STATISTICAL ANALYSIS PLAN

Protocol title: A Phase II randomised, double blind, parallel group dose-ranging study of oral RV3-BB Rotavirus Vaccine administered at a titre of $1x10^7$, 3×10^6 or 1×10^6 as a 3 dose neonate schedule or administered at a titre of $1x10^7$as a 3 dose infant schedule.	
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Glossary of abbreviations

ABBREVIATION	DESCRIPTION
AE	Adverse event
CI	Confidence interval
CV	Coefficient of variation
DBL	Database lock
eCRF	Electronic case report form
ICH	International Conference on Harmonisation
IP	Investigational product
ITT	Intention-to-treat
MedDRA	Medical dictionary for regulatory activities
MIMS	Monthly Index of Medical Specialities
N	Sample size
ND	Not done
ODS	Output delivery system
PP	Per-protocol
PT	Preferred term
RTF	Rich text format
SAE	Serious adverse event
SAF	Safety
SAP	Statistical Analysis Plan
SD	Standard deviation
SOC	System organ class
TLFs	Tables, data listings and figures

1. Overview

1.1.1 Introduction

This document describes the rules and conventions to be used in the presentation and analysis of a phase II randomised, double blind, parallel group dose-ranging study of oral RV3-BB Rotavirus Vaccine administered at a titre of $1x10^7$, $3x10^6$ or $1x10^6$ as a 3 dose neonate schedule or administered at a titre of $1x10^7$as a 3 dose infant schedule.

This statistical analysis plan (SAP), is based on protocol MCRI-RV3-BB-004, version 04, dated 11-Jan-2019 and CRF, Version 05, Dated 22-Jul-2019.

2. Trial objectives

The following objectives are those stated in the protocol.

2.1.1 Primary objectives

The primary objective of this study is to assess a cumulative anti-rotavirus serum IgA response (defined as a ≥ 3-fold increase from baseline) 4 weeks after 3 doses of RV3-BB administered in a neonatal schedule at a vaccine titre of $1x10^7$, $3x10^6$ or $1x10^6$.

2.1.2 Secondary objectives

The secondary objectives of this study are:

- To assess the cumulative anti-rotavirus serum IgA response (defined as a ≥3-fold increase from baseline) 4 weeks after 3 doses of RV3-BB administered in an infant schedule at a vaccine titre of $1x10^7$.
- To assess cumulative vaccine take and components of vaccine take after 3 doses of RV3-BB (titre of $1x10^7$) administered in a neonatal schedule versus three doses of RV3-BB in an infant schedule at a vaccine titre of $1x10^7$.
- To assess cumulative vaccine take and the components of RV3-BB vaccine take after 3 doses of RV3-BB administered as a neonatal schedule at a vaccine titre of $1x10^7$, $3x10^6$ or $1x10^6$.
- To assess cumulative vaccine take and components of vaccine take after 2 doses of RV3-BB administered as a neonatal schedule at a vaccine titre of $1x10^7$, $3x10^6$ or $1x10^6$ or 2 doses as an infant schedule at a vaccine titre of $1x10^7$.
- To assess cumulative vaccine take and components of vaccine take after the first dose of RV3-BB administered as a neonatal schedule at a vaccine titre of $1x10^7$, $3x10^6$ or $1x10^6$ compared to placebo (1st dose of IP in the infant schedule).
- To assess cumulative vaccine take and components of vaccine take after the first dose of RV3-BB at a vaccine titre of $1x10^7$, $3x10^6$ or $1x10^6$ in the neonatal schedule compared with the first dose of RV3-BB at a vaccine titre of $1x10^7$ in the infant schedule.
- To describe the geometric mean titre of the anti-rotavirus serum IgA response after 3 doses of RV3-BB administered as a neonatal schedule or an infant schedule.

- To describe the safety and tolerability of RV3-BB when administered as an infant or as a neonatal schedule.
- To describe the occurrence of diarrhoea episodes in participants, according to severity and detection of wild-type rotavirus.

2.1.3 Exploratory objectives

The basis of the exploratory objectives is to further understand potential barriers to rotavirus vaccine efficacy in the region. The exploratory objectives of this study are:

- To describe Histo-blood Group Antigens (Lewis and Secretor) status of participants in association with anti-rotavirus sero-conversion and cumulative vaccine take after 3 doses RV3-BB administered in either the neonatal schedule (any titre) or infant schedule.
- To assess maternal anti-rotavirus IgA and IgG levels in association with anti-rotavirus IgA seroconversion and cumulative vaccine take in participants following receipt of RV3-BB administered in either the neonatal schedule (at any vaccine titre) or infant schedule at a vaccine titre of $1x10^7$.
- To describe the gut microbiome in participants receiving RV3-BB administered in the neonatal schedule (at any vaccine titre) or infant schedule at a vaccine titre of $1x10^7$.
- To describe innate and adaptive immune responses in infants vaccinated with RV3-BB administered in the neonatal vaccine schedule (any titre) or in the infant schedule at a vaccine titre of $1x10^7$.

3. Endpoints

3.1.1 Immunogenicity endpoints

Immunogenicity will be assessed through:

- Serum anti-rotavirus IgA response (defined as a ≥3 fold increase from baseline) at each serum collection time-point to 4 weeks after 3 doses of RV3-BB.
- Serum anti-rotavirus IgA levels at each serum collection time point.
- Vaccine take defined as at least a threefold increase in serum anti-rotavirus immunoglobulin A (IgA) from baseline to post IP dosing, or detectable RV3 shedding in stools (by ELISA or PCR) any day from day 3 to 5 following administration of IP.
- Cumulative vaccine take defined as vaccine take observed at the current assessment time point or following any previous dose.
- Anti-rotavirus IgA response (defined as at least a threefold increase from baseline to post IP dosing) at each serum collection time point.
- Presence of RV3 shedding in stools (by ELISA or PCR) from day 3 to 5 following each dose of IP.
- Anti-rotavirus IgA titres defined as geometric mean titres at each serum collection time point.
- Episodes of diarrhoea, from the time of randomisation to 28 days following the last dose of IP. Episodes will be confirmed by Rotavirus antigen ELISA on samples.

3.1.2 Safety and tolerability endpoints

Safety and tolerability will be assessed through:

- Unsolicited Adverse Events (AE) in the days 0-28 following each dose of IP.
- Serious Adverse Events (SAE) from the first dose of IP until 28 days post last dose of IP.
- Review of episodes of blood in stool.

3.1.3 Exploratory endpoints

Exploratory endpoints will be assessed through:

HBGA Lewis and Secretor status will be determined by ELISA/PCR on Saliva samples collected at Visits 1, 3, 5 and 7 and whole blood collected at Visit 9 for non secretor participants.

- Maternal serum IgA and IgG response will be determined on maternal serum sample collected pre study or at Visit 1.
- Bacterial taxonomic and genetic functional profiling of stool microbiome will be conducted on stool samples collected post administration of IP and at birth (prior to first IP dose).
- B and T cell function will be measured by flow cytometry and innate and adaptive responses measured by whole killed pathogen assay and Toll like receptor ligand assays from whole blood collected at birth (cord), Visit 3 and Visit 9.

4. Trial design

4.1.1 Design overview

This is a Phase II, randomised, double blind, four arm parallel group study. Approximately 688 participants will be randomised to the study; 172 to each treatment arm.

Pregnant women will be invited to provide preliminary consent for the study prior to labour and delivery. A maternal blood sample and a stool will be collected. Following delivery, those who remain interested will be invited to provide written informed consent for their baby to participate in the study.

Following confirmation of eligibility for the trial post-birth, participants will be randomised in a 1:1:1:1 ratio to one of four treatment arms; high-dose, mid-dose or low-dose neonatal schedule, or a high-dose infant schedule. Each study participant will receive four oral doses of IP, with each administration consisting of 1 mL RV3-BB Rotavirus Vaccine or 1mL of placebo. All participants will receive a total of 3 doses of RV3-BB vaccine, one dose of placebo, and one dose of Rotarix®.

A baseline stool sample will be collected prior to the administration of the first dose of IP. A stool sample will also be collected on either Day 3, 4 or 5 following each dose of IP. Samples will also be collected for any cases of diarrhoea (defined as 3 or more stools that are looser than normal for that participant).

A saliva sample collected at birth and prior to IP doses 2, 3 and 4.

Cord blood will be collected at birth (or a venous blood sample if cord not available), and blood samples taken prior to IP doses 2, 3 and 4, and prior to the Rotarix® dose.

Participants will be followed until 18 weeks of age. Face to face visits will be conducted at least monthly. Adverse events, including blood-in-stool occurrences, and diarrhoea episodes will be recorded for the duration of the study.

An independent Data Safety Monitoring Board (DSMB) will review safety data at agreed recruitment and progression milestones throughout the study, as agreed and documented in the DSMB Charter. The DSMB will recommend whether the study

continues unchanged, continues with amendments, or is stopped. Recruitment will continue during the DSMB review periods.

4.1.2 Study duration

The recruitment period is estimated at 9 months. The study period for each participant will be 18 weeks, and the total duration of the study approximately 15 months.

4.1.3 Schedule of Procedures

Visit	Pre-study	1	2	3	4	5	6	7	8	9[9]
Timing	Second trimester to birth	Day 1	Day 8	Week 6	Week 7	Week 10	Week 11	Week 14	Week 15	Week 18
Window		Birth to 144 hrs old	7±1 days post V1	+7 days	7±1 days post V3	±7 days and at least 21 days post V3	7±1 days post V5	±7 days and at least 21 days post V5	7±1 days post V7	±7 days and at least 21 days post V7
Preliminary consent	X									
Cord blood collection	X									
Maternal blood sample	X									
Maternal stool sample	X									
Study consent		X								
Eligibility confirmation		X								
Demographics and medical history		X								
Physical exam		X								
Vital signs[1]		X	X	X	X	X	X	X	X	X
Stool sample	X[5]	X[6]		X[6]		X[6]		X[6]		
Saliva sample		X		X		X		X		
Randomisation		X								
IP administration		X		X		X		X		
Antacid administration[2]				X		X		X		
EPI vaccine administration[9]		X		X		X		X		X
IPV administration				X		X		X		
Rotarix® administration										X[7]
Pre dose blood sample		X[10]		X		X		X		X
Issue/review diarrhoea log[3]		X		X		X		X		X
Issue/review study reminder Card[3]		X		X		X		X		X
Issue stool sample kits[3]		X		X		X		X		
Feeding review[4]		X	X	X	X	X	X	X	X	X
AE recording		X	X	X	X	X	X	X	X	X
Con med recording		X	X	X	X	X	X	X	X	X
Follow up contacts			Weekly contact (clinic, home or telephone) throughout the study							

1. Temperature, pulse rate, respiratory rate. At dosing visits vital signs will be measured within one hour pre-dose and 15 and 30 mins post dose.

2. 2mL of Mylanta Original® will be administered within the 10 minutes prior to the IP dose.
3. Logs, reminder card and kits will be issued and reviewed as required.
4. Record percentage of baby's intake that is breast milk, formula, or other.
5. A pre-dose stool sample will be collected at any time before the first dose of IP.
6. A stool sample will be collected 3 – 5 days post dose.
7. If the participant withdraws prior to receiving all IP doses they will be given a Rotarix® dose one month following their last dose of IP.
8. Day 1 pre-dose sample will be cord blood.
9. EPI vaccines will be provided via usual supplier (not study) but administered by study staff at appropriate time points according to standard schedule. The exceptions are OPV and other rotavirus vaccines, which will not be given (see Section 5.4 of the protocol).
10. A 1.0mL blood sample will be collected if cord blood is not able to be collected.

5. Changes/deviations from the planned analysis

Analysis and reporting of some exploratory objectives of this study will be completed outside of this analysis plan and the clinical study report. The analysis for the immune development and microbiome endpoints will take a significant amount of time to complete and the complex nature and large amounts of data generated for each will impact on the time to reporting for the primary endpoint. The disposition of samples collected specifically for these exploratory objectives will be included as listings in the clinical study report.

This relates specifically to the below objectives and endpoints of the study. For each objective an analysis plan will be pre-defined and documented prior to starting analysis and an addendum report or publication and will be added to the clinical study report when all analysis is complete.

Objectives:

- To describe the gut microbiome in participants receiving RV3-BB administered in the neonatal schedule (at any vaccine titre) or infant schedule at a vaccine titre of $1x10^7$.
- To describe innate and adaptive immune responses in infants vaccinated with RV3-BB administered in the neonatal vaccine schedule (any titre) or in the infant schedule at a vaccine titre of $1x10^7$.

Endpoints:

- Bacterial taxonomic and genetic functional profiling of stool microbiome will be conducted on stool samples collected post administration of IP and at birth (prior to first IP dose).
- B and T cell function will be measured by flow cytometry and innate and adaptive responses measured by whole killed pathogen assay and Toll like receptor ligand assays from whole blood collected at birth (cord), Visit 3 and Visit 9.

Based on established data on cord blood serum IgA in 100 mother baby pairs in Indonesia (Chan et al 2011) and also in New Zealand in the Phase IIa study (77 cord blood tested for serum IgA) (Bines et al 2015) and reported elsewhere, we anticipate that all participants should have a cord blood serum IgA concentration lower than the limit of detection (20 units). This is consistent with biology and if serum IgA is detected in cord blood above the definition of baseline it is not biologically plausible and could be an error at the time of collection or processing of the sample. During sample testing and analysis the laboratory reported that a higher than previously observed rate of collection error for cord samples with approximately 15-20% of samples swapped or contaminated at the time of collection. It has been reported elsewhere that up to 20% of samples are often contaminated or invalid at the time of collection (Tong et al 2002).

In previous studies, samples with an IgA result greater than 30 were excluded from analysis and handled as missing data (assigned a value of 10) (Bines et al 2018). In response to the observation of increased collection/ handling error (in previous studies the total samples excluded based on sampling or collection error was approximately 5%) it is planned to assign all baseline cord values greater than 30 to lower than the limit of detection (assigned a value of 10) for analysis for serum IgA primary and secondary analyses. A sensitivity analysis for the primary analysis using the reported cord values for serum IgA as the baseline value for the calculation of a serum IgA response will also be presented (note, this is only relevant for comparisons involving the neonatal schedule where cord blood is used as the baseline).

6. Analysis populations

Agreement and authorization of participants included/excluded from each analysis population will be reached and documented prior to final database hard lock. TCD will provide the sponsor with a list of all potential participants to be excluded from the relevant analysis populations, including the reason(s) for exclusion from the analysis populations. Decisions about these potential exclusions from specific analysis populations will be made at a Blinded Data Review Meeting [to include at least the sponsor, responsible TCD statistician and the responsible project manager(s)], the minutes of which will document any decisions about exclusions from analysis populations. The Blinded Data Review Meeting will occur prior to database lock to ensure that any final potential queries can be answered before the final lock. Any reclassification/recategorization of protocol deviations during the Blinded Data Review Meeting will be considered final to align with decisions reached on exclusions from analysis populations.

The following sets will be used for the statistical analyses:

6.1.1 Full Analysis Dataset (FAS)

All participants randomised into the study. Participants will be analysed according to treatment to which they were randomised. Immunogenicity analyses performed in the FAS are considered supportive.

6.1.2 Safety Analysis Dataset (SAF)

All randomised participants who received at least one dose of IP. Participants will be analysed according to the treatment received.

6.1.3 Intention-to-Treat (ITT) Analysis Dataset

All randomised participants who received at least one dose of IP and have an evaluable baseline and post-baseline blood sample (at least one post baseline dose of RV3-BB). Participants will be analysed according to the treatment to which they were randomised. All analyses performed using the ITT set will be considered supportive.

6.1.4 Per-protocol (PP) Analysis Dataset

All participants from the ITT population who completed the study in compliance with the protocol and who reported no major violation of the study protocol which may have effect on the primary endpoint. Participants will be analysed according to the treatment to which they were randomised. Primary analyses will be performed on the PP set.

7. General considerations

7.1.1 Visit and date conventions

Visit day will be calculated from the reference treatment start date which will be used to present start/stop day of assessments and events. The reference treatment start date is defined as the date of first IP administration. The following conventions will be used for visit references:

- $Visit\ day = date\ of\ event - reference\ treatment\ start\ date$
- $Visit\ week = \frac{visit\ day}{7},\ rounding\ up\ to\ next\ whole\ number$

No visit windowing (i.e. remapping of visits based on visit windows) will be performed for this trial. The assigned nominal visit will be used for by-visit summaries. In the situation where the assessment/event date is partial or missing, visit day/week/month, and any corresponding durations will appear missing in the data listings. Unscheduled measurements will not be included in by-visit summaries. Trial visit will be assigned as below (see section 4.3, Schedule of Events):

Pre-study: Second trimester to birth

Preliminary consent, cord blood collection and maternal blood sampling will happen during this period.

Visit 1 (Day 1; birth to 144 hours old)

Participants will return to the trial site for Day 1 procedures and maternal blood samples will be collected.

Visit 2 (Day 8; 7±1 days post visit 1), Visit 4 (Week 7; 7±1 days post visit 3), Visit 6 (Week 11; 7±1 days post visit 5), Visit 8 (Week 15; 7±1 days post visit 7)

Participants will return to the trial site for vital sign measurements, feeding review, AE and concomitant medication recording.

Visit 3 (Week 6; + 7 days), Visit 5 (Week 10; ±7 days and at least 21 days post visit 3), Visit 7 (Week 14; ±7 days and at least 21 days post visit 5), Visit 9 (Week 18; ±7 days and at least 21 days post visit 7)

Participants will return to the trial site for Week 6/10/14/18 procedures.

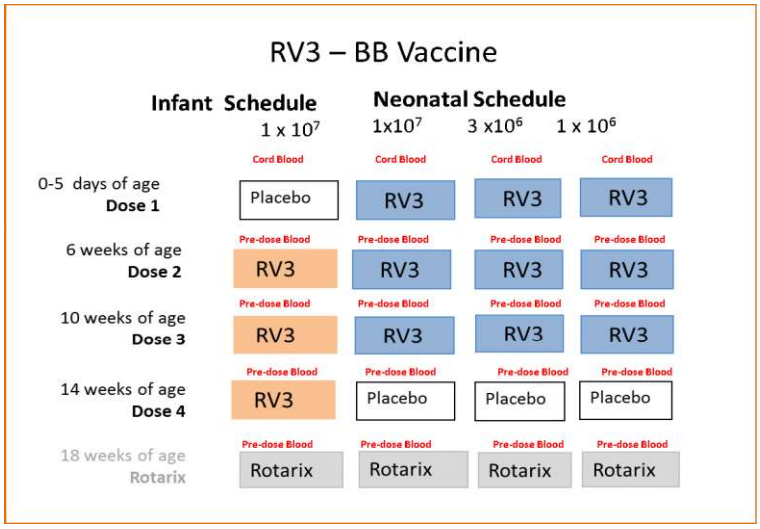
7.1.2 Baseline

Unless otherwise specified, Baseline is defined as the last non-missing observation taken prior to IP administration.

Due to the timing of the blood tests, baselines for serum anti-rotavirus IgA are defined differently for participants in the neonatal vaccine schedule and the infant vaccine schedule. Baseline serum anti-rotavirus IgA in the neonatal vaccine schedule is defined as the measurement from the cord blood. In the infant vaccine schedule baseline is defined as the measurement from analysis of serum collected prior to the second dose, although the measurement from the cord blood/visit 1 venous blood may be used if there are no data on IgA following the first dose of IP. Note that where cord blood is unavailable for collection, a venous blood sample can be collected at Visit 1 prior to IP administration. See section 8.1.3 for details.

7.1.3 Stratifications

For analysis purposes, participants may be sub-classified into the following stratification levels, where applicable:



- Gender
  - Male
  - Female
- Delivery route
  - NVD
  - Caesarean Section
- Race/predominant ethnicity
  - American Indian or Alaska Native
  - Asian
  - Black or African American
  - Native Hawaiian or Other Pacific Islander
  - White
  - Other
  - Unknown
  - Not reported

7.1.4 Statistical tests

A single analysis will be performed at the end of the trial. No interim analyses are planned.

Primary Analysis:

The primary analysis and primary sensitivity analysis are based on the per protocol population defined as participants who receive all 3 doses of RV3-BB and have no major protocol violations (after reviewed and agreed at the end of the study). Thirty percent of participants are expected to be excluded from the PP population due to death, study withdrawal, loss to follow-up, or study non-compliance.

Non-inferiority of the lower titre vaccine will also be assessed in the intention to treat population. The cumulative probability of response by 18 weeks of age will be estimated by the Kaplan-Meier method for each vaccine arm. The difference between high and low titre arms in the cumulative probabilities and its 95% confidence interval will be calculated. If the upper bound of the confidence interval is below 20%, non-inferiority of the lower titre vaccine will be demonstrated.

Additionally, we will calculate the 95% CI of the binomial proportions using the method of Newcombe-Wilson without continuity correction and generalized estimating equations (GEE) after dose 3 on the PP and ITT analyses set.

All the primary and primary sensitivity analysis will be based on PP and ITT analysis set.

See section 10 for additional details.

Secondary Analyses:

Immunogenicity and vaccine-take results will be (inferentially) analysed as done for the primary analysis. Descriptive analyses are described in section 10.

Safety

No formal statistical testing will be performed on the safety data.

7.1.5 Software

All analyses will be conducted using SAS® Version 9.4 (or above).

8. Statistical considerations

8.1.1 Sample size

With the high titre RV3-BB neonatal vaccine schedule as the active control arm, the sample size is calculated to demonstrate the non-inferiority of the lower titre vaccine arms with respect to the proportion of participants who have a serum IgA response, 4 weeks after 3 doses of vaccine. A non-inferiority margin of 20% for the difference between arms will be used.

The primary analysis is based on the per protocol population defined as participants who receive all 3 doses of RV3-BB and have no major protocol violations. Thirty percent of participants are expected to be excluded from the PP population due to death, study withdrawal, loss to follow-up, or study non-compliance. Among those in the PP population, a 50% response probability is assumed for the active controls. Based on a one-sided 0.025 level score test with 90% power under the alternative of no difference in response probabilities, 172 participants per arm are required for a total sample size of 688 participants.

8.1.2 Multicentre studies

Approximately 688 babies will be randomised across three sites in Malawi. Study visits will be conducted at the sites or at participant homes.

8.1.3 Missing data

Where possible, data from participants who withdraw prematurely from the study will be included in any analysis.

Participants with missing baseline IgA measurements will be assigned a value below the lower limit of detection (i.e assigned a value of 10) in the calculation of serum immune response (although the cord blood IgA value will still be acknowledged as missing when summarising titre). Partial date imputation of adverse events and concomitant medications are mentioned in respective sections.

9. Primary and Secondary Analyses

In order to be included in the analysis of cumulative vaccine take, participants must have at least one of IgA response or shedding data following any dose of IP

Where:

1. An IgA response is defined as a 3-fold rise from baseline, where baseline is defined as cord blood for neonatal comparison and post dose 1 blood for infant comparison (or cord if post dose 1 data is missing). It will be assumed that cord blood is 10 if cord blood is missing).
2. Shedding data is defined as having stool data for at least 1 time point from days 3-5 post vaccine.

All available data on IgA response, shedding at all time points (post dose 1, 2 and 3 for the neonatal comparison and post doses 2, 3 and 4 for the infant comparison) will be used to determine whether vaccine take is positive or negative for each individual, with participants classified as having a positive vaccine take if any response (IgA, or shedding days 3-5) at any time point is positive, and classified as negative for vaccine take otherwise (i.e. if all known measurements at all time points are negative).

9.1.1 Variables and derivations

Primary endpoint:

A *serum IgA response* in the *neonatal* schedule, is defined as a ≥ 3-fold increase from baseline, where baseline is defined as stated in sections 7.1.2 and 8.1.3 for handling of missing baseline IgA measurements of this analysis plan.

Secondary endpoints:

- A *serum IgA response* in the *infant* schedule, is defined as a ≥ 3-fold increase from baseline, where baseline is defined as stated in sections 7.1.2 and 8.1.3 for handling of missing baseline IgA measurements of this analysis plan.

- *Cumulative vaccine take* is defined as vaccine take observed at the current assessment time point or following any previous dose, where *vaccine take* is

defined as ≥ 3-fold increase in serum anti-rotavirus IgA from baseline to post IP dosing, or detectable RV3 shedding in stools (by ELISA or PCR) any day from day 3 to 5 following administration of IP.

- *Shedding of RV3-BB* is defined as presence of RV3-BB shedding in stool from days 3-5 following each dose of IP.

- *Geometric mean titre* of the anti-rotavirus serum IgA response after 3 doses of RV3-BB.
- *Safety and tolerability* (as per sections 15 to 16 of this analysis plan) of RV3-BB.
- *Occurrence of diarrhoea episodes* in participants, according to severity and detection of wild-type rotavirus.

Derivations:

A participant will, at each vaccine titre, be defined a "responder" if he/she has a serum *IgA response* (≥ 3-fold increase in serum anti-rotavirus IgA from baseline) for neonatal arms IP dose 1 - 3 and infant arm IP dose 2 - 4 of RV3-BB. The response rate will be dichotomised (0 = non-responder, 1 = responder).

Geometric means (and associated summary statistics) will be determined as follows:

1. Log-transform the titre data (if not already log-transformed)
2. Determine the arithmetic mean and other descriptive statistics of the transformed data using the PROC MEANS procedure
3. Exponentiate the output obtained from the PROC MEANS procedure, depending on data to be presented (log-transfer vs not log-transformed)

9.1.2 Analysis

Non-inferiority of lower titre vaccines (primary as well as secondary endpoints):

The difference between high and low titre arms in the response rate and its 95% confidence interval will be calculated. The cumulative probability of anti-rotavirus serum IgA response (as defined above) by 18 weeks of age will be estimated by the Kaplan-Meier method for each vaccine arm. PROC LIFETEST (SAS® Version 9.4 or above) will

be used to analyse the response rates (non-responder *vs.* responder). With the CENSOR variable, 1 is commonly used to indicate that an event occurred (subject is a responder) and 0 indicates that the event did not occur (the subject was censored/is a non-responder).

Non-inferiority of the lower titre vaccine will be demonstrated if the upper bound of the confidence interval is below 20%.

Additionally, we will calculate the 95% CI of the difference between vaccine groups in the serum IgA response rate after dose 3 using the method of Newcombe-Wilson without continuity correction for the difference between binomial proportions as implemented in PROC FREQ (SAS® Version 9.4 or above).

Generalized estimating equations (GEE) will be used to analyse the response rates (non-responder vs. responder) after dose 3 using PROC GEE (SAS® Version 9.4 or above). We will calculate a binary outcome (1 = ≥ 3-fold increase, 0 = < 3-fold increase from baseline) after dose 1, 2, and 3 for each participant who received vaccine according to the neonatal schedule. After that, we will fit a model with dose as a discrete/categorical/factor variable, vaccine group (low, medium, high dose) as a discrete variable, and dose by vaccine group interaction term. The model will use the binomial distribution for the outcome variable and the exchangeable correlation matrix to account for the correlation between a participant who received vaccine according to the neonatal schedule outcomes. Model will be selected based on quasi information criteria (QIC) and the model with less QIC value will be used for the analysis. Once the model is fit, contrast statements will be used in PROC GENMOD (SAS® Version 9.4 or above) to calculate the difference in the response rate after dose 3 for the high versus low dose and so forth.

In the table, (Estimate, standard error, 95% CI) will be presented.

The general linear model to be fit by GEE is

$$\text{logit}(p_{ij}) = \beta_{ij}$$

where p_{ij} is the response rate after the i-th dose in the j-th vaccine group. Differences between the βs represent the log odds ratio in response rates.

The cumulative vaccine take and the components of vaccine take (i.e. secondary endpoints) will be estimated similarly,

All summary and listing of immunogenicity data will be based on the FAS, ITT and PP populations. All the primary endpoint, primary sensitivity and secondary endpoint efficacy analysis will be based on the PP and ITT populations.

9.1.3 Serum IgA

Serum IgA titres will be summarised at each serum collection time point, by treatment group using geometric means and the mean and standard deviation on the log scale. Measurements below the lower limit of detection will be assigned a value of half of limit for analysis e.g., if IgA is <20, this will be assigned a value of 10. This will be performed by the laboratory personnel prior to data transfer. The number of participants (and proportion) with measurements below the lower limit of detection will also be presented, using frequency tabulations. Infant and neonatal Serum IgA titres will also be listed on log scale by treatment group on FAS population.

9.1.4 Cumulative serum Anti-Rotavirus IgA Response

The number and proportion of participants with a positive serum anti-IgA rotavirus response (defined as ≥3 fold increase in titre from baseline in anti-rotavirus IgA, to post investigational product dosing) at each serum collection time point will be summarised, by treatment group for the neonatal vaccine schedule group and the infant vaccine schedule group, using frequency tabulations.

9.1.5 Cumulative Vaccine Take After Three Doses of RV3-BB

Baseline values of IgA measurement for the neonatal schedule will be obtained from analysis of cord blood. Baseline values of IgA measurements for the infant schedule will be obtained from analysis of sera collected prior to the second dose of investigational product.

Neonatal comparison - a positive cumulative vaccine take corresponds to a serum immune response post investigational product doses 1, 2 or 3 or detectable RV3-BB virus shedding in stool (days 3, 4 or 5) post investigational product doses 1, 2 or 3.

Infant comparison - a positive cumulative vaccine take corresponds to a serum immune response post investigational product doses 2, 3 or 4 or detectable RV3-BB virus shedding in stool (days 3, 4 or 5)) post investigational product doses 2, 3 or 4.

9.1.6 Cumulative Vaccine Take After Two RV3-BB Doses

The number and proportion of participants with cumulative vaccine take after two RV3-BB doses (i.e., infant schedule with positive serum immune response post investigational product dose 2 or 3 or RV3-BB virus shedding in stool post investigational product dose 2 or 3 or neonatal schedule with positive serum immune response post investigational product dose 1 or 2 or RV3-BB virus shedding in stool post investigational product dose 1 or 2) will be summarised, by treatment group for the neonatal and infant schedule groups, using frequency tabulations.

9.1.7 Cumulative Vaccine Take After one RV3-BB Doses

The number and proportion of participants with cumulative vaccine take after the first RV3-BB dose (i.e., neonatal schedule with positive serum immune response post investigational product dose 1 or RV3-BB virus shedding in stool post investigational product dose 1 compared to “placebo” infant schedule with positive serum immune response post investigational product dose 1 or RV3-BB virus shedding in stool post investigational product dose 1 will be summarised, by treatment group for the neonatal schedule group and the “placebo” infant group, using frequency tabulations.

9.1.8 Shedding of RV3-BB

Only rotavirus VP6 PCR positive and “RV3 vaccine-like” responses will be summarised for RV3-BB shedding. Positive PCR samples that are sequenced as “wild type-like” by sequence analysis will not be included in RV3-BB shedding but will be listed.

The number and proportion of participants shedding RV3-BB on one of days 3-5 following each dose of investigational product will be summarised for each dose number, by treatment group using frequency tabulations. In addition, the number and proportion of participants shedding according to NSP3 PCR on one of days 3-5 following each dose

of investigational product will be summarised for each dose number, by treatment group using frequency tabulations.

9.1.9 New shedders

(VP6 PCR positive and “RV3 vaccine-like” responses) will be summarised in frequency tabulations for the number and proportion of participants who are “new shedders”, at doses 2, 3 and 4.

9.1.10 Diarrhoea endpoint

The presence of rotavirus and the severity of episodes in diarrhoea samples will be summarised (n, %) and listed by treatment group. Description of the occurrence of diarrhoea episodes in participants, according to severity and strain of wild-type rotavirus will be listed.

9.1.11 Other endpoints:

- Comparison of the geometric mean titre of the anti-rotavirus serum IgA response after 3 doses of RV3-BB between the neonatal schedules versus the infant schedule.

- Description of safety and tolerability (as per sections 15 to 16 of this analysis plan) of RV3-BB, for the neonatal schedules versus the infant schedule.

9.1.12 Exploratory endpoints:

- Histo-blood group antigens (HBGA) status of participants in association with anti-rotavirus sero-conversion and cumulative vaccine take after 3 doses RV3-BB administered in either the neonatal schedule (any titre) or infant schedule.
  The number and proportion of participants for HBGA status (HBGA phenotype) will be presented overall for treatment group and by dose. HBGA phenotyping is determined by detection of antigens A, B, H, and Lewis a and b in saliva by enzyme-linked immunosorbent assay (ELISA). Infants with detectable salivary A, B, or H antigen are classified as secretors. Where detection of A, B, and H antigens was negative or borderline, secretor status was confirmed by ELISA to

detect lectin antigen. Infants who were positive for either Lewis a or Lewis b antigen were classed as Lewis positive, and those negative for both Lewis antigens as Lewis negative. *FUT2* genotype is determined for infants of nonsecretor phenotype (ie ABH negative). FUT2 genotyping results are "non secretor" (se/se) "secretor" (Se/Se or secretor (se/Se).

The HBGA phenotype status will be listed by treatment group on the FAS population.

Vaccine take and components of, shedding and IgA seroconversion will be summarised by HBGA phenotype for the neonatal (any titre) and infant schedules after three doses of RV3-BB.

HBGA Phenotyping criteria:

A, B, H	Lewis b	Lewis a	Lectin	HBGA Phenotype
At least 1 positive	Positive	Negative	Not required	Secretor Lewis positive
At least 1 positive	Negative	Negative	Not required	Secretor Lewis negative
At least 1 positive	Positive	Positive	Positive/weak positive	Partial secretor Lewis positive
All negative	Positive or negative	Positive	Negative	Non-secretor Lewis positive
All negative	Negative	Negative	Negative	Non-secretor Lewis negative

The number and proportion of ABO blood type will be presented overall by treatment group. ABO typing is deduced in participants that are secretor negative (ie negative to ABH) have been typed using "whole blood typing" to determine ABO blood type as blood type cannot be deduced by the A,B,H ELISA results. "wholebloodtyping"result present the blood type as A, AB, B and 0. For secretor positive the blood type is determined as follows;

Group A result	**Group B result**	**Group 0(H) result**	**Blood type**
Neg	pos		A

Pos	neg		B
Pos	pos		O
Neg	neg		AB

The ABO blood type will be listed by treatment group on the FAS population.

- To assess maternal anti-rotavirus IgA and IgG levels in association with anti-rotavirus IgA seroconversion and cumulative vaccine take in participants following receipt of RV3-BB administered in either the neonatal schedule (at any vaccine titre) or infant schedule at a vaccine titre of $1x10^7$.

  Maternal Serum IgA and IgG titres will be summarised, by treatment group using geometric means titres and the mean and standard deviation on the log scale. Measurements below the lower limit of detection will be assigned a value of half of the limit of detection for analysis e.g., if IgA or IgG is <20, this will be assigned a value of 10. This will be performed by the laboratory personnel prior to data transfer. The number of participants (and proportion) with measurements below the lower limit of detection will also be presented, using frequency tabulations. Maternal Serum IgA and IgG titres will also be listed on log scale by treatment group on FAS population.

- Participants in the FAS with samples included in the microbiome subset for exploratory analyses will be listed.

- Participants in the FAS with samples included in the immune development subset for exploratory analyses will be listed.

10. Participant disposition and withdrawal

10.1.1 Variables and derivations

End of trial classifications are defined as follows:

- Early withdrawal: Participants who exited the trial prior to completion for any reason other than being lost to follow-up, such as adverse event, protocol deviation/violation, death, withdrawal by investigator, withdrawal of consent or any other reason.

  Note: Participants who withdraw from study or IP will continue to receive IPV until the course is complete. They will receive Rotarix® at standard scheduled time points according to the National Immunisation Program.

- Completed trial: Participants that completed all trial visits, as indicated in the 'End of Study' (CRF page 55).

- Lost to follow-up: Participants who exited the trial prior to completion due to being lost to follow-up (CRF page 55).

10.1.2 Analysis

If the study is prematurely discontinued, all available data will be listed, and a review will be carried out to determine which statistical analyses are considered appropriate.

Participant disposition and reason for withdrawal will be summarized and presented in data listings for the FAS population. The number of participants included in the relevant analysis populations, as well as the number of participants excluded with reasons for exclusion from the relevant analysis populations (Section 6) will be summarized and presented in data listings for the FAS population.

Protocol violations/deviations will be summarized for the SAF and ITT population and listed for FAS population.

11. Participant demographics and baseline characteristics

11.1.1 Variables and derivations

Trial completion, trial withdrawals, exclusions and protocol non-compliances will be summarized. Data for background and demographic variables will be listed by participant. Descriptive statistics will be provided. Medical history, current medical conditions, results of laboratory screening and baseline tests and any other relevant baseline information will be listed by participant. Previous and concomitant medication taken at any stage from birth and during the trial period will be listed by participant. Baseline for every participant will be defined as the last non-missing observation prior to the first exposure to IP.

The following demographic and baseline characteristics will be reported, to be collected from birth to 144 hours old (Day 1 of the study):

- Demographic data including date of birth, gender, ethnicity, gestational age and birth weight, age at IP administration date and height/length.
- Medical history (see section 14, page 39).
- All medications, supplements, traditional/complementary/herbal products and immunisations administered to the baby since birth (see section 14, page 39).
- All medications, supplements, traditional/complementary/herbal products and immunisations administered to the mother from 4 weeks pre-delivery (see section 14, page 39).
- Vital signs (see section 17, page 47).
- Physical examination (see section 18, page 48).

11.1.2 Analysis

Demographic data and baseline characteristics will be summarized for each treatment group for the FAS, SAF, ITT and PP populations and presented in data listings treatment group, participant number and visit for the FAS population. To see if certain participants are being excluded from PP analysis population, the Logistic regression will be performed on the demographic and baseline characteristics associated parameters as

covariate with exclusion from the PP population and will be presented for the SAF population.

The Logistic regression model without categorical variable is as follows:

$Y= \alpha + \beta (X)$

Where,

Y= The Per Protocol exclusion flag (Binary response).

α =Intercept

β =slope

X= Covariate i.e. Gender, Ethnicity, Gestational age (weeks), Birth weight (grams), Weight at Visit 1 (grams) and Age at IP administration (day)

The hypothesis is as follows

H0: $\beta = 0$

i.e. There is no statistically significant effect of respective demographics and baseline characteristics parameters on Per Protocol exclusion set

V/s

H1: $\beta \neq 0$

i.e. There is statistically significant effect of respective demographics and baseline characteristics parameters on Per Protocol exclusion set.

The above hypothesis will be tested based on α (alpha)=0.05, if the p-value ≥ 0.05 then we will conclude that there is no statistically significant effect of respective demographics and baseline characteristics parameters on Per Protocol exclusion set i.e. Ho is accepted.

Note: All continuous variables will fit into one model and all (binary and ordinary) variables will fit into other model separately.

12. Exposure to IP

12.1.1 Variables and derivations

In eCRF IP Administration form we are capturing the treatment dosing related data and the dates of first IP administration will be derived as the first date of dosing from the IP Administration form in eCRF. The date of last IP administration will be derived as the date of last dose of IP from the End of the Study form in eCRF page.

12.1.2 Analysis

Based on the completion of the IP Administration pages in the CRFs, IP administration data will be summarised and presented in data for the FAS and ITT population.

13. Treatment Compliance

13.1.1 Variables and derivations

Treatment compliance will be derived as a dichotomous variable. A participant will be considered compliant with study treatment if they received the scheduled administration of all doses of the IP, within the protocol specified time windows. Any incomplete administration of IP will be recorded in the participant notes and the CRF.

Administration of any dose of IP can be delayed at the discretion of the investigator or Medical Monitor however, if the second, third or fourth doses fall outside of the protocol specified window it will be recorded as a protocol deviation.

Additional protocol specified time windows were included to assist in keeping participants compliant with local authority regulations for the receipt of vaccines in the National immunisation program.

Assessment windows for treatment compliance relating to IP administration are

- Visit 1 (Day 1; <=144 hours of age)
- *Visit 3 (Week 6; +7 days),*
- *Visit 5 (Week 10; ±7 days),*
- *Visit 7 (Week 14; ±7 days),*

13.1.2 Analysis

Based on the completion of the IP Administration pages in the CRFs, compliance for each participant at each administration visit will be reported. IP administration data as well as compliance data will be summarised and presented in data listings for the FAS and ITT population.

14. Medical and treatment history

14.1.1 Variables and derivations

Medical history will be coded using the MedDRA central coding dictionary, Version (refer Data Management Plan (DMP) for version number).

14.1.2 Analysis

Medical history will not be analysed but will be presented as data listings for the FAS.

15. Previous and concomitant medications

15.1.1 Variables and derivations

Infant and maternal concomitant medications will be documented in the participant's CRF. This will include all prescription and non-prescription medication, traditional, herbal and complementary medicines given to the participant from birth until end of study will be recorded.

In addition, prescription and non-prescription medication including traditional, herbal and complementary medicines taken by the mother will be recorded from 4 weeks prior to delivery, during delivery, and post-delivery until end of study (or until cessation of breastfeeding).

Concomitant medication records will include the drug name, total daily dose, route of administration, start and stop date of administration, and indication (for participants only).

Indication List: Anaemia, Anaemia in pregnancy, Anaemia prevention in pregnancy, Anaemia prophylaxis, Anaesthesia, Antenatal, Anthelmintic, Anti biotic, Anti malaria drug, Anti retro viral therapy,Antibiotic, Anti-emetic, Antiemetic post ceaserian section, Antiretroviral therapy, ART, ARVS, Asthma, C/S pain, Contrimoxazole preventive therapy, Cough, CPT, Diarrhoeam, Elevated blood pressure, For rehydration, HAART, HIV related neuropathy, Indication anaemia, Infection prevention after returned placent removal, Infection prevention post ceaserian section, Infection prevention post evacuation, IPT Iron supplement, Isonazide preventive therapy, Isoniazid prophylaxis therapy, Lower abdominal pain treatment, Malaria, Malaria prophylaxis, Malaria prophylaxis in pregnancy, Mastitis, Minimise bleeding post delivery, MOH antenatal protocol, Neuropathy prophylaxis, Oesophageal reflux, Pain, PCP prophylaxis, Peripheral neuropathy, Pneropathy prophlaxis and treatment, Pneumocystis carinii pneumonia (CPC) prophylaxis, Pneumonia cariri prophylaxis, Pneumonia prophylaxis, PNP, Post caeser, Post delivery arrest of haemorrage, Post delivery pain, Post op prophylaxis, Post op surgery, Post partum pain killer, Post sugical-mastitis, Postpartum hahituage (p.p.h.20 cervical tear), Pre and post surgery antibiotics, Precaution of anaemia in pregnancy, Prevention of anaemia, Prevention of anaemia during pregnancy,

Prevention of anaemia in pre and post delivery, Prevention of infection, Prevention of opportunistic infections, Prevention of peripheral neuropathy, Promote uterine contraction (prevents bleeding), Prophylaxis after ceaserian section, Prophylaxis after ceaserian section (pain), Prophylaxis after ceaserian section(antibiotic), Prophylaxis and treatment for neuropathy, Prophylaxis for neuropathy, Prophylaxis for PCP antibiotic, Prophylaxis for tuberculosis antibiotic, Prophylaxis of anaemia in pregnancy, Prophylaxis pinaemia, Prophylaxis therapy for co-infection, Puerperial sepsis, Rash, Sepsis, Sexually transmitted infection treatment, Supplementation, Syphilis treatment, TB prophylaxis, to prevent anemia in pregnancy, Treatment of HIV and AIDS, Treatment of infection, Tuberculosis prophylaxis, Upper respiratory tract infection, Urinary tract infection treatment, UTRI, Vitamin supplement.

'*Previous medications*' are defined as any medication received as well as stopped prior to first administration of the IP.

'*Previous/concomitant medications*' are defined as any medication received prior to first administration of the IP however, only stopped after first administration of the IP, during the trial until Visit 9.

'*Concomitant medications*' are defined as any medication where the start date is after first administration of the IP, during the trial until Visit 9.

Where necessary, start and end dates for concomitant medications will be imputed as follows:

The imputations derived for partial dates will be as follows:

- Imputation on the concomitant medication receive date:
    - if only the day-part is missing, and the month and year are equal to treatment start month and treatment start year OR if both the day- and month-part are missing, and the year is equal to treatment start year, then the minimum of treatment start date and concomitant medication end date is used;
    - in all other cases the missing day-part becomes the 1st day of the month, and the missing month-part becomes the 1st month of the year.

- Imputation on the concomitant medication end date:
    - if only the day part is missing then the last day of the month is used if this does not result in a date after the subject's trial exit date (e.g. death) in which case the trial exit date will be used;
    - if the day and month part are missing then the last day of the month and Last month of the year (i.e. 31DEC) are used if this does not result in a date after the subject's trial exit date (e.g. death) in which case the trial exit date will be used;
    - in all other cases the concomitant medication end date will not be imputed.

All medications will be coded using WHO-DD (refer Data Management Plan (DMP) for version number).

15.1.2 Analysis

Previous- and concomitant medications will be presented in data listings for the FAS populations. All medications captured in the eCRF will be categorised as either '*Previous medications*', '*Previous/concomitant medications*' or '*Concomitant medications*' and separate listings for infant and maternal. Listing will be presented by Standardized Medication Name and Reported Term.

16. Adverse events

All safety parameters (adverse events (AEs), and the occurrence of blood in the stool) will be summarized descriptively by treatment group. The descriptive statistics will include the number of observations, mean, standard deviation, median, minimum and maximum for continuous variables and number of observations and their percentages and number of participants for categorical variables.

AEs will be coded using MedDRA version (refer Data Management Plan (DMP) for version number) and will be presented by system organ class and preferred term for each treatment group. Coding details are available in the Data Management Plan.

16.1.1 Variables and derivations

Adverse Events (AEs) will be defined as events that started at the time of, or after the, first IP administration, as well as those events that started prior to the first study drug administration, but which worsened after the first study drug administration.

Imputations will only be performed where at least the year is provided. The imputations derived for partial dates will be as follows:

- Imputation on the AE start date:
    - if only the day-part is missing, and the month and year are equal to treatment start month and treatment start year OR if both the day-part and month-part are missing, and the year is equal to treatment start year, then the minimum of treatment start date and AE end date is used;
    - otherwise the missing day-part becomes the 1st day of the month, and the missing month-part becomes the 1st month of the year.
- Imputation on the AE end date:
    - if only the day part is missing then the last day of the month is used if this does not result in a date after the subject's trial exit date (e.g. death) in which case the trial exit date will be used;
    - if the day and month part are missing then the last day of the month and Last month of the year (i.e. 31DEC) are used if this does not result in a

date after the subject's trial exit date (e.g. death) in which case the trial exit date will be used;
    - in all other cases the AE end date will not be imputed.
- There will be no default for a missing year field.

16.1.2 Analysis

All summaries and listings for safety parameters (adverse events (AEs) will be based on the SAF population.

In order to be able to compare the neonatal vaccine schedule to the infant vaccine schedule after 3 doses of RV3-BB, due to the different time points of vaccination between the two different schedules, summaries of AEs are presented based on doses 1-3 of investigational product for the neonatal schedule and doses 2-4 of investigational product for the infant schedule. Hence, only AEs that started after the time of administration of the investigational product up to 28 days following each dose will be summarised for doses 1, 2 and 3 for the neonatal comparison and for doses 2, 3 and 4 for the infant comparison. In addition, some summaries will be presented by dose with an overall summary of AE (as presented for the DSMB).

Summaries (and listings where applicable) will be presented for all participants. For summaries, all safety data will be presented by dose number unless otherwise specified.

16.1.3 Incidence of AEs and SAEs

AEs defined in section 16.1.1 and Serious AEs is defined as AEs for which seriousness is indicated as “Yes”. In cases where seriousness criteria are missing, the worst-case scenario will be assumed. The incidence of all SAEs and AEs by MedDRA System Organ Class (SOC) and Preferred Term (PT) will be presented by dose number and treatment group. In addition, an overall summary will be presented indicating the following counts:

- Participants with at least one AE
- Participants with at least one SAE
- Participants with at least one grade 3/4 AE

- Participants with AEs leading to death
- Participants with AEs leading to early withdrawal from trial
- Participants with at least one IP related AE
- Participants with at least one IP related grade 3/4 AE
- Total number of AEs
- Total number of grades 3/4 AEs
- Total number of IP related AEs (assessed as possibly, probably or definitely related to IP)
- Total number of Investigational Product-related grade 3/4 AE (assessed as possibly, probably or definitely related to IP)

16.1.4 AEs by severity

The incidence (%) and number of events of all AEs will be presented by SOC, PT, severity and dose number for each treatment group.

16.1.5 Investigational Product-related AEs

AEs relatedness to IP (determined by the principal investigator) will be summarized per the following categories:

- Related (“Definitely related”, “Probably related”, “Possibly related”)
- Not related (“Unlikely related”, “Not related”)

If relatedness is missing, the worst case will be assumed, i.e. related. The incidence (%) and number of events of all drug-related AEs will be presented by SOC, PT, relatedness and dose number for each treatment group.

16.1.6 Serious AEs

Serious AEs are defined in section 16.1.3. In cases where seriousness criteria are missing, the worst-case scenario will be assumed.

The incidence (%) and number of events of all serious AEs will be presented by SOC, PT and dose number for each treatment group. A data listing of serious adverse events (SAEs) will also be presented.

16.1.7 Investigational Product-related serious AEs

Serious Investigational Product-related AEs are defined as AEs for which seriousness is indicated as 'yes' and the relatedness defined as 'related' (see section 16.1.5). In cases where seriousness/relatedness criteria are missing, the worst-case scenario will be assumed, i.e. serious related AE.

The incidence (%) and number of events of all serious AEs will be presented by SOC, PT, relatedness and dose number for each treatment. A data listing of serious adverse events (SAEs) will also be presented.

16.1.8 AEs and related AEs leading to early withdrawal from study

The incidence (%) and number of events of all AEs and related AEs leading to early withdrawal will be presented by SOC, PT and dose number for each treatment. A data listing of AEs leading to early withdrawal from study will be presented.

16.1.9 AEs leading to death

The incidence (%) and number of events of all AEs leading to death will be presented by SOC, PT and dose number for each treatment group. A data listing of all AEs for deceased participants will be presented.

16.1.10 Other AEs of special interest

Cases of blood in stool will be listed for the FAS population.

17. Vital signs

17.1.1 Variables and derivations

The following vital signs parameters will be examined at study visits mentioned in the Schedule of Procedures (section 4.1.3).

- Pulse Rate (beats/min)
- Respiratory Rate (breaths/min)
- Temperature (C)

17.1.2 Analysis

All summaries for vital signs will be based on the SAF population and will be listed for the FAS population.

For each vital sign parameter pre-specified in the protocol, summary statistics for continuous parameters will be presented by treatment group for all pre- and post-vaccination assessments.

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18. Physical examination

18.1.1 Variables and derivations

A complete physical examination including the following body systems will be examined at study entry by a clinician prior to randomisation - at Visit 1 (Day 1):

- general appearance,
- HEENM (head, ears, eyes, nose and mouth),
- skin,
- cardiovascular system,
- musculoskeletal system,
- respiratory system,
- gastrointestinal system
- nervous system
- other physical examination

These measurements will be categorised as:

- Normal
- Abnormal, clinically significant
- Abnormal, clinically not significant
- Not done.

New findings or clinically significant changes noted in any physical examinations conducted post dosing will be recorded as AEs.

18.1.2 Analysis

Physical examination at screening will be listed for the FAS population.

19. Other Safety Assessments

19.1.1 Variables and derivations

A feeding assessment will be examined at study visits mentioned in the Schedule of Procedures (section 4.1.3)

19.1.2 Analysis

Feeding assessment (categorised as receiving breast milk exclusively or not) will be summarised by treatment group and by visit, using frequency tabulations for the FAS and ITT population. In addition, all feeding data will be listed for the FAS.

20. Appendix 1: Programming Conventions for Tables, Data Listings and Figures (TLFs)

In general, if we have no response or record for respective parameter in data (i.e. n=0) then while presenting statistics in table, only n will be presented as “0” and other statistics will be left blank. In case, we have only one response or record for respective parameter (i.e. n=1) in data then statistic (S.D) in table will be presented “ND”.

20.1.1 Paper Size, Orientation and Margins

The margin, page size and line size specifications as stipulated in Table 20.1.1 will be used for the presentation of all TLFs.

Table 20.1.1: Output margin, page size and line size specifications

	Landscape
Margins (Inches):	
Top	1
Bottom	1
Left	1
Right	1
Header (Inches)	0.5
Footer (Inches)	0.5
SAS® specifications:	
PAGE SIZE	46
LINE SIZE	134

20.1.2 Fonts

The font type “Courier New” or “Arial” should be used as default for tables and data listings, with a font size of 9. The font color should be black. No bolding, underlining and italics are permitted.

Figures should have a default font of “Times Roman”, “Arial”, “Helvetica” or “Courier New”.

20.1.3 Header and Footer Information

Headers and Footer should be defined as follows:

- The header should be placed at the top of the page (same place on each page).
- The sponsor name should appear in row 1, left-aligned in header.
- The word “CONFIDENTIAL” should appear in row 1, centered aligned at footer in compiled file only.
- The protocol number should appear in row 2, left-aligned in header.
- The page identification in the format Page X of Y (where Y is the total number of pages for the TLF) should appear in row 2, right-aligned at Footer.
- The TLF identification number should appear in row 3, centered in header.
- The TLF title should start in row 4, centered in header.
- The TLF population should appear in row 5, centered in header. The population should be spelled out in full, e.g. *Safety analysis population* in preference to *SAF analysis population*.
- Row 6 in header should be a continuous row of underscores (‘_’) (the number of underscores should equal the line size).
- Row 7 in header should be a blank line.
- Proper/Mixed case should be used for titles in header.
- Titles should not contain quotation marks or footnote references.
- The column headings should be underlined with a row of underscores (‘_’).
- Column headings spanning more than one column should be underlined and have underscores on either side of the title and should be centered.
- Column headings should be in mixed case.
- In general, the analysis population count should appear in the column header in the form “(N=XX)” and Column headings containing numbers should be centered (eg. N=XX).

20.1.4 Table and Data Listing Table, Listing and Figure (TLF) Conventions

20.1.5 General

- The first row in the body of the table or data listing should be blank.

- The left-hand column should start in Column 1. No indenting or centering of the TLF should occur.
- Rounding should be done with the SAS® function ROUND.
- Numerical values in tables should be rounded, not truncated as per section (19.1.6) for respective statistics.
- Numerical values should be center point aligned.
- Text values should be left aligned.
- The first letter of a text entry should be capitalized.
- The study investigational drug should appear first in tables followed by reference drug in columns.
- All variables contained on the eCRF (which have data present) should appear in the data listings, along with all derived data appearing in the corresponding tables.
- The width of the TLF should match the line size.

20.1.6 Univariate statistics

- Statistics should be presented in the same order across tables (i.e., n, mean, SD, SE, minimum, median and maximum).
- If the original data has N decimal places, then the summary statistics should have the following decimal places:
  - Minimum, maximum: N.
  - CV (%): N+1.
  - Mean and median: N +1.
  - SD: N + 2.
  - SE: N+1.

20.1.7 Frequencies and percentages [n (%) m]

- Percent values should be reported inside parentheses, with one space between the count and the left parenthesis of the Percent values. An example is given below:
  - 124 (64.5)

- Mentions values should be reported, with one space from the right parenthesis of the percentage. Parentheses should be justified to accept a maximum of 100.0 as a value and padded with blank space if the percent is less than 100.0. and percentage should be reported inside parentheses, with one space between the count and the left parenthesis of the percentage and same for the right parenthesis. An example is given below:
  - 77 (100.0) 156
  - 50 (64.9) 56
  - 0 (0.0) 0
- Percentages will be reported to one decimal place, except percentages <100.0 but >99.9 will be presented as '>99.9' (e.g., 99.99 is presented as >99.9); and percentages <0.1 will be presented as '<0.1' (e.g., 0.08 is presented as <0.1). Rounding will be applied after the <0.1 and >99.9 rule.
  - (<0.1)
  - (6.8)
  - (>99.9)
- Percentages may be reported to 0 decimal places as appropriate (for example, where the denominator is relatively small).
- Where counts are zero, mentions of 0 and percentages of 0.0 should appear in the table.

20.1.8 Confidence intervals (CIs)

- CIs should be presented with one additional decimal place as that of the raw data.
- CIs should be justified so that parentheses displayed on consecutive lines of a table "line up".

20.1.9 P-values

- P-values should be reported to four decimal places.

20.1.10 Ratios

- Ratios should be reported with one additional decimal place as that of the raw data.

20.1.11 Spacing

- There should be a minimum of 1 blank space between columns (preferably 2).

20.1.12 Missing values

- A “0” should be used to indicate a zero frequency.
- A blank will be used to indicate missing data in data listings.

20.1.13 Tables, Listings and Figure output conventions

The compiled final file will be presented in PDF format with TOC for listing and table/figures separately. The Table, Listing and Figures will be provided in RTF and PDF files using the SAS® Output Delivery System (ODS).

Summary tables will be stratified by treatment group, unless otherwise specified.

The templates provided in the separate output templates document describe the format and content for presentation of tables, listings and figures (TLFs).

All percentages (%) for a specific summary are calculated using the total number of participants included in the relevant analysis population as the denominator, unless otherwise specified.

Data listings will be based on all participants randomized to IP in the FAS, unless otherwise specified by treatment group, Participant ID and visit number (if applicable).

By default, descriptive statistics for quantitative measurements will include the number of participants (n), mean, standard deviation (SD), minimum, median and maximum and qualitative measurements will include the number of participants (n), percentage (%).

20.1.14 Dates and times

In footnote, depending on data available, dates and times will take the form ddMMMyyyy and hh:mm.

20.1.15 Spelling format

The spelling format to be used is English US.

20.1.16 Presentation of visits

- IP Dose 1 Visit 1 (Day 1)
- Post IP Dose 1 Visit 2 (Day 8)
- IP Dose 2 Visit 3 (Week 6)
- Post IP Dose 2 Visit 4 (Week 7)
- IP Dose 3 Visit 5 (Week 10)
- Post IP Dose 3 Visit 6 (Week 11)
- IP Dose 4 Visit 7 (Week 14)
- Post IP Dose 4 Visit 8 (Week 15)
- Post IP Dose 4 Visit 9 (Week 18)

21. Revision history

Version	Date	Change
1.0	16-Mar-2020	Initial version
2.0	28-May-2020	• "Not done" abbreviation added as "ND" • Exploratory endpoint updated in section 3.1.3 • ITT and PP definition updated in section 6.1.3 and 6.1.4 respectively • All the primary, primary sensitivity and secondary efficacy analysis population updated in section 9.1.2 and also updated All the primary, primary sensitivity analysis population in section 7.1.4 • "Cohort" word replaced by "Treatment Group" in respective section. • Serum IgA listings added for Neonatal and infant as well as for mother in section 9.1.3 and 9.1.12 respectively. • Definition of a positive cumulative vaccine take are updated in section 9.1.5 and 9.1.6. • Diarrhea Table and listing variable updated in section 9.1.10 • Protocol violations/deviations listing population updated in section 10.1.2 • Demographic table and listing population updated, and Population information added for the Logistic regression model table in section 11.1.2 • Exposure to IP table and listing population updated in section 12.1.2 • IP administration data as well as compliance table and listing population updated in section 13.1.2 • Medical history listing population added in section 14.1.2 • Previous- and concomitant medications listing sorting order added in section 15.1.2. • Treatment emergent adverse events (TEAEs) word deleted from the SAP. • Adverse events listing population added in section 16.1.2 • blood in stool listing population added in section 16.1.10 • Vital sign parameter name added , Listing population

		added and deleted “change from pre- and post-vaccination assessment” line. • New section 19 added for the feeding assessment • Section 20 updated based on mock shells requirement as appropriate.

22. Reference

- Biometrics, Vol. 57, No. 1. (Mar., 2001), pp. 120-125
- dx.doi.org/10.1016/j.vaccine.2014.08.011
- Chan J, Nirwati H, Triasih R, Bogdanovic-Sakran N, Soenarto Y, Hakimi M, Duke T, Buttery JP, Bines JE, Bishop RF, Kirkwood CD, Danchin MD. Maternal antibodies to rotavirus: could they interfere with live rotavirus vaccines in developing countries? Vaccine. 2011 Feb 1;29(6):1242-7. doi: 10.1016/j.vaccine.2010.11.087. Epub 2010 Dec 13.
- Tong et al 2002; BJOG: an International Journal of Obstetrics and Gynaecology October 2002, Vol. 109, pp. 1175–1177
- Bines JE, Danchin M, Jackson P, Handley A, Watts E, Lee KJ, West A, Cowley D, Chen MY, Barnes GL, Justice F, Buttery JP, Carlin JB, Bishop RF, Taylor B, Kirkwood CD; RV3 Rotavirus Vaccine Program. Safety and immunogenicity of RV3-BB human neonatal rotavirus vaccine administered at birth or in infancy: a randomised, double-blind, placebo-controlled trial. Lancet Infect Dis. 2015 Dec;15(12):1389-97. doi: 10.1016/S1473-3099(15)00227-3. Epub 2015 Aug 26.
- Bines JE, At Thobari J, Satria CD, Handley A, Watts E, Cowley D, Nirwati H, Ackland J, Standish J, Justice F, Byars G, Lee KJ, Barnes GL, Bachtiar NS, Viska Icanervilia A, Boniface K, Bogdanovic-Sakran N, Pavlic D, Bishop RF, Kirkwood CD, Buttery JP, Soenarto Y. Human Neonatal Rotavirus Vaccine (RV3-BB) to Target Rotavirus from Birth. N Engl J Med. 2018 Feb 22;378(8):719-730. doi: 10.1056/NEJMoa1706804.