy South African adults, toddlers and infants

Immunogenicity

- To evaluate the immunogenicity of three doses of the trivalent P2-VP8 subunit rotavirus vaccine at different dose levels in healthy South African infants

Secondary Objectives:

Safety

- To evaluate the longer term safety (through 6 months after the last vaccination) of the trivalent P2-VP8 subunit rotavirus vaccine at escalating dose levels in healthy South African adults, toddlers and infants

Immunogenicity

- To evaluate the immunogenicity of two doses of the trivalent P2-VP8 subunit rotavirus vaccine at different dose levels in healthy South African infants
- To evaluate the immunogenicity of the trivalent P2-VP8 subunit rotavirus vaccine at different dose levels in healthy South African adults and toddlers

Exploratory Objective:

Efficacy

- To evaluate the impact of the trivalent P2-VP8 subunit rotavirus vaccination on shedding of Rotarix subsequently administered in healthy South African infants as a test of concept

STUDY ENDPOINTS

Primary Endpoints:

Safety (all 3 age-groups)

- Number of SAEs through 28 days after the last study injection
- Number of AEs through 28 days after the last study injection
- Number of vaccine-induced local and systemic reactions

Immunogenicity (infants):

- Proportion of infants with anti-P2-VP8 IgG & IgA seroresponses by ELISA (four-fold increase in antibody titers between baseline and 4-weeks post-third study injection) in assays using each of the three vaccine antigens (P[4], P[6] and P[8])
- Proportion of infants with neutralizing antibody responses (2.7-fold and 4-fold increase in antibody titers between baseline and 4-weeks post-third study injection) to each of the three rotavirus strains from which the vaccine antigens are derived, as well as heterologous strains
- Anti-P2-VP8 IgG & IgA geometric mean titers (GMT) (baseline and 4 weeks third study injection) in ELISA assays using each of the three vaccine antigens (P[4], P[6] and P[8])
- Neutralizing antibody GMT (baseline and 4 weeks post-third study injection) to each of the three rotavirus strains from which the vaccine antigens are derived, as well as heterologous strains
- Proportion of infants with neutralizing antibody responses to at least two of the three strains from which the vaccine antigens are derived

Secondary Endpoints:

Safety (all 3 age-groups)

- Number of SAEs at any time during the study
- Number of AEs at any time during the study

Immunogenicity (infants):

- Proportion of infants with anti-P2-VP8 IgG & IgA seroresponses by ELISA (four-fold increase in antibody titers between baseline and 4 weeks post-second study injection) in assays using each of the three vaccine antigens (P[4], P[6] and P[8])
- Proportion of infants with neutralizing antibody responses (2.7-fold increase in antibody titers between baseline and 4 weeks post-second study injection) to each of the three rotavirus strains from which the vaccine antigens are derived, as well as heterologous strains
- Anti-P2-VP8 IgG & IgA geometric mean titers (GMT) (baseline and 4 weeks post-second study injection) in ELISA assays using each of the three vaccine antigens (P[4], P[6] and P[8])
- Neutralizing antibody GMT (baseline and 4 weeks post-second study injection) to each of the three rotavirus strains from which the vaccine antigens are derived, as well as heterologous strains
- Proportion of infants with neutralizing antibody responses to at least two of the three strains from which the vaccine antigens are derived (4 weeks post-second study injection)

Immunogenicity (adults and toddlers)

- Proportion of adults and toddlers with anti-P2-VP8 IgG & IgA seroresponses by ELISA (four-fold increase in antibody titers between baseline and 4-weeks post-final study injection) in assays using each of the three vaccine antigens (P[4], P[6] and P[8])
- Proportion of adults and toddlers with neutralizing antibody responses (2.7-fold increases in antibody titers between baseline and 4-weeks post-final study injection) to each of the three rotavirus strains from which the vaccine antigens are derived, as well as heterologous strains
- Anti-P2-VP8 IgG & IgA geometric mean titers (GMT) (baseline and 4 weeks post final study injection) in ELISA assays using each of the three vaccine antigens (P[4], P[6] and P[8])

- Neutralizing antibody GMT (baseline and 4 weeks post-final study injection) to each of the three rotavirus strains from which the vaccine antigens are derived, as well as heterologous strains
- Proportion of adults and toddlers with neutralizing antibody responses to at least two of the three strains from which the vaccine antigens are derived

Exploratory Endpoint

- Proportion of participants shedding Rotarix after administration of Rotarix "challenge"

STUDY RATIONALE

Live oral rotavirus vaccines have not performed optimally in developing-country populations, similar to the discrepancy in performance observed between developed versus developing countries for other oral, live attenuated enteric vaccines such as those targeted against cholera and poliomyelitis. Several direct and indirect observations support the possibility that parenterally-administered non-replicating rotavirus vaccines may be successful, including observations derived from natural rotavirus infection studies in infants, rotavirus challenge studies in adult volunteers, human rotavirus vaccine trials, and animal rotavirus vaccine studies. The P2-VP8 vaccine contains a truncated rotavirus VP8 subunit fused to the tetanus toxin P2 CD4 epitope. It is parenterally administered, which may overcome limitations of oral vaccines in developing countries. A monovalent formulation containing the VP8 subunit from a P[8] rotavirus strain has been demonstrated to be well-tolerated and immunogenic in US adults (VAC 009) and is currently being tested in toddlers and infants in South Africa (VAC 013), where it has been generally well-tolerated. In the study in adults, the monovalent vaccine elicited robust neutralizing antibody responses to several homologous P[8] strains of rotavirus, but modest response to P[4] strains and meager responses to P[6] strains. Thus, the trivalent formulation to be tested in this study, containing VP8 subunits from P[4], P[6] and P[8] strains was developed, with the goal to optimize responses to those strains that account for the vast majority of the burden of rotavirus disease globally. Preclinical studies of the trivalent vaccine indicate improved responses to P[4] and P[6] strains. Preliminary analysis of serologic responses and impact of the monovalent P2-VP8 vaccine on shedding of Rotarix, as a proxy model for efficacy, in infants in VAC 013 has provided promising results. Those results have also provided the basis for the selection of the dose levels of the trivalent vaccine to be tested in this protocol.

INVESTIGATIONAL PRODUCTS

Vaccine: The trivalent P2-VP8 subunit rotavirus vaccine produced in E. coli is adsorbed onto aluminum hydroxide (0.5625 mg/dose). Three dose levels of vaccine will be tested: 15 µg, 30 µg and 90 µg (consisting of equal amounts of VP8 antigen derived from a P[4], a P[6] and a P[8] strain of rotavirus), all to be administered in 0.5 mL volumes intramuscularly. The three antigens are derived from DS1, 1076 and Wa strains, respectively.

Placebo: Normal Saline (NS)

STUDY POPULATION	• 30 healthy South African adults (18-45 years old, inclusive, at time of first vaccination) • 30 healthy South African toddlers (≥2 & < 3 years old at time of first vaccination) • 600 healthy South African infants (≥6 & <8 weeks old at the time of first vaccination)
STUDY DURATION	Adult and infant participants will be followed for approximately 8 months after randomization (6 months following last [3rd] injection). Toddler participants will be followed for approximately 6 months after randomization (6 months following the single injection).

STUDY DESIGN

The trial will be a double-blind, randomized, placebo-controlled, descending-age, dose-escalation study in which two dose-levels of vaccine will be tested in South African adults and toddlers, and three dose-levels will be tested in infants, with progression from one dose-level to the next and one age-group to the next based on assessment of safety and tolerability.

The adult cohorts (Cohort A1 and A2) will consist of 15 participants (12 vaccine recipients and 3 placebo recipients) per dose level, who will receive three intramuscular injections four weeks apart. The two dose levels of vaccine to be tested will be 30 µg (consisting of 10 µg each of the P[4], P[6] and P[8] P2-VP8 subunit proteins) and 90 µg (consisting of 30 µg each of the P[4], P[6] and P[8] P2-VP8 subunit proteins). Progression from low- to high-dose in adults, and to low-dose in toddlers, will be based on assessment of safety and tolerability during the week after the first injection in the adult low-dose cohort. The adult cohorts will receive a series of 3 study injections to allow optimal comparison with the results of the VAC 009 study, in which US adults received a series of 3 study injections of the monovalent vaccine (containing P[8] only, up to 60 µg).

Similarly, the toddler cohorts (Cohorts B1 and B2) will consist of 15 participants (12 vaccine recipients and 3 placebo recipients) per dose level. Participants will receive a single intramuscular injection. If found to be safe in the adult cohorts, the two dose levels of vaccine to be tested will be 30 µg (consisting of 10 µg each of the P[4], P[6] and P[8] P2-VP8 subunit proteins) and 90 µg (consisting of 30 µg each of the P[4], P[6] and P[8] P2-VP8 subunit proteins). Initiation of the toddler low-dose cohort will be based on assessment of safety and tolerability of that dose during the week after the first injection in the adult low-dose cohort. Progression from low- to high-dose in the toddlers, and to low-dose in infants, will be based on assessment of safety and tolerability during the week after the first injection in the toddler low-dose cohort. Further, progression to the high-dose in toddlers will also be based on assessment of safety and tolerability during the week after first injection in the adult high-dose cohort. The toddler cohorts will receive only one study injection, as was the case for toddlers in VAC 013, as it is the safety data following this single dose that will be assessed to determine whether to proceed to the infant cohorts.

Initial assessment in infants will consist of 16 participants (12 vaccine recipients and 4 placebo recipients) per dose level (Cohorts C1, C2 and C3). Participants will receive three intramuscular injections four weeks apart. If found to be safe in the toddler cohorts, the three dose levels of vaccine to be tested will be 15 µg (consisting of 5 µg each of the P[4], P[6] and P[8] P2-VP8 subunit proteins), 30 µg (consisting of 10 µg each of the P[4], P[6] and P[8] P2-VP8 subunit proteins) and 90 µg (consisting of 30 µg each of the P[4], P[6] and P[8] P2-VP8 subunit proteins). Initiation of the infant low-dose (15 µg) cohort will be based on assessment of safety and tolerability during the week after first injection in the toddler low-dose (30 µg) cohort. Progression from the 15 µg to the 30 µg dose cohort in the infants will be based on assessment of safety and tolerability during the week after the first injection both in the infant 15 µg -dose cohort and the toddler 30 µg dose cohort. Progression from the 30 µg to the 90 µg dose cohort in the infants will be based on assessment of safety and tolerability during the week after the first injection in the infant 30 µg dose cohort.

Should all three doses be tolerated in the initial assessment in infants, testing in infants will be expanded (Group D) to obtain both (a) greater safety experience and (b) more robust immunogenicity data for comparison of the three doses to support planning of subsequent, advanced phase testing. Group D will consist of 4 groups of 138 participants each: 15 µg, 30 µg and 90 µg of vaccine and placebo.

All infants will receive 3 doses of Rotarix, at monthly intervals, starting a month after the final study injection (on study Day 84, at the time the post-vaccination blood sample is obtained), and fecal shedding of Rotarix will be assessed during the week after the first dose on a subset of infants.

This study was performed in compliance with Good Clinical Practice.

SIGNATURE PAGE

SPONSOR: PATH

STUDY TITLE: A Phase I/II double-blind, randomized, placebo-controlled, descending-age, dose-escalation study to examine the safety, tolerability and immunogenicity of the trivalent P2-VP8 subunit rotavirus vaccine in healthy South African adults, toddlers and infants.

PROTOCOL: VAC 041

PATH Medical Officer: (Alan Fix, MD, MS)

Signed: _____ Date: _____

The Emmes Corporation Statistician: (Len Dally, M.Sc)

Signed: _____ Date: _____

TABLE OF CONTENTS

List of Abbreviations

AE	Adverse Event
CI	Confidence interval
cm	Centimeter
CRF	Case Report Form
CRO	Contract Research Organization
CSR	Clinical Study Report
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
EDC	Electronic data capture
ELISA	Enzyme linked immunosorbent assay
EPI	Expanded Program on Immunization
ERC	Ethics Review Committee
FA	Full analysis
FAM-CRU	Family Clinical Research Unit, Tygerberg Hospital
FDA	Food and Drug Administration
GMFR	Geometric mean fold rise
GMT	Geometric Mean Titer
HBV	Hepatitis B virus
HCG	Human chorionic gonadotropin
HCV	Hepatitis C virus
HgB	Hemoglobin
HIV	Human immunodeficiency virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation
Ig	Immunoglobulin
IM	Intramuscular
IPTG	Isopropyl β-D-1-thiogalactopyranoside
LLOQ	Lower Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities
N	Number (typically refers to participants)
PCR	Polymerase chain reaction
PI	Principal Investigator
PP	Per Protocol
PS	Polysaccharide

PT	Preferred Term
PVS	PATH Vaccine Solutions
RMPRU	Respiratory and Meningeal Pathogens Research Unit
SAE	Serious Adverse Event
SDCC	Statistical Data Management Center
SRC	Safety Review Committee
ULOQ	Upper Limit of Quantification
USA	United States of America
WBC	White blood cells

1 INTRODUCTION

This is a Phase I/II double-blind, randomized, placebo-controlled, descending-age, dose-escalation study to examine the safety, tolerability and immunogenicity of the trivalent P2-VP8 subunit rotavirus vaccine in healthy South African adults, toddlers and infants.

1.1 Purpose of the Analyses

This Statistical Analysis Plan describes the statistical methodology and summaries required to assess the safety, tolerability and immunogenicity of 3 dose-levels of the trivalent P2-VP8 subunit rotavirus vaccine when administered to healthy South African toddlers and infants. The primary study hypotheses are as follows:

Safety

The trivalent P2-VP8 subunit rotavirus vaccine is safe and well-tolerated in healthy South African adults, toddlers and infants.

Immunogenicity

The trivalent P2-VP8 subunit rotavirus vaccine is immunogenic in healthy South African infants and will induce neutralizing immune responses to at least 2 of the 3 strains from which vaccine antigens are derived in 60% or more of participants in at least one of the study groups

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Objectives

Safety

- To evaluate the safety and tolerability of the trivalent P2-VP8 subunit rotavirus vaccine at escalating dose levels in healthy South African adults, toddlers and infants

Immunogenicity

- To evaluate the immunogenicity of three doses of the trivalent P2-VP8 subunit rotavirus vaccine at different dose levels in healthy South African infants

2.1.2 Secondary Objectives

Safety

- To evaluate the longer term safety (through 6 months after the last vaccination) of the trivalent P2-VP8 subunit rotavirus vaccine at escalating dose levels in healthy South African adults, toddlers and infants

Immunogenicity

- To evaluate the immunogenicity of two doses of the trivalent P2-VP8 subunit rotavirus vaccine at different dose levels in healthy South African infants
- To evaluate the immunogenicity of the trivalent P2-VP8 subunit rotavirus vaccine at different dose levels in healthy South African adults and toddlers

2.1.3 Exploratory Objectives

Efficacy

- To evaluate the impact of the trivalent P2-VP8 subunit rotavirus vaccination on shedding of Rotarix subsequently administered in healthy South African infants as a test of concept.

2.2 Endpoints

2.2.1 Primary Endpoints

Safety

- Number of SAEs through 28 days after the last study injection
- Number of AEs through 28 days after the last study injection
- Number of vaccine-induced local and systemic reactions

Immunogenicity (infants):

- Proportion of infants with anti-P2-VP8 IgG & IgA seroresponses by ELISA (four-fold increase in antibody titers between baseline and 4-weeks post-third study injection) in assays using each of the three vaccine antigens (P[4], P[6] and P[8])
- Proportion of infants with neutralizing antibody responses (2.7-fold increases in antibody titers between baseline and 4-weeks post-third study injection) to each of the three rotavirus strains from which the vaccine antigens are derived, as well as heterologous strains
- Anti-P2-VP8 IgG & IgA geometric mean titers (GMT) (baseline and 4 weeks post third study injection) in ELISA assays using each of the three vaccine antigens (P[4], P[6] and P[8])
- Neutralizing antibody GMT (baseline and 4 weeks post-third study injection) to each of the three rotavirus strains from which the vaccine antigens are derived, as well as heterologous strains
- Proportion of infants with neutralizing antibody responses to at least two of the three strains from which the vaccine antigens are derived (4-weeks post-third study injection)

2.2.2 Secondary Endpoints

Safety (all 3 age-groups)

- Number of SAEs at any time during the study
- Number of AEs at any time during the study

Immunogenicity (infants)

- Proportion of infants with anti-P2-VP8 IgG & IgA seroresponses by ELISA (four-fold increase in antibody titers between baseline and 4-weeks post-second study injection) in assays using each of the three vaccine antigens (P[4], P[6] and P[8])
- Proportion of infants with neutralizing antibody responses (2.7-fold increases in antibody titers between baseline and 4-weeks post-second study injection) to each of the three rotavirus strains from which the vaccine antigens are derived, as well as heterologous strains
- Anti-P2-VP8 IgG & IgA geometric mean titers (GMT) (baseline and 4 weeks post-second study injection) in ELISA assays using each of the three vaccine antigens (P[4], P[6] and P[8])
- Neutralizing antibody GMT (baseline and 4 weeks post-second study injection) to each of the three rotavirus strains from which the vaccine antigens are derived, as well as heterologous strains

Immunogenicity (adults and toddlers)

- Proportion of adults and toddlers with anti-P2-VP8 IgG & IgA seroresponses by ELISA (four-fold increase in antibody titers between baseline and 4-weeks post-final study injection) in assays using each of the three vaccine antigens (P[4], P[6] and P[8])
- Proportion of adults and toddlers with neutralizing antibody responses (2.7-fold increases in antibody titers between baseline and 4-weeks post-final study injection) to each of the three rotavirus strains from which the vaccine antigens are derived, as well as heterologous strains
- Anti-P2-VP8 IgG & IgA geometric mean titers (GMT) (baseline and 4 weeks post final study injection) in ELISA assays using each of the three vaccine antigens (P[4], P[6] and P[8])
- Neutralizing antibody GMT (baseline and 4 weeks post-final study injection) to each of the three rotavirus strains from which the vaccine antigens are derived, as well as heterologous strains
- Proportion of adults and toddlers with neutralizing antibody responses to at least two of the three strains from which the vaccine antigens are derived

2.2.3 Exploratory Endpoint

- Proportion of participants shedding Rotarix after administration of Rotarix "challenge"

2.3 Study Definitions and Derived Variables

2.3.1 Adverse Event (AE) or Medical Event

An adverse event is any untoward medical occurrence in humans, whether or not considered related to study product, which occurs during the conduct of a clinical trial. Any change from baseline assessment of clinical status, ECGs, routine laboratory tests, x-rays, physical examinations, etc., that is considered clinically significant by the site PI is considered an AE.

Suspected adverse drug reaction is any AE for which there is a reasonable possibility that the study product caused the AE. A reasonable possibility implies that there is evidence that the study product caused the event.

Adverse reaction is any AE caused by the vaccine.

2.3.2 Serious Adverse Events (Defined as Serious Adverse Events, Serious Suspected Adverse Reactions or Serious Adverse Reactions)

An SAE, including a serious suspected adverse reaction or serious adverse reaction as determined by the site PI or the Sponsor, is any event that results in any of the following outcomes:

1. Death
2. Life-threatening AE (Life-threatening means that the study participant was, in the opinion of the site PI or Sponsor, at immediate risk of death from the event as it occurred.)
3. Inpatient hospitalization or prolongation of existing hospitalization
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital abnormality or birth defect
6. Important medical event that may not result in one of the above outcomes but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious event

2.3.3 Unexpected Adverse Event

An AE is "unexpected" when its nature (specificity) or severity is not consistent with applicable product information, such as safety information provided in the Investigators' Brochure (IB), the investigational plan or the protocol.

2.3.4 Definitions and Derivations

The following definitions and derivations will be used in this study:

- Diarrhea is defined as ≥ 3 looser than normal stools in a 24 hour period.
- A baseline value will be defined as the last value obtained prior to the first vaccination of study product.
- Age will be calculated from the date of enrollment and will be presented in whole years for adults, months for toddlers and days for infants (truncated integer).
- The calculations for GMTs will be performed by taking the anti-log of the arithmetic mean of the $\log_{10}$-transformed titers.
- Fold increase/rise from baseline will be calculated as the following:

  Ratio of: $\frac{post-immunization\ titer}{pre-immunization\ titer}$

- Calculation of geometric mean fold rise (GMFR) will be performed by taking the anti-log of the arithmetic mean of the difference in $\log_{10}$ titers, where

  Difference = (*post-immunization* log_{10} *titer*) – (*pre-immunization* log_{10} *titer*)

- Antibody responses are defined as:
    - **Anti-P2-VP8 IgG & IgA seroresponses by ELISA**: 4-fold increase from baseline in antibody titers in assays using each of the three vaccine antigens (P[4], P[6] and P[8]).
    - **Neutralizing antibody responses**: 2.7-fold increase from baseline in antibody titers to each of the three rotavirus strains from which the vaccine antigens are derived, as well as heterologous strains.
- Prior medications are those medications that started and stopped prior to study vaccination.

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

The trial is a double-blind, randomized, placebo-controlled dose-escalation study in which two dose-levels (30 μg and 90 μg) of vaccine will be tested in adults and toddlers and then three dose-levels will be assessed in infants (15 μg, 30 μg and 90 μg).

In **Group A**, cohorts of 15 adults (12 vaccine and 3 placebo recipients) per dose level will receive three study injections at 4 week intervals (consistent with the regimen in the adult study of the monovalent vaccine), advancing from the first to the second dose-level after assessment of safety data through the first week after the first injection (A1).

Based on the safety data in the adult group, **Group B** cohorts of 15 toddlers (12 vaccine recipients and 3 placebo recipients) per dose level will receive a single intramuscular injection (consistent with the regimen administered to toddlers in the current study of the monovalent vaccine in toddlers), advancing from the first to the second dose-level after assessment of safety data through the first week after the injection in the lower dose toddler group (B1) and all available data for the adult group.

Based on the safety data in group B toddler cohorts, **Group C** cohorts of 16 infants (12 vaccine recipients and 4 placebo recipients) per dose level will receive three study injections at 4 week intervals (concomitant with EPI vaccinations, with the exception of rotavirus vaccination), starting at ≥6 and <8 weeks of age. Advancing from the first to the second dose-level in Group C will occur after assessment of safety data through the first week after the first injection (C1), as well as all available data for toddlers and adults. Advancing from the second to the third dose-level (90 μg) in Group C will occur after assessment of safety data through the first week after the first injection of the second dose-level (30 μg).

If all three dose levels are tolerated in the group C infant dose-escalation phase they will be assessed in an expanded infant cohort (**Group D**) of 552 infants (138 at each dose level and 138 placebo recipients). All infants will receive Rotarix at 4, 8 and 12 weeks after the third study injection (study Days 84, 112 and 140), and **fecal shedding** of Rotarix will be assessed during the week after the first dose in a subset of infants. Three doses of Rotarix are to be administered rather than two to counterbalance the remote theoretical possibility that the P2-VP8 subunit vaccine could reduce response to Rotarix.

3.2 STUDY SCHEMA

Group		Trivalent P2-VP8 Dose	N	-7[a] to -1	0	7	28	56	84	89, 91, 93	168	224
A Adult	A1	30 µg	12	B(S,I)	X	B(S)	X, B(I)	X, B(I)	B(I)			F
		Placebo	3	B(S,I)	P	B(S)	P, B(I)	P, B(I)	B(I)			F
	A2	90 µg	12	B(S,I)	X	B(S)	X, B(I)	X, B(I)	B(I)			F
		Placebo	3	B(S,I)	P	B(S)	P, B(I)	P, B(I)	B(I)			F
A Total			**30**									
B Toddler	B1	30 µg	12	B(S,I)	X	B(S)	B(I)				F	
		Placebo	3	B(S,I)	P	B(S)	B(I)				F	
	B2	90 µg	12	B(S,I)	X	B(S)	B(I)				F	
		Placebo	3	B(S,I)	P	B(S)	B(I)				F	
B Total			**30**									
C Infant	C1	15 µg	12	B(S,I)	X	B(S)	X	X, B(I)	B(I)	Sh		F
		Placebo	4	B(S,I)	P	B(S)	P	P, B(I)	B(I)	Sh		F
	C2	30 µg	12	B(S,I)	X	B(S)	X	X, B(I)	B(I)	Sh		F
		Placebo	4	B(S,I)	P	B(S)	P	P, B(I)	B(I)	Sh		F
	C3	90 µg	12	B(S,I)	X	B(S)	X	X, B(I)	B(I)	Sh		F
		Placebo	4	B(S,I)	P	B(S)	P	P, B(I)	B(I)	Sh		F
C Total			**48**									
D Infant		15 µg	138	B(S,I)	X		X	X, B(I)	B(I)	Sh		F
		30 µg	138	B(S,I)	X		X	X, B(I)	B(I)	Sh		F
		90 µg	138	B(S,I)	X		X	X, B(I)	B(I)	Sh		F
		Placebo	138	B(S,I)	P		P	P, B(I)	B(I)	Sh		F
D Total			**552**									
All Cohorts Total			**660**									

X = Trivalent P2-VP8 vaccine
P = placebo
B = blood sample for (S) safety and/or (I) immunogenicity testing
F = final study f/u visit/call
Sh = stool samples to assess Rotarix shedding. Only at RMPRU (Soweto).
[a] For adult and toddler cohorts, -28 to -1

For each age group, progression from one dose level to the next will require review of clinical and safety lab data through 7 days after the first dose at the lower dose level. Safety laboratory blood specimens will be drawn at baseline for all participants (screening) and 7 days after first study injection in Groups A, B and C.

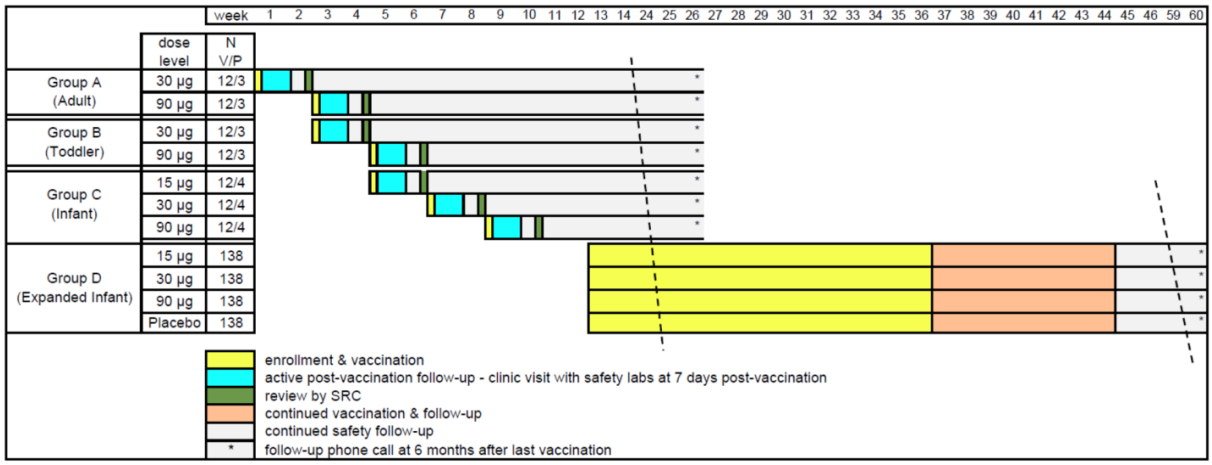
Blood samples for immunogenicity analysis will be obtained at baseline for all participants, Days 28, 56 and 84 for Group A (adults), Day 28 for Group B (toddlers), and Days 56 and 84 for Groups C and D (infants).

Stool samples to assess Rotarix shedding will be obtained from infants (Groups C and D) on days 89, 91 and 93 (i.e., 5, 7 and 9 days after the first Rotarix dose on day 84) in the subset of infants enrolled at the Respiratory and Meningeal Pathogens Research Unit (RMPRU).

For each dose level, progression from adults in Group A to toddlers in Group B and then to infants in Group C will require review of clinical and safety laboratory data through 7 days after study injection in the older group. (see Figure 1)

These determinations will be made by the Safety Review Committee (SRC).

Figure 1: Study overview and timeline



Note:
Time line does not take into account delay of enrollment of infant groups until after peak of rotavirus season.

3.3 Selection of Study Population

This study is a multi-site clinical trial, to be performed in South Africa at the Family Clinical Research Unit (FAM-CRU) at Tygerberg Children's Hospital, the Respiratory and Meningeal Pathogens Research Unit (RMPRU) in Soweto, and the Shandukani Research Centre in Hillbrow, Johannesburg. The study population will be recruited, screened and qualified by site staff under the direction of the PIs at each site. Adult and toddler participants will be enrolled only at RMPRU.

Participants are in general good health: in the adult group, participants will be ≥18 and ≤45 years of age; in the toddler cohort, participants will be ≥2 and <3 years of age; and the infant cohorts will be ≥6 and <8 weeks old at the time of enrollment. Potential volunteer families of infants may be contacted from before the babies were born through the noted targeted age. Final eligibility determination depended on the results of the medical history, clinical examination, screening laboratory tests, fulfillment of all the inclusion criteria, absence of any of the exclusion criteria, appropriate understanding of the study and completion of the consent process by adult participants and parents of toddler and infant participants.

Investigators 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 though they met all inclusion/exclusion criteria. For instance, medical, occupational or other conditions present in the parents may have made safety evaluations difficult or made toddlers and infants poor candidates for retention. The parents of all toddlers and infants targeted for enrollment have to comprehend the purpose of the study and provide written informed consent. In addition, the adult participants and families of toddler and infant participants should be resident in the area without plans to leave the study site during the course of the study.

A sufficient number of healthy adults, toddlers and infants are to be screened, with consent, to enroll 30 adults, 30 toddlers and 600 infants in the study.

3.3.1 Inclusion Criteria

Fulfillment of all of the following criteria is required to accept an adult, toddler or infant in the study:

1. Healthy adults, toddlers and infants as established by medical history and clinical examination before entering the study
2. Age:
   Adults: ≥18 and ≤45 years old at time of enrollment
   Toddlers: ≥2 and <3 years old at the time of enrollment
   Infants: ≥6 and <8 weeks at the time of enrollment
3. Participant (adults) or parental (toddlers and infants) ability and willingness to provide written informed consent
4. Intention for the participant (and for toddlers and infants, the parent) to remain in the area with the child during the study period
5. If female and of childbearing potential, be not breastfeeding and not pregnant (based on a negative serum pregnancy test at screening and a negative urine pregnancy test at each study injection visit, prior to injection), planning to avoid pregnancy for at least 4 weeks after the last injection, and willing to use an adequate method of contraception consistently and have repeated pregnancy tests prior to second and third injections.

3.3.2 Exclusion Criteria

Any of the following will exclude an adult, toddler or infant from the study:

1. Presence of fever on the day of enrollment (axillary or oral temperature >37.6ºC)
2. Acute disease at the time of enrollment
3. Concurrent participation in another clinical trial throughout the entire timeframe for this study
4. Presence of malnutrition or 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 that would compromise the participant's health or is likely to result in nonconformance to the protocol
5. For infant cohort, history of premature birth (<37 weeks gestation)
6. History of congenital abdominal disorders, intussusception, or abdominal surgery
7. Known or suspected impairment of immunological function based on medical history and physical examination
8. For infant cohort only, prior receipt of rotavirus vaccine
9. A known sensitivity or allergy to any components of the study vaccine
10. History of anaphylactic reaction
11. Major congenital or genetic defect

12. Parents of participating toddlers or infants not able, available or willing to accept active weekly follow-up by the study staff
13. Receipt of any immunoglobulin therapy and/or blood products in the last 6 months or planned administration during the study period
14. History of chronic administration (defined as more than 14 days) of immunosuppressant medications, including corticosteroids, in the last 6 months (those on inhaled or topical steroids may be permitted to participate in the study)
15. Any medical condition in the participant (or parents of toddler and infant participants) that, in the judgment of the investigator, would interfere with or serves as a contraindication to protocol adherence or a participant's (or parents') ability to give informed consent
16. Clinically significant screening laboratory value*
17. HIV infection
    a. For adults and toddlers, to be assessed by HIV ELISA
    b. For infants, to be assessed by PCR, if mother is not known to be negative (negative test result between 24 weeks gestation and screening).

*Grade 1 laboratory abnormalities (see toxicity table in Appendix I) will not be considered to be exclusionary at screening unless judged to be clinically significant by the PI. Potential participants with laboratory values of grade 2 or higher are not to be randomized without concurrence of the Emmes medical monitor and PATH medical officer.

After informed consent has been obtained and the potential participant is identified as meeting inclusion and exclusion criteria for enrollment, the (s)he will be enrolled in the study and assigned a randomization number.

3.3.3 Treatments Administered

3.3.3.1 Product Description

The P2-VP8 subunit rotavirus vaccine is made by inserting a codon optimized synthetic gene encoding the VP8 region of rotavirus VP4 fused to the P2 T-cell epitope coding sequence of tetanus toxin into the Pj411 proprietary cloning vector developed by DNA 2.0, Menlo Park, CA. The vector carries a kanamycin resistance gene as a selection marker. The vector was transfected into the BL21 strain of E. coli. The fusion protein is purified from Isopropyl β-D-1-thiogalactopyranoside (IPTG)-induced and physically lysed cultures using standard column chromatographic techniques employing Q-Sepharose and Butyl 650 as resins in addition to ultrafiltration and diafiltration. The purified protein is co-formulated with aluminum hydroxide [$Al(OH)_3$].

3.3.3.2 Presentation and Formulation

The trivalent P2-VP8 vaccine is formulated as a sterile suspension containing a total of 360 µg of protein (120 µg of each P type) per mL adsorbed to aluminum hydroxide (1.125 mg of aluminum per mL in a phosphate buffer, pH 7). The vaccine is contained within a 2 mL borosilicate glass vial sealed with a butyl rubber stopper and a crimped metal collar. The vaccine is stored at 2-8°C. To produce the 15, 30 and 90 µg doses (containing 5, 10 and 30 µg of each P2-VP8 vaccine component, respectively), the vaccine is diluted with the aluminum hydroxide adjuvant diluent within 6 hours of administration (aluminum hydroxide diluted to a concentration of 1.125 mg/mL before being used to dilute the vaccine).

The doses of the trivalent P2-VP8 subunit vaccine will be prepared in the pharmacy. Blinded study syringes will be prepared for the study coordinator for administration to the volunteers

3.3.4 Method of Assigning Participants to Treatment Groups (Randomization)

Enrollment is sequential in the order given below and in the group sample sizes given in Table 3.3.4.

Cohort A1: Adults were randomly assigned to 30 µg P2-VP8 or Placebo in a 4:1 ratio.
Cohort A2: Adults were randomly assigned to 90 µg P2-VP8 or Placebo in a 4:1 ratio.

Cohort B1: Toddlers were randomly assigned to 30 µg P2-VP8 or Placebo in a 4:1 ratio.
Cohort B2: Toddlers were randomly assigned to 90 µg P2-VP8 or Placebo in a 4:1 ratio.

Cohort C1: Infants were randomly assigned to 15 µg P2-VP8 or Placebo in a 3:1 ratio.
Cohort C2: Infants were randomly assigned to 30 µg P2-VP8 or Placebo in a 3:1 ratio.
Cohort C3: Infants were randomly assigned to 90 µg P2-VP8 or Placebo in a 3:1 ratio.

Cohort D: Infants were randomly assigned to 15 µg, 30 µg or 90 µg of P2-VP8, or to Placebo in a 1:1:1:1 ratio.

Table 3.3.4 Dosing Schedule

Group	P2-VP8 dose	N	Study Day		
			0	28	56
A Adults	30 µg	12	X	X	X
	Placebo	3	X	X	X
	90 µg	12	X	X	X
	Placebo	3	X	X	X
B Toddlers	30 µg	12	X		
	Placebo	3	X		
	90 µg	12	X		
	Placebo	3	X		
C Infants	15 µg	12	X	X	X
	Placebo	4	X	X	X
	30 µg	12	X	X	X
	Placebo	4	X	X	X
	90 µg	12	X	X	X
	Placebo	4	X	X	X
D Infants	15 µg*	138	X	X	X
	30 µg*	138	X	X	X
	90 µg*	138	X	X	X
	Placebo	138	X	X	X

X = Injection day
* If dose tolerated in Group C

3.3.5 Blinding

The randomization scheme was generated and maintained by the Statistical Data Coordinating Center (SDCC) at the Emmes Corporation, Rockville, MD. Randomization and assignment of treatment codes is performed using the enrollment module of the Emmes AdvantageEDC[SM] electronic data capture system on the day participants received their first study vaccination, after confirmation of eligibility and immediately prior to immunization. The blinded investigator provides the treatment assignment code to the unblinded study pharmacist or member of the IP formulation team for preparation of the vaccine or placebo to be given to the participant. The unblinded pharmacist refers to a Treatment Key Listing, provided for the trial by Emmes, to determine the treatment for the participant. The pharmacist maintains the Treatment Key Listing under locked/secured conditions and does not reveal the randomization code to any other study staff member, participant or parent. Investigational study product was prepared by a qualified unblinded research pharmacist and witnessed by another unblinded study staff member then dispensed in a masked syringe (opaque sheath over the syringe barrel) with administration needle. All follow-up safety and efficacy evaluations are performed by blinded clinic staff.

3.4 Immunogenicity and Safety Variables

The following section describes the collection of immunogenicity and safety variables. For a detailed schedule of activities refer to Table 14.1.3 of the protocol. For a list of the primary and secondary immunogenicity and safety variables, refer to Section 2.2 of this report.

3.4.1 Safety Variables

The safety variables to be assessed are unsolicited adverse events (AEs), serious adverse events (SAEs), multiple clinical safety laboratory measurements [including white blood cells (WBC), hemoglobin (Hgb), platelets, alanine aminotransferase (ALT), albumin, total bilirubin and serum creatinine], physical exam, and vital sign parameters. Solicited systemic reactogenicity events within 7 days of each vaccination are also collected. If a solicited sign or symptom starts during the seven days post-vaccination and continues, it is still reported as a reactogenicity symptom. Any symptom starting after seven days post-vaccination is recorded as an AE. Safety events are collected from the time of the first study injection through completion of the study at 6 months after the final injection (as above). For participants who withdraw or were withdrawn from the study, an early termination visit should occur 5-7 days after the last injection or study visit to elicit occurrence of AEs (serious and non-serious).

3.4.1.1 Reactogenicity Events

Reactogenicity events are adverse events that are common and known to occur or are of particular interest following the administration of the study vaccine. These events are collected in a standard, systematic format using a graded scale based on functional assessment or magnitude of reaction. The reactogenicity adverse events are solicited systemic reactions collected by interviews with the participant/parent/guardian, memory aids and assessed by the site at clinic visits. Reactogenicity assessments include the following:

Local reactogenicity (adults, toddlers and infants)

- Pain
- Tenderness
- Erythema
- Induration
- Pruritus

Systemic Reactogenicity (Adults)

- Fever
- Headache
- Vomiting
- Nausea
- Fatigue
- Chills
- Myalgia

Systemic Reactogenicity (Toddlers and infants)

- Fever
- Vomiting
- Irritability
- Decreased activity
- Decreased appetite

3.4.1.2 Unsolicited Adverse Events

An AE is defined as any untoward medical occurrence associated with the use of a vaccine in humans, whether or not considered vaccine related, that occurs during the conduct of a clinical trial. Any change from baseline assessment of clinical status, routine laboratory tests, X-rays, physical examinations, etc., that is considered clinically significant by the PI is considered an AE.

AEs, including non-solicited injection site and systemic reactions not meeting the criteria for "SAEs" were captured on the appropriate case report forms. All AEs are followed until resolution or stability. AEs are graded for severity and relationship to study product. AEs are also coded by MedDRA® version 19.1 or higher for preferred term and system organ class.

The study site assigns severity grades based on the severity grading criteria provided in Appendix II of the Protocol. Possible grades are from Mild (Grade 1) to Life Threatening (Grade 4). Any AEs leading to death are Grade 5 events. AEs are graded with the most severe grade during the illness/symptom.

When assessing causality of an AE to study product, the PI considers whether there was a reasonable possibility that the study product caused the event. Reasonable possibility implies there is evidence to suggest that the study product caused the reported event. An affirmative answer designates the event as a suspected adverse reaction, and the AE is considered "related". If the answer is no, then the AE is considered "unrelated".

3.4.1.3 Serious Adverse Events

An SAE, including a serious suspected adverse reaction or serious adverse reaction as determined by the PI or the Sponsor, is any event that results in any of the following outcomes:

- Death;
- Life-threatening adverse event (Life-threatening means that the study participant was, in the opinion of the PI or Sponsor, at immediate risk of death from the event as it occurred);
- Inpatient hospitalization greater than 24 hours or prolongation of existing hospitalization;

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life function, or;
- Congenital abnormality or birth defect;
- Important medical events that may not result in one of the above outcomes but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious event.

All SAEs are:

- Recorded on a multi-page SAE form set and sent to the Emmes Coordination Center within 24 hours of the site's knowledge of the event.
- Reviewed by the Emmes Medical Monitor and a report written and provided to the PI.
- The site PIs ensure the timely dissemination of required SAE information, including expedited reports, to their respective local ethics committees in compliance with applicable local regulations and guidelines and are responsible for submitting the safety report (initial and follow up SAE reports) or other safety information (e.g., revised IB) to their respective ethics committees and for retaining a copy in the site's study file.
- The Emmes Coordinating Center notifies the Sponsor and the CRO performing site monitoring simultaneously with the reporting to the clinical database. They also provide the SRC, the PATH Medical Officer and the Emmes Medical Monitor with listings of all SAEs on an ongoing basis.
- Reviewed by the site PI who applies clinical judgment to determine whether the SAE is of sufficient severity to require discontinuation of administration of study injection for that participant. If necessary, the site PI will suspend any trial procedures and institute the necessary medical therapy to protect the participant from any immediate danger.
- Followed to resolution by the PI or a study physician.
- Reported to the WIRB by PVS according to the WIRB guidelines and using the WIRB Ten Day Adverse Event Form.

SAEs are collected on each subject through 6 months after the final study vaccination.

3.4.2 Immunogenicity Variables

Antibody (IgG and IgA by ELISA) to P2-VP8 and Rotavirus neutralization analyses will be performed on sera from the following visits:

	Screening	**4 Weeks Post Vac.1** Day 28	**4 Weeks Post Vac.2** Day 56	**4 Weeks Post Vac.3** Day 84
Adults:	X	X	X	X
Toddlers:	X	X		
Infants:	X		X	X

Anti-rotavirus IgA (whole viral lysate) by ELISA will be determined in serum samples collected at baseline and one month after the final study injection (i.e., day 84).

Additional immunogenicity assays may be performed by research laboratories to further evaluate post-study injection immune responses and to explore whether correlates of protection may be identified

For immunogenicity variables, data inferior to the Lower Limit of Quantification (LLOQ) will be replaced by half the detection limit; data superior to the Upper Limit of Quantification (ULOQ) will be replaced by the limit (truncated data). No search for outliers will be performed. Prior to statistical analysis, all immunology data will be $\log_{10}$ transformed to improve the distributional properties of the data and reduce the impact of potential outliers.

4 SAMPLE SIZE CONSIDERATIONS

4.1.1 Primary Safety Endpoints

Safety will be assessed by analyses of the following primary endpoints (events), where the unit of analysis in each case will be the proportion of participants with at least one event:

- Moderate or greater solicited local reactions
- Moderate or greater solicited systemic reactions
- Moderate or greater adverse events
- Moderate or greater adverse events where there is a reasonable possibility that the study product caused the event, i.e., are suspected adverse reactions
- Serious adverse events

It was assumed that almost all participants enrolled would provide data for safety analysis and at least 90% of enrolled participants would be evaluable for immunogenicity assessments.

Safety

The sample size for this study was selected to detect frequent adverse events. Given a planned sample size of 12 vaccine recipients per dose group and 24 vaccine recipients for the two dose groups combined in both adults and toddlers (A and B), the study will have an approximately 90% chance of observing at least one AE that occurs at a rate of 17.5% and 9.2%, respectively. For the two infant cohorts combined (C and D), 150 vaccine recipients per dose group and 450 vaccine recipients for the three dose groups combined will provide at least 90% chance of observing an AE that has a 1.6% and 0.5% rate of occurrence, respectively.

Conversely, if no SAEs are observed in 24 and 450 vaccine recipients, the study will be able to rule out SAEs occurring at a rate of approximately 11.7% and 0.7%, respectively based on the upper bounds of the one-sided 95% confidence.

Primary Immunogenicity Endpoints

Based on the results in South African infants who received monovalent P2-VP8 vaccine or placebo (VAC-013), the strain specific seroresponse rates for both P2-VP8 vaccine doses were expected to be ≥80% for at least one of the three P2-VP8 vaccine doses and <20% for the placebo group. For the two infant cohorts combined, with 135 evaluable recipients per dose level (assuming 10% loss), this study was designed to provide at least 74% and 95% power (Fisher's exact 2-tailed test) to detect 15 and 20 percentage points

difference (e.g. 65% vs. 80% and 60% vs. 80%), respectively, in seroresponse rates between any two P2-VP8 dose groups. For comparisons with the combined placebo groups, this study was designed to provide ≥99% power (Fisher's exact 1-tailed test) to detect a ≥30 percentage point increase in the P2-VP8 group compared with placebo (e.g. 50% vs. 20%).

For the sample calculations of the Geometric Mean Titer (GMT) endpoint based on the VAC 013 study, the LOG10 standard deviations were estimated to be <0.6 (range: 0.24 – 0.56) for the P2-VP8 doses and the placebo group. Using a conservative LOG_{10} standard deviation (SD) of 0.6, this study with 135 evaluable infants per group (assuming 10% loss) was designed to provide at least 98% power to detect as low as a 2-fold difference between any two P2-VP8 dose groups or between any P2-VP8 vaccine dose (15 μg, 30 μg or 90 μg dose) and the placebo group.

i.e., for 2 groups A & B, with A > B:

Require $GMT(A) / GMT(B) \geq 2$

i.e., $Log_{10}A - Log_{10}B \geq Log_{10}(2) \approx 0.301$

Therefore, $A \sim N(\mu, 0.6)$ and $B \sim N(\mu - 0.301, 0.6)$

Under this assumption using a student t-test, a sample size of N=135 (per group) will give 98% power to detect a significant difference at the 5% level in mean Log_{10} titer between 2 groups of at least 0.301.

5 GENERAL STATISTICAL CONSIDERATIONS

5.1 General Principles

All continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, median, quartiles and range (maximum and minimum). Where appropriate (e.g., immunogenicity), geometric means and corresponding 95% confidence intervals will be included. The number and percent of participants (based on the population sample size) of observed levels will be reported for all categorical measures. Where appropriate (e.g., immunogenicity and safety outcomes), 95% confidence intervals for the proportion of participants with an event will be included.

In general, all data will be listed, sorted by treatment and participant, and when appropriate by visit number within participant.

5.2 Timing of Analyses

The primary analysis of safety and immunogenicity data through 28 days (day 84) post-final vaccination and the analysis of shedding data from days 5, 7 and 9 post first Rotarix dose on day 84 will be performed when all relevant safety, immunogenicity and shedding data have been received, all associated data queries have been resolved and the only remaining data to be collected are long-term safety data. A copy of the database will be made at that time and 'frozen' for the primary analyses prior to final database lock. The final clinical study report (CSR) will include all the primary analyses (which will not be repeated) as well as all secondary and exploratory analyses. These will be performed once the study is completed (final visit is day 224 after the last enrolled infant) and the database has been cleaned and locked.

5.3 Analysis Populations

A tabular listing of all participants, visits, and observations excluded from the safety, full analysis and per protocol populations will be provided in the CSR (Appendix 16.2.3).

5.3.1 Enrolled Population

All screened participants who provide informed consent (IC), regardless of the participant's randomization and treatment status in the trial, will be included in the enrolled population.

5.3.2 Safety Population

All participants in the enrolled population who receive a study vaccination and have safety data available will be included in the safety population. Treatment groups for safety analysis after a particular vaccination (1st, 2nd or 3rd) will be assigned according to the actual treatment received. For the analysis of accumulated safety data, participants will be grouped according to the first dose received and if a participant received mixed doses (e.g., 30 µg and 90 µg of P2-VP8 at Dose 1 & 2), the safety data collected after the start of the mixed dosing (e.g. post Dose 2) will be excluded. All excluded data will be presented in the data listings. All safety analyses will be performed using this population.

5.3.3 Full Analysis (FA) Population (Modified Intent To Treat Population)

All participants in the enrolled population who were randomized, received a study vaccination, and have post-vaccination immunogenicity measurement(s). For the viral shedding analysis, the FA population will include the enrolled population who were randomized, received a study vaccination, and have any post-Rotarix shedding measurement(s). The analysis based on this population will serve as supportive results for the primary immunogenicity and viral shedding objectives. Participants will be analyzed as randomized.

5.3.4 Per Protocol Population

All randomized participants who adhere to the protocol, complete all their scheduled visits and vaccinations and present no major protocol violations prior to database lock. A “major” violation is defined as a protocol violation that is considered to have an impact on the immunogenicity results of the study. Volunteers are analyzed according to randomized treatment arm. For the viral shedding analysis, the per protocol (PP) population will include participants meeting all the above criteria with the addition that participants must have provided at least one stool sample between 5 and 9 days following the first dose of Rotarix.

The specific criteria for exclusion of participants from the PP population will be established before breaking the blind and will be based on the blinded review of protocol violations.

5.4 Covariates and Subgroups

There is no *a priori* plan to summarize the immunogenicity or safety endpoints for covariates. The protocol is set up as a descending age-group study, so each age-group will be summarized separately. The 3 age groups to be analyzed are adults (18-45 years), toddlers (2 to <3 years) and infants (6 to <8 weeks). Safety endpoints for infants will be presented for each site and for all sites combined.

5.5 Missing Data

In general, all missing data will be treated as missing completely at random and no imputation will be performed except for the safety endpoints as described below. Non-analyzable data (e.g., due to major protocol violations) will be documented in the deviations.

5.6 Interim Analyses and Data Monitoring

The primary analysis of safety and immunogenicity data through 28 days (day 84) post-final vaccination and the analysis of shedding data from days 5, 7 and 9 post first Rotarix dose on day 84 will be performed prior to final database lock (see Section 5.2). Review of all blinded safety data by the Safety Review Committee (SRC) occurs on a regular basis. The site PIs and/or designated site staff are responsible for continuous close safety monitoring of all study participants and for alerting the Sponsor if unexpected concerns arise or stopping criteria are met. The Emmes medical monitor is also automatically alerted via email as soon as any unexpected SAE or event contributing to any stopping criteria was reported.

5.6.1 Safety Review Committee

A SRC, comprised of the site PIs from each study site, the Sponsor medical officer, the Emmes medical monitor and two independent local medical experts, has monitored safety throughout the duration of the study (in addition to the Data Safety Monitoring Board described in section 8.2 of the protocol). The responsibilities and procedures of the SRC were defined in the SRC Charter. The study statistician with assistance of the data management staff prepares safety reports as needed for SRC discussions. In addition to routine review of safety information, a central role of the SRC was the review of safety data for the recommendation of whether to progress to the next dose level in each age cohort and to the next age cohort. Cumulative safety data are available continuously for review by SRC members, and safety reports are prepared for each SRC deliberation of dose escalation and progression to subsequent cohorts (adults to toddlers and toddlers to infants).

The SRC members review the safety data throughout the study to determine whether pause criteria had been met.

5.6.2 Study Pause

Study pause is defined as a decision to cease, temporarily or permanently, enrollment and all study injections. Study pause would not eliminate any safety follow-up procedures specified by the protocol. The Sponsor would pause the study if the SRC determined that study pause criteria had been met. The DSMB would be convened by teleconference to review study pauses and provide recommendations to the Sponsor regarding continuation or permanent discontinuation of study vaccinations, or study modification.

Meeting one or more of the following criteria would automatically pause or halt further study injections in the study. These pause rules refer to suspected adverse reactions across all cohorts, as defined below:

- One participant with a vaccine-related serious AE (SAE);
- Two participants with ≥grade 3 (severe) localized inflammatory reaction at the injection site;

- The same objective ≥grade 3 (severe) systemic reactogenicity signs or symptoms (fever, vomiting), within seven days following study injection in three participants in Groups A, B or C, and ≥5% of participants in Group D;
- Two participants with a systemic rash, including, but not limited to, generalized urticaria, generalized petechiae, or erythema multiforme, within seven days following study injection;
- Two participants with the same ≥grade 3 (severe) vaccine-related abnormal clinical laboratory evaluation, within seven days following study injection.

5.7 Multicenter Studies

This study is being conducted at 3 sites: the Respiratory and Meningeal Pathogens Research Unit (RMPRU) in Soweto enrolled all participants in groups A to C. Group D infants were enrolled at 3 sites: RMPRU, SRC (Shandukani Research Centre) and FAM-CRU (Family Clinical Research Unit, Tygerberg). All summary tables, graphs and listings for infants in groups C and D will be reported by site and for all 3 sites combined.

5.8 Multiple Comparisons/Multiplicity

For each event/endpoint, a multiplicity adjustment for 4 treatment groups will be applied using a sequential testing approach by performing an overall comparison of all treatment groups prior to pairwise comparisons. No multiplicity adjustment for multiple immunogenicity endpoints is planned, since the dose selection for the Phase 3 will be based on comprehensive statistical and clinical evaluation of all immunogenicity endpoints including safety results.

5.9 Software to be used for Analyses

All analyses performed by Emmes will use SAS® Version 9.3 or higher software.

6 STUDY SUBJECTS

6.1 Participant Disposition

The disposition of participants and exposure to study vaccinations will be tabulated by site and treatment group.

6.2 Protocol Deviations

A summary of participant-specific protocol deviations will be presented by the reason for the deviation, the deviation category, and treatment group for all participants. All participant-specific protocol deviations and non-participant-specific protocol deviations will be included as data listings. Protocol deviations will not necessarily always lead to exclusion from the analysis population. Determination of exclusion will be established before breaking the blind and based on the blinded review of protocol violations.

7 STATISTICAL ANALYSIS

Descriptive statistics (mean, standard deviation, median, range) for continuous measures (e.g., age) and proportions for categorical measures (e.g., gender) will be summarized by treatment group. All percentages will be presented to one decimal place. Means, medians, standard deviations, confidence intervals, etc., will be presented as integers if the absolute value is greater than or equal to 100 in magnitude, to one decimal place if greater than or equal to 10, and 2 decimal places if less than 10. P-values will be reported to 4 decimal places.

For both safety and immunogenicity, when the use of descriptive statistics to assess group characteristics or differences is required, the following methods will be used: for categorical variables/responses, the number, proportion (% and exact 95% confidence interval) of participants in each category. For continuous variables/responses, the mean, median, standard deviation, quartiles and range, as well as the geometric mean and 95% confidence interval for immunogenicity outcomes. A non-parametric method (e.g., bootstrap or Wilcoxon rank sum test) will be used for continuous variables if the data are not consistent with the normal distribution.

The following methods will be used for formal testing of differences between study groups: for binomial response variables logistic regression will be used to test for an overall treatment effect and, if statistically significant, p-values from all pair-wise comparisons obtained from the regression model will be reported. Where multiple results are available for the same participant (e.g., reactogenicity after each vaccination), the correlation between results from the same participant will be taken into account by using a generalized mixed effects model, with treatment group as a fixed effect and participant as a random effect. For continuous variables that are not normally distributed, the overall treatment effect will be assessed by the Kruskal-Wallis test and if statistically significant, pair-wise comparisons will be performed using the Wilcoxon 2-sample test. For $\log_{10}$ immunogenicity values the overall treatment effect will be assessed by ANOVA and if statistically significant, estimates of all pair-wise comparisons from the model will be reported.

All testing and inference will be performed with a 2-sided p-value of 0.05. For each immunogenicity endpoint, a multiplicity adjustment for 4 treatment groups will be applied using a sequential testing approach by performing an overall comparison of all treatment groups prior to pair-wise comparisons. No multiplicity adjustment for multiple immunogenicity endpoints is planned, since the dose selection for the Phase 3 will be based on comprehensive statistical and clinical evaluation of all immunogenicity endpoints including safety results.

7.1 Study Objectives

The primary objectives of the study are to assess the safety, tolerability and immunogenicity of the study vaccine.

7.1.1 Safety (all 3 age groups)

All unsolicited AEs and SAEs reported after participants are exposed to the study product and up to 28 days post last study injection are included in the primary analysis of safety.

To assess safety, the number and percentage of participants experiencing at least one AE, and the number and percentage of participants experiencing each specific AE, categorized by body system and preferred term, is tabulated by study cohort and product received along with their corresponding exact binomial (Clopper-Pearson) 95% confidence intervals. Overall summaries by cohort and by product received include the number and percentage of participants experiencing: (1) any SAE; (2) any adverse experience; (3) any Grade 2 or greater AE; (4) any AE judged related to study product; (5) any Grade 2 or greater AE judged related to study product (**Table 14.3.2.1**). In addition, AEs will be summarized by grade. P-values from statistical analyses will be reported only for the primary endpoints listed in section 4.1.1.

7.1.2 Tolerability

All local reactions and systemic reactogenicity reported up to 7 days post vaccination are included. To assess tolerability, the number and percentage of participants with Grade 2 or higher local or systemic reactions is tabulated by study cohort and by product received along with their corresponding 95% confidence intervals. Local and systemic reactions are tabulated separately. Overall summaries by cohort and by product received will include the number and percentage of participants experiencing: (1) each specific reaction at Grade 2 or greater after each injection; (2) each specific reaction at Grade 2 or greater after any injection; (3) any reaction at Grade 2 or greater after each injection; and (4) any reaction at Grade 2 or greater after any injection (**Table 14.3.1.1 for local** and **Table 14.3.1.2 for systemic**). Descriptive statistics will be used to summarize the duration of local and systemic reactions. In addition, local and systemic reactions will be summarized by grade.

7.1.3 Clinical laboratory values

Changes in clinical laboratory values between baseline and one week after vaccination will be analyzed descriptively.

7.1.4 Immunogenicity Objectives

Immunogenicity analyses will compare the rates of responses and geometric mean titers (GMTs) between the various treatment groups based on injections received per protocol. These analyses will be conducted for both per-protocol and full analysis populations, provided the two populations differ.

To adjust for maternal IgG and neutralizing antibody in the infant population, an analysis of the adjusted sero-response rates utilizing the exponential decay function based on the estimated half-life of the maternal antibody will be performed in addition to the analysis based on the unadjusted raw values. These adjusted analyses will be performed on the combined infant cohorts for day 56 and day 84 versus pre-vaccination responses.

For each assay, an estimated maternal antibody half-life will be derived from linear regression of Log_2 transformed titers in placebo recipients from the combined infant cohorts. Any placebo recipient with a suspected community acquired rotavirus infection as identified by a post vaccination titer higher than baseline, or any placebo recipient with a baseline titer below the detectable limit of the assay will be excluded from the regression analysis.

Half-Life (in days) calculation

For each participant calculate:

- d = actual days post dose one
- $V_0 = Log_2(T_0)$ …… T_0 = baseline titer
- $V_1 = Log_2(T_d)$ …… T_d = titer on day d
- $GMdiff = V_1 - V_0$

Then perform:

- Linear regression of GMdiff = d
- Par = parameter estimate for Day (d)
- MH = -1 / Par …… MH = Maternal Half-Life (days)

Calculation of adjusted titers and adjusted fold-rise

$FRMA_d = (T_d / T_{0)} \times 2^{(d/MH)}$

where:

- $FRMA_d$ = maternal antibody adjusted fold-rise

Analysis of Anti-P2-VP8 Responses

Each of the three types of analysis below will be

Descriptive statistics and analysis of Anti-P2-VP8 titers will be presented by treatment group and time-point. This analysis will be separately presented for adults, toddlers and infants; IgG & IgA seroresponses; and the three vaccine antigens (P[2], P[4], P[6]). An ANOVA or Kruskal-Wallis test will be conducted to assess the overall difference between treatment groups in mean log (base10) titer. Separate p-values will be presented at each time-point. Two-sided 95% CI for the geometric mean titer (GMT) for each treatment group will be obtained using a t-distribution on log transformed titers/levels.

Anti-P2-VP8 Fold-Rise (FR) from Pre-Vaccination will be presented by treatment and time-point. This analysis will be separately presented for adults, toddlers and infants; IgG & IgA seroresponses; and three vaccine antigens (P[2], P[4], P[6]). An ANOVA or Kruskal-Wallis test will be conducted to assess the difference between treatment groups in mean log (base10) FR. Separate p-values will be presented at each time-point. Two-sided 95% CI for the geometric mean fold rise for each treatment group will be obtained using a t-distribution on log transformed fold rise measurements. For infants, analysis of IgG will be conducted two ways 1) Unadjusted for Maternal Antibodies 2) Adjusted for Maternal Antibodies.

Number and Proportion of subjects with Anti-P2-VP8 seroresponse (at least a 4-fold rise in anti P2-VP8 titer from baseline) will be presented by treatment and time-point. This analysis will be separately presented for adults, toddlers and infants; IgG & IgA seroresponses; the three vaccine antigens (P[2], P[4], P[6]); subjects with seroresponses to all 3 vaccine antigens and to each of the 3 specific pairs of vaccine antigens. The proportion of participants with a positive response at a specific time point will be evaluated using the 2-sided exact binomial (Clopper-Pearson) 95% CI for each treatment group. A logistic regression analysis will be performed to test the overall treatment effect. Separate p-values will be presented at each time-point. For infants, analysis of IgG will be conducted two ways 1) Unadjusted for Maternal Antibodies 2) Adjusted for Maternal Antibodies.

Analysis of Neutralizing Antibody Responses

Descriptive statistics and analysis of neutralizing antibody titers will be presented by treatment and time-point. This analysis will be separately presented for adults, toddlers and infants and each rotavirus strain (Wa, DS-1 and 1076) as well as heterologous strains. An ANOVA or Kruskal-Wallis test will be conducted to assess the difference between treatment groups in mean log (base10) titer. Separate p-values will be presented at each time-point. Two-sided 95% CI for the geometric mean titer (GMT) for each treatment group will be obtained using a t-distribution on log transformed titers/levels.

Neutralizing antibody Fold-Rise (FR) from Pre-Vaccination will be presented by treatment and time-point. This analysis will be separately presented for adults, toddlers and infants and each rotavirus strain (Wa, DS-1 and 1076) as well as heterologous strains. An ANOVA or Kruskal-Wallis test will be conducted to assess the difference between treatment groups in mean log (base10) FR. Separate p-values will be presented at each time-point. Two-sided 95% CI for the geometric mean fold rise for each treatment group will be obtained using a t-distribution on log transformed fold rise measurements. For infants, analysis will be conducted two ways 1) Unadjusted for Maternal Antibodies 2) Adjusted for Maternal Antibodies.

Number and Proportion of subjects with neutralizing antibody response (at least a 2.7-fold and a 4-fold rise in neutralizing antibody titers from baseline) will be presented by treatment and time-point. This analysis will be separately presented for adults, toddlers and infants; and each of the three rotavirus strains (Wa, DS1, 1076) as well as heterologous strains. The proportion of participants with a positive response at a specific time point will be evaluated using the 2-sided exact binomial (Clopper-Pearson) 95% CI for each treatment group. A logistic regression analysis will be performed to test the overall treatment effect. Separate p-values will be presented at each time-point. For infants, analysis will be conducted two ways 1) Unadjusted for Maternal Antibodies 2) Adjusted for Maternal Antibodies.

Number and Proportion of subjects with a neutralizing antibody response against at least **2 of the 3** rotavirus strains (Wa, DS1, 1076), against all 3 rotavirus strains and against each of the 3 specific pairs of rotavirus strains will be presented by treatment group and time-point. The proportion of participants with a positive response at a specific time point will be evaluated using the 2-sided exact binomial (Clopper-Pearson) 95% CI for each treatment group. A logistic regression analysis will be performed to test the overall treatment effect. Separate p-values, estimates and 95% confidence intervals from the logistic regression will be presented at each time-point. For infants, analysis will be conducted two ways 1) Unadjusted for Maternal Antibodies 2) Adjusted for Maternal Antibodies.

Analysis of Anti-rotavirus IgA Responses to Whole Viral Lysate

Descriptive statistics and analysis of anti-rotavirus to whole lysate will be presented by treatment and time-point for infants only. An ANOVA or Kruskal-Wallis test will be conducted to assess the difference between treatment groups in mean log (base10) response. Separate p-values will be presented at each time-point. Two-sided 95% CI for the geometric mean titer (GMT) for each treatment group will be obtained using a t-distribution on log transformed titers/levels.

Fold-Rise (FR) from Pre-Vaccination in anti-rotavirus to whole lysate will be presented by treatment at 4 weeks post final vaccination. An ANOVA or Kruskal-Wallis test will be conducted to assess the difference between treatment groups in mean log (base10) FR. Two-sided 95% CI for the geometric mean fold rise for each treatment group will be obtained using a t-distribution on log transformed fold rise measurements.

Number and Proportion of subjects with an anti-rotavirus to whole lysate response (at least a 4-fold rise from baseline) will be presented by treatment at 4 weeks post final vaccination. The proportion of participants with a positive response will be evaluated using the 2-sided exact binomial (Clopper-Pearson) 95% CI for each treatment group. A logistic regression analysis will be performed to test the overall treatment effect.

7.1.5 Rotarix Shedding

Assessment of shedding of Rotarix virus is performed for each of the three specified post-vaccination days (Days 89, 91 and 93, i.e., 5, 7 and 9 days after the first Rotarix administration) and for shedding on any of the three days. The primary of these analyses will be for shedding on any of the three days. Additionally, the effect of IgA, IgG and Nab responses (2.7-fold and 4-fold rise on day 84) on shedding of Rotarix virus (at any time) will be analyzed, by each of the 3 antigens (for IgA and IgG) and 3 rotavirus strains (NAb). The proportion of participants with shedding at a specific time point and at any time point will be evaluated using the 2-sided exact binomial (Clopper-Pearson) 95% CI for each treatment group. A logistic regression analysis will be performed to test the overall treatment effect and separate p-values will be presented for each time-point and overall.