GlaxoSmithKline	**Statistical Analysis Plan**
Detailed Title:	A phase III, randomized, open study to assess the immunogenicity, reactogenicity and safety of two different formulations of GlaxoSmithKline (GSK) Biologicals' oral live attenuated human rotavirus (HRV) vaccine, Rotarix, when given as a two-dose primary vaccination, in healthy infants with no previous history of rotavirus illness or vaccination.
eTrack study number and Abbreviated Title	l116566 (ROTA-083)
Scope:	All data pertaining to the above study.
Date of Statistical Analysis Plan	Amendment 2 Final 23 June 2020

APP 9000058193 Statistical Analysis Plan Template V4 (Effective date: 3 June 2019)

TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

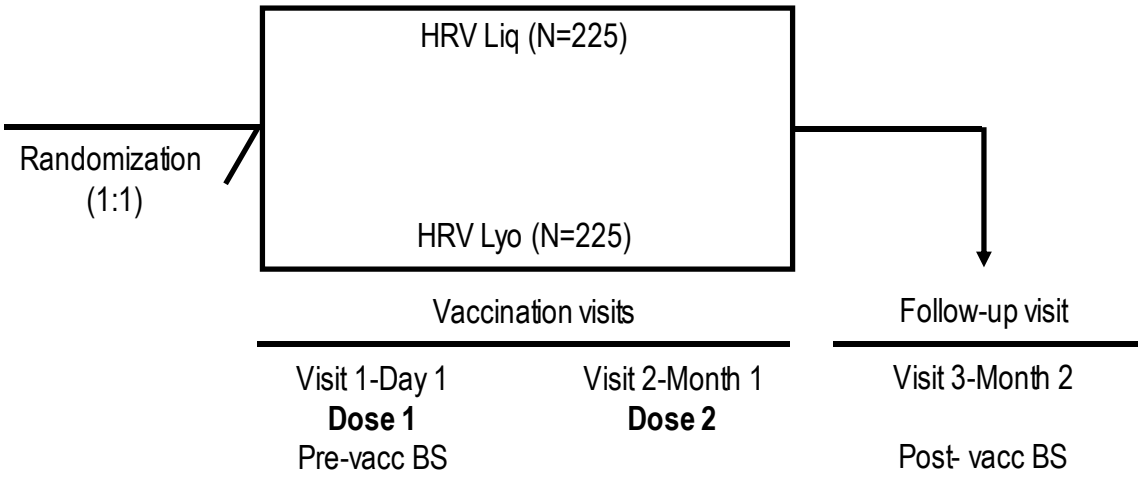
LIST OF ABBREVIATIONS

AE	Adverse event
CDISC	Clinical Data Interchange Standards Consortium
CI	Confidence Interval
CRF	Case Report Form
CTRS	Clinical Trial Registry Summary
eCRF	electronic Case Report Form
EoS	End of Study
ES	Exposed Set
GMC	Geometric mean antibody concentration
GMT	Geometric mean antibody titer
GSK	GlaxoSmithKline
HRV	Human Rotavirus
IgA	Immunoglobulin A
kg	Kilograms
LL	Lower Limit of the confidence interval
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligrams
mL	Milliliter
PPS	Per Protocol Set
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SBIR	GSK Biological's Internet Randomization System
SD	Standard Deviation
SR	Study Report
TFL	Tables Figures and Listings
TOC	Table of Content
UL	Upper Limit of the confidence interval

1. DOCUMENT HISTORY

Date	Description	Protocol Version
29 AUG 2018	Final version	Amendment 3 – 31 OCT 2017
11 NOV 2019	Amendment 1: The SAP was amended to be aligned with the protocol amendment. – Seroconversion was redefined in section 3.2 of Secondary Objectives – Between group assessment of immunogenicity was changed to an ANCOVA model – Code 2020 was redefined in section 5.2.2.1 – Within group assessment will include a antibody concentrations fold increase calculation in section 6.3.1.1	Amendment 4 – 30 OCT 2019
23 JUN 2020	Amendment 2: – Additional considerations were added in the SAP section 6.3.2, to include analyses planned in Protocol Amendment 3 as requested by the Indian regulatory agency – Because the summary of subjects withdrawn from the study includes SAE and AE as separate reason categories and such SAE/AE are described by narrative in the study report, the summary by preferred term planned for AEs or SAEs leading to withdrawal won't be generated (see additional considerations 6.4.2) – The summary of subjects excluded from PPS analyses will be tabulated by reason for exclusion, including deviations from age and intervals between study visits. Therefore, no separate summary of deviation from age and intervals between study visits will be generated (see additional considerations 6.1.2)	Amendment 4 – 30 OCT 2019

2. STUDY DESIGN



N= number of subjects planned to be enrolled; HRV= Human Rotavirus; Pre-Vacc= Pre-vaccination; Post-Vacc=Post-Vaccination; BS=Blood sample

Protocol waivers or exemptions are not allowed unless necessary for the management of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures (Section 5.5 in the protocol), are essential and required for study conduct.

- **Experimental design**: Phase III, open-label, randomized (1:1), multi-centric, single-country study with two parallel groups.
- **Duration of the study**: The intended duration of the study, per subject, is approximately two months.
  - Epoch 001: Primary starting at Visit 1 (Day 1) and ending at Visit 3 (Month 2).
- **Primary Completion Date (PCD)**: Last subject attending Visit 3.
- **End of Study (EoS)**: Last testing results released for samples collected at Visit 3.
- **Study groups**: The study groups and epoch foreseen in the study are provided in Table 1.

Table 1 Study groups and epoch foreseen in the study

Study groups	Number of subjects	Age at Dose 1 (Min/Max)	Epoch 001
HRV Liq	225	6 weeks-10 weeks	●
HRV Lyo	225	6 weeks- 10 weeks	●

- **Treatment groups**: Table 2 presents the study groups and the vaccine to be administered in the study.
  - HRV liquid vaccine group (also referred to as HRV Liq).
  - HRV lyophilized vaccine group (also referred to as HRV Lyo).

Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/Product name	Study Groups	
		HRV Liq	HRV Lyo
HRV liquid	GSK Biologicals' HRV liquid vaccine	x	
HRV lyophilized	GSK Biologicals' HRV lyophilized vaccine		x
	GSK Biologicals' calcium carbonate buffer		x

- **Control**: active control (Lyophilized HRV vaccine)
- **Vaccination schedule**:
  - Two oral doses of the HRV vaccine to be given according to a 0, 1 month schedule.
  - All subjects are allowed to receive routine childhood vaccinations according to the local immunization practice. Administration of all routine childhood vaccinations given since birth will be recorded in the electronic Case Report Form (eCRF).
- **Treatment allocation**: Randomized (1:1). Treatment number will be allocated using GSK Biologicals' Randomization System on Internet (SBIR).
- **Blinding:** open

The blinding of study epoch is provided in Table 3.

Table 3 Blinding of the study epoch

Study Epoch	Blinding
Epoch 001	open

- **Sampling schedule**: Details of the samples to be collected are as follows:
  - Blood samples (approximately 2 ml) will be collected from all subjects at Visit 1 and Visit 3 to measure serum anti-RV Immunoglobulin A (IgA) antibody concentrations using Enzyme Linked Immunosorbent Assay (ELISA).
- **Type of study**: self-contained
- **Data collection**: eCRF.
- **Intervals between study visits:** Intervals between study visits to define vaccination and blood sample schedule intervals for Per Protocol Set (PPS) are as follows:

Table 4 Intervals between study visits

Interval	Optimal length of interval	Allowed interval
Visit 1 → Visit 2	1 month	28-48 days after Dose 1
Visit 2 → Visit 3	1 month	31-48 days after Dose 2

Note: The date of the previous visit serves as the reference date for the intervals between the study visits.

3. OBJECTIVES

3.1. Primary objective

- To evaluate non-inferiority of GSK Biologicals' HRV liquid vaccine compared to GSK Biologicals' HRV lyophilized vaccine in terms of geometric mean concentrations (GMCs) for anti-RV antibodies, one month post dose 2 of HRV liquid vaccine and HRV lyophilized vaccine.
  - Criterion: Non-inferiority will be stated if the lower limit of the two-sided 95% confidence interval (CI) for the ratio of anti RV IgA antibody GMCs between HRV liquid vaccine over the HRV lyophilized vaccine, one month after dose 2 is greater than or equal to 0.5.

Refer to Section 4.1 for the definition of the primary endpoint.

3.2. Secondary objectives

- To assess the immunogenicity of the HRV liquid vaccine and HRV lyophilized vaccine, in terms of seroconversion* rates, one month post dose 2 of HRV vaccine.

  **** Seroconversion is defined as:***

  - ***for subjects with a pre-vaccination anti-RV IgA antibody concentration <20 U/mL, seroconversion is achieved when the post-vaccination concentration is ≥20 U/mL.***
  - ***for subjects with a pre-vaccination anti-RV IgA antibody concentration ≥20 U/mL, seroconversion is achieved when the post-vaccination concentration is ≥2 times the pre-vaccination concentration.***
- To assess the reactogenicity of the HRV liquid vaccine and the HRV lyophilized vaccine in terms of solicited adverse events (AEs), during the 8-day (Day 1–Day 8) follow-up period after each vaccination.
- To assess the safety of the HRV liquid vaccine and the HRV lyophilized vaccine in terms of unsolicited AEs, during the 31-day (Day 1–Day 31) follow-up period after each vaccination and serious adverse events (SAEs), during the entire study period.

Refer to Section 4.2 for the definition of the secondary endpoints.

4. ENDPOINTS

4.1. Primary Endpoint

- Anti-RV IgA antibody concentrations
  - Serum anti-RV IgA antibody concentrations, expressed as GMCs, one month post dose 2 of HRV vaccine.

4.2. Secondary Endpoints

- Anti-RV IgA antibody concentrations
  - Anti-RV IgA antibody seroconversion rate, one month post dose 2 of HRV vaccine.
- Solicited general symptoms
  - Occurrence of each type of solicited general symptom within the 8-day (Day1-Day 8) solicited follow-up period, after each dose of HRV vaccine.
- Unsolicited adverse events
  - Occurrence of unsolicited AEs within 31 days (Day 1-Day 31) after any dose of HRV vaccine according to Medical Dictionary for Regulatory Activities (MedDRA) classification.
- Serious adverse events
  - Occurrence of SAEs from dose 1 of HRV vaccine up to study end.

5. ANALYSIS SETS

5.1. Definition

5.1.1. Exposed Set

The Exposed Set (ES) will include all subjects with at least one study vaccine administration documented.

- A safety analysis based on the ES will include all vaccinated subjects.
- An immunogenicity analysis based on the ES will include all vaccinated subjects for whom immunogenicity data is available.

5.1.2. Per-Protocol Set for analysis of immunogenicity

The PPS for immunogenicity will include all eligible subjects from the ES:

- who have received both doses of study vaccine according to their random assignment.
- for whom the HRV vaccine, liquid or lyophilized formulation, is administered according to protocol. Note that the subjects who regurgitate after vaccination and receive a replacement dose are to be retained in the PPS,
- who comply with the vaccination schedule for HRV vaccine (liquid or lyophilized formulation), as per Table 4,
- who have not received a vaccine prohibited by the protocol up to Visit 3 blood sample,

- who have not received medication prohibited by the protocol up to Visit 3 blood sample,
- whose underlying medical condition(s) was (were) not prohibited by the protocol up to Visit 3 blood sample,
- with no protocol violation of demographics (unknown age at study entry or outside protocol defined age-interval),
- who comply with blood sampling schedule,
- for whom immunogenicity data are available at pre- and post-vaccination sampling time points,
- for whom the post-vaccination immunogenicity data are within the 21-48 days interval after the second dose,
- who have no other concomitant infection up to Visit 3 blood sample, which may influence the immune response.

5.2. Criteria for eliminating data from Analysis Sets

Elimination codes are used to identify subjects to be eliminated from analysis. Detail is provided below for each sets.

5.2.1. Elimination from Exposed Set (ES)

Code 1030 (Study vaccine not administered at all) and code 900 (invalid informed consent or fraud data) will be used for identifying subjects eliminated from ES.

5.2.2. Elimination from Per-protocol analysis Set (PPS)

5.2.2.1. Excluded subjects

A subject will be excluded from the PPS analysis under the following conditions

Code	Decode => Condition under which the code is used
900	Questionable subject => Invalid informed consent or fraud data
1030	Study vaccine dose not administrated but subject number allocated => subjects enrolled but not vaccinated
1040	Administration of vaccine(s) forbidden in the protocol => • Administration of a vaccine not foreseen by the study protocol and administered during the period starting from 30 days before the first vaccination and ending at Visit 3, with the exception of the inactivated influenza vaccine, which is allowed at any time during the study, and other licensed routine childhood vaccinations.

Code	Decode => Condition under which the code is used
1070	Study vaccine dose not administered according to protocol => • Subjects for whom the second administered dose is not aligned with the first dose (e.g. lyo as second dose after a first dose with liquid) • Subject who did not receive the second dose • Subjects orally vaccinated with the correct vaccine but who regurgitated during the same visit, without replacement dose • Route of vaccination which is not oral for HRV study vaccines • Wrong reconstitution of administered vaccine
1080	Vaccine temperature deviation => vaccine administered despite a Good Manufacturing Practices (GMP) no-go temperature deviation
1090	Expired vaccine administered => Subjects who received an expired vaccine
2010	Protocol violation (inclusion/exclusion criteria) => ineligible subject: • A male or female infant is not between and including 6 and 10 weeks (42-70 days) of age at the time of the first study vaccination • Infant born less than or equal to 28 weeks of gestation • Previous vaccination against HRV • Other considerations as stated in section 4.2 – 4.3 in the protocol
2020	Initially unknown antibody status = > Unknown antibody status at pre-dose 1 blood sample.
2040	Administration of any medication forbidden by the protocol => • Any investigational or non-registered product (drug or vaccine) other than the study vaccines used during the study period between the first vaccination at Visit 1 to the blood sampling at Visit 3. • Immunosuppressants or other immune-modifying drugs administered chronically (i.e., more than 14 days) during the study period between Visit 1 to Visit 3. For corticosteroids, this will mean prednisone $\geq$ 0.5 mg/kg/day, or equivalent. Inhaled and topical steroids are allowed. • Immunoglobulins and/or any blood products administered during the study period between the first vaccination at Visit 1 and the blood sampling at Visit 3. • Administration of long-acting immune-modifying drugs between the first vaccination at Visit 1 to the blood sampling at Visit 3 (e.g., infliximab)

Code	Decode => Condition under which the code is used
2060	Concomitant infection related to the vaccine which may influence immune response => Subjects with non-vaccine type RV detected in GE stool samples that may impact immunogenicity at Visit 3.
2070	Concomitant infection not related to the vaccine which may influence immune response = > Condition that has the capability of altering their immune response at visit 3 such autoimmune disease.
2080	Non-compliance with vaccination schedule (including wrong and unknown dates) => Subjects who did not comply with the interval for dose 2 (Dose 2 should be between 28-48 days after Dose 1).
2090	Non-compliance with blood sampling schedule (including wrong and unknown dates => Subjects who did not comply with the blood sample interval (blood sample post dose 2 should be between 21-48 days after Dose 2).
2100	Essential serological data missing => Anti-HRV results not available post-vaccination.
2120	Obvious incoherence or abnormality or error in data => E.g. Sample mismanagement impacting results.

5.2.2.2. Right censored Data

Not applicable.

5.2.2.3. Visit-specific censored Data

Not applicable.

6. STATISTICAL ANALYSES

Note that standard data derivation rule and stat methods are described in Section 11 and will not be repeated below.

6.1. Demography

6.1.1. Analysis of demographics/baseline characteristics planned in the protocol

The distribution of subjects enrolled among the study centers will be tabulated as a whole and for each group.

The number of subjects who withdraw from the study will be tabulated by group according to the reason for drop-out.

The number of subjects enrolled into the study as well as the number of subjects excluded from PPS analyses will be tabulated as a whole and for each group.

The deviations from specifications for age and intervals between study visits will be tabulated by group.

The median, mean, range and standard deviation of age (in weeks) at each HRV vaccine dose will be computed by group. The median, mean and standard deviation of height in centimeter (cm) and weight in kilograms (kg) at Visit 1 will be computed by group. The geographical ancestry and sex composition will be presented.

Summary of co-administered vaccinations (i.e., vaccinations given on the day of each HRV vaccine dose) and intercurrent vaccinations (i.e., vaccinations other than the HRV lyophilized and HRV liquid vaccine administered from birth up to Visit 3, excluding vaccination given on the day of HRV vaccine doses) will be summarized by group for the ES.

For a given subject and a given demographic variable, missing measurements will not be replaced.

6.1.2. Additional considerations

All demography summaries will be generated for the ES. The summary of age, height, weight, race and sex will also be provided for the PPS.

Number and reason for elimination from PPS will be tabulated by group. Since the reasons ‘deviations from specification for age’ and ‘deviations from specifications for interval between study visits’ will be part of this table, no separate table will be generated for these 2 reasons for elimination from PPS.

6.2. Exposure

6.2.1. Analysis of exposure planned in the protocol

Not applicable.

6.2.2. Additional considerations

The number of doses administered will be tabulated for each group.

6.3. Immunogenicity

6.3.1. Analysis of immunogenicity planned in the protocol

The primary analysis will be based on the PPS for analysis of immunogenicity. If, in any vaccine group, the percentage of vaccinated subjects with serological results excluded from the PPS for analysis of immunogenicity is 5% or more, a second analysis based on the ES will be performed to complement the PPS analysis.

6.3.1.1. Within group assessment

The following calculations will be performed for each group

- For each group, at each time point that anti-rotavirus IgA is measured,
  - GMCs and their 95% CIs will be computed.
  - Seropositivity/seroconversion rates and their exact 95% CI will be computed,
  - The distribution of anti-RV IgA antibody concentrations at Visit 1 and Visit 3 will be displayed using Reverse Cumulative Curves (RCCs).

6.3.1.2. Between groups assessment

- The 95% CI for the ratio of anti-RV IgA antibody GMCs at Visit 3 between the HRV liquid vaccine over the HRV lyophilized vaccine will be computed using an ANCOVA model on the logarithm-transformed concentrations. This model will include the vaccine group and the logarithm of the baseline concentration as covariables. The GMC ratio and their 95% CI will be derived by exponential transformation of the corresponding group contrast in the model (primary objective).
- The asymptotic standardized 95% CI for the difference in seroconversion rate at Visit 3 between the HRV liquid vaccine and HRV lyophilized vaccine will be computed (secondary objective) as described in Section 11.1.

Refer to the Section 3.1 for the success criteria of the primary objective.

6.3.1.2.1. Definitions related with between groups assessment

- A seronegative subject is a subject whose anti-RV IgA antibody concentration is below the clinical meaningful threshold of <20 U/ml*.
- A seropositive subject is a subject whose anti-RV IgA antibody concentration is greater than or equal to the clinical meaningful threshold of 20 U/ml.
- Seroconversion is defined as:
  - ***for subjects with a pre-vaccination anti-RV IgA antibody concentration <20 U/mL, seroconversion is achieved when the post-vaccination concentration is ≥20 U/mL.***
  - ***for subjects with a pre-vaccination anti-RV IgA antibody concentration ≥20 U/mL, seroconversion is achieved when the post-vaccination concentration is ≥2 times the pre-vaccination concentration.***
- The GMCs calculations are performed by taking the anti-log of the mean of the log concentrations transformations. Antibody concentrations below the technical cut-off (<13 U/ml*) of the assay will be given an arbitrary value of half the cut-off for the purpose of GMC calculation.

* Note: 20 U/ml corresponds to the clinical meaningful threshold to define seroconversion rate, while 13 U/ml corresponds to technical cut-off of revalidated laboratory assay.

An immunogenicity analysis based on the ES will include all vaccinated subjects for whom immunogenicity data is available. For the immunogenicity measurement, missing or non-evaluable measurements will not be replaced.

6.3.2. Additional considerations

Complementary to the within group assessment of immunogenicity; fold increase from pre-vaccination to one month post-dose 2 of anti-RV antibody concentrations will be computed for each group.

The protocol amendment 3 required an additional condition to be part of the PPS. This condition was to have pre-vaccination anti-RV IgA antibody concentration <20 U/mL. As per request from the Indian Regulatory Authorities (DCGI), the PPS analyses addressing the primary and secondary objectives planned in the protocol amendment 3 will be generated, namely for the PPS limited to subjects with available pre-vaccination anti-RV IgA antibody concentrations <20 U/mL.

- The 95% CI for the ratio of anti-RV IgA antibody GMCs at Visit 3 between the HRV liquid vaccine over the HRV lyophilized vaccine will be computed using an ANOVA model on the logarithm-transformed concentrations. This model will include the vaccine group as covariables. The GMC ratio and their 95% CI will be derived by exponential transformation of the corresponding group contrast in the model (primary objective from protocol amendment 3).
- The asymptotic standardized 95% CI for the difference in seroconversion rate at Visit 3 between the HRV liquid vaccine and HRV lyophilized vaccine will be computed (secondary objective from protocol amendment 3).

6.4. Analysis of safety

The ES will be used for the analysis of safety.

6.4.1. Analysis of safety planned in the protocol

6.4.1.1. Within groups assessment

The following calculations will be performed for each group:

- The percentage of doses and of subjects reporting at least one symptom (solicited or unsolicited) during the 8-day (Day 1-Day 8) solicited follow-up period will be computed, along with exact 95% CI. The same calculations will be done for symptoms (solicited or unsolicited) rated as grade 3 in intensity, for symptoms (solicited or unsolicited) assessed as causally related to vaccination and for symptoms resulting in medically attended visit.

- The percentage of doses and of subjects reporting each individual solicited general symptom will be computed, over the 8-day (Day 1-Day 8) solicited follow-up period, post vaccination, along with exact 95% CI. The same calculations will be done for each individual solicited general symptom rated as grade 3 in intensity, for each individual solicited general symptom assessed as causally related to vaccination and for symptoms resulting in medically attended visit.
- The verbatim reports of unsolicited AEs will be reviewed by a physician and the signs and symptoms will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). Every verbatim term will be matched with the appropriate Preferred Term. The percentage of subjects with unsolicited AEs occurring within 31-day (Day 1-Day 31) follow-up period after any dose with its exact 95% CI will be tabulated by preferred term. Similar tabulation will be done for unsolicited AEs rated as grade 3, for unsolicited AEs with causal relationship to vaccination, for unsolicited AE resulting in medically attended visit and for AEs or SAEs leading to drop out.
- The percentages of subjects who started taking at least one concomitant medication, by type, from Day 1 to Day 8 after vaccinations will be tabulated with exact 95% CI. The percentages of subjects who started taking at least one concomitant medication, by type, during the study period will also be tabulated with exact 95% CI.

SAEs reported during the study period will be described in detail.

6.4.2. Additional considerations

The percentage of doses with unsolicited AEs occurring within 31-day (Day 1-Day 31) follow-up period after any dose with its exact 95% CI will be tabulated by preferred term. Similar tabulation will be done for unsolicited AEs rated as grade 3, for unsolicited AEs with causal relationship to vaccination, for unsolicited AE resulting in medically attended visit.

Safety analysis will be done per administered dose. Subjects who missed reporting symptoms (solicited/unsolicited or concomitant medications) will be treated as subjects without symptoms (solicited/unsolicited or concomitant medications, respectively). A sensitivity analysis will be performed on documented doses i.e. accounting only for doses where solicited symptoms are indicated as being absent/present in case more than 5% of the doses are not documented.

Because the summary of subjects withdrawn from the study mentioned in Section 6.1.1 includes SAE and AE as separate reason categories and such SAE/AE are described by narrative in the study report, the summary by preferred term planned for AEs or SAEs leading to drop out in Section 6.4.1.1 will not be generated.

Summary of temperature will be provided by 0.5° increment (i.e. ≥ 38.0°C, >38.5; > 39, > 39.5, > 40). Fever, defined as a body temperature of ≥38°C irrespective of route of measurement, will be integrated to the summaries as a general symptom.

7. ANALYSIS INTERPRETATION

Except for analyses addressing criteria specified in the primary objective, all the analyses will be descriptive/exploratory in nature. The use of these descriptive/exploratory analyses should be limited to support the confirmatory analyses or to generate hypothesis.

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

Description	Analysis ID	Disclosure Purpose (CTRS=public posting, SR=study report, internal)	Dry run review needed (Y/N)	*Study Headline Summary (SHS)*requiring expedited communication to upper management (Yes/No)	Reference for TFL
Final	E1_01	SR	Yes	Yes	All tables from TFL TOC

8.2. Statistical considerations for interim analyses

Not applicable.

9. CHANGES FROM PLANNED ANALYSES

Not applicable.

10. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES (TFL)

The TFL Table Of Content (TOC) is as separate document. It provides the list of tables/listings and figures needed for the study report, and It also identifies the tables eligible for each analysis and their role (synopsis, in-text, post-text, SHS, CTRS).

The following group names will be used in the TFLs, to be in line with the T-domains:

Group order in tables	Group label in tables	Group definition for footnote
1	HRV Liq	HRV liquid vaccine
2	HRV Lyo	HRV lyophilised vaccine

11. ANNEX 1 STANDARD DATA DERIVATION RULE AND STATISTICAL METHODS

11.1. Statistical Method References

The exact two-sided 95% CIs for a proportion within a group will be the Clopper-Pearson exact CI [Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of binomial. *Biometrika*. [1934;26:404-413].

The standardised asymptotic two-sided 95% CI for the group difference in proportions is based on the method described in the following paper: Robert G. Newcombe, interval estimation for the difference between independent proportions: comparison of eleven methods, *Statist Med.* 1998; 17, 873-890]. The standardised asymptotic method used is the method six.

The GMC group ratio will be obtained using an ANCOVA model on the logarithm-transformed concentrations. This model will include the vaccine group and the logarithm of the baseline concentration as covariables. The ANCOVA model will include the vaccine group. The GMC ratio and their 95% CI will be derived by exponential transformation of the corresponding group contrast in the model.

11.2. Standard data derivation

11.2.1. Date derivation

- SAS date derived from a character date: in case day is missing, 15 is used. In case day & month are missing, 30June is used.
- Onset day for an event (AE, medication, vaccination, …): the onset day is the number of days between the last study vaccination & the onset/start date of the event. This is 1 for an event starting on the same day as a vaccination. See SAS date derived in case the start date of the event is incomplete.
- Duration: Duration of an event is expressed in days. It is the number of days between the start & the stop dates + 1. Therefore duration is 1 day for an event starting & ending on the same day.

11.2.2. Dose number

- The study dose number is defined in reference to the number of study visits at which vaccination occurred. More specifically dose 1 refers to all vaccines administered at the first vaccination visit while dose 2 corresponds to all vaccinations administered at the second vaccination visit even if this is the first time a product is administered to the subject.

- Relative dose: the relative dose for an event (AE, medication, vaccination) is the most recent study dose given before an event. In case the event takes place on the day a study dose is given, the related dose will be that of the study dose, even if the event actually took place before vaccination. For instance, if an adverse event begins on the day of the study vaccination but prior to administration of the vaccine, it will be assigned to this dose. In case a study dose is not administered and an event occurs after the subsequent study dose (e.g. 3rd study dose), the relative dose of the event will be study dose associated to the subsequent study dose (e.g. dose 3).
- The number of doses for a product is the number of time the product was administered to a subject.
- The incidence per dose is the number of vaccination visits at which an event was reported among all vaccination visits.

11.2.3. Demography

- Age: Age at the reference activity, computed as the number of complete weeks between the date of birth and the reference activity.

11.2.4. Immunogenicity

- For a given subject and given immunogenicity measurement, missing or non-evaluable measurements will not be replaced.

  The GMCs calculations are performed by taking the anti-log of the mean of the log concentration transformations. Antibody concentrations below the cut-off of the assay will be given an arbitrary value of half the cut-off of the assay for the purpose of GMC calculation.
- In general, the assay cut-off is the value under which there is no quantifiable result available. For an assay with a specific ‘assay cut_off’, numerical immuno result is derived from a character field (rawres):
  - If rawres is ‘NEG’ or ‘-’ or ‘(-)’, numeric result= assay cut_off/2,
  - if rawres is ‘POS’ or ‘+’ or ‘(+)’, numeric result = assay cut_off,
  - if rawres is ‘< value’ and value<=assay cut_off, numeric result =assay cut_off/2,
  - if rawres is ‘< value’ and value>assay cut_off, numeric result =value,
  - if rawres is ‘> value’ and value<assay cut_off, numeric result =assay cut_off/2,
  - if rawres is ‘> value’ and value>=assay cut_off, numeric result =value,
  - if rawres is ‘<= value’ or ‘>= value’ and value<assay cut_off, numeric result =assay cut_off/2,
  - if rawres is ‘<= value’ or ‘>= value’ and value>=assay cut_off, numeric result =value,
  - if rawres is a value < assay cut_off, numeric result = assay cut_off/2,

- if rawres is a value >= assay cut_off, numeric result = rawres,
- else numeric result is left blank.

11.2.5. Safety

- For analysis of solicited, unsolicited adverse events (such as serious adverse events or adverse events by primary MedDRA term) and for the analysis of concomitant medications, all vaccinated subjects will be considered. Subjects who did not report the event or the concomitant medication will be considered as subjects without the event or the concomitant medication respectively.
- In case there will be more than 5% of subjects without documented dose for solicited symptoms (i.e., symptom screen not completed), sensitivity analysis will include only vaccinated subjects for doses with documented safety data (i.e., symptom screen completed).
- The following rules will be used for the analysis of solicited symptoms:
  - Subject who didn't document the presence or absence of a solicited symptom after one dose will be considered not having that symptom after that dose in the analysis done on "administrated dose".
  - Subjects who documented the absence of a solicited symptom after one dose will be considered not having that symptom after that dose.
  - Subjects who documented the presence of a solicited symptom and fully or partially recorded daily measurement over the solicited period will be included in the summaries at that dose and classified according to their maximum observed daily recording over the solicited period.
  - Subjects who documented the presence of a solicited symptom after one dose without having recorded any daily measurement will be considered as having that symptom after that dose).
- Intensity of the following solicited AEs will be assessed as described in Table 5 and Table 6.

Table 5 Intensity scales to be used by the parent(s)/LAR(s) for solicited symptoms during the solicited follow-up period

Adverse Event	Intensity grade	Parameter
Fever*		Record temperature in °C/°F using any age-appropriate route.
Irritability/Fussiness	0	Behavior as usual
	1	Crying more than usual/no effect on normal activity
	2	Crying more than usual/interferes with normal activity
	3	Crying that cannot be comforted/prevents normal activity
Diarrhea$		Record the number of looser than normal stools/day
Vomiting§		Record the number of vomiting episodes/day
Loss of appetite	0	Appetite as usual
	1	Eating less than usual/no effect on normal activity
	2	Eating less than usual/interferes with normal activity
	3	Not eating at all
Cough/runny nose	0	Normal
	1	Cough/runny nose which is easily tolerated
	2	Cough/runny nose which interferes with daily activity
	3	Cough/runny nose which prevents daily activity

*Fever is defined as temperature ≥ 38.0°C/100.4°F. The preferred location for measuring temperature in this study will be the oral cavity, the axilla and the rectum.
$ Diarrhea is defined as passage of three or more looser than normal stools within a day.
§ Vomiting is defined as one or more episodes of forceful emptying of partially digested stomach contents ≥1 hour after feeding within a day.

Table 6 Intensity scales for diarrhea, vomiting and fever occurring during the solicited period

Adverse Event	Intensity grade	Parameter
Diarrhea $	0	Normal (0-2 looser than normal stools/day)
	1	3 looser than normal stools/day
	2	4-5 looser than normal stools/day
	3	≥ 6 looser than normal stools/day
Vomiting §	0	Normal (no emesis)
	1	1 episode of vomiting/day
	2	2 episodes of vomiting/day
	3	≥ 3 episodes of vomiting/day
Fever	0	temperature < 38.0°C/100.4° F
	1	temperature ≥ 38.0°C/100.4° F – ≤ 38.5°C/101.3 F
	2	temperature > 38.5°C/101.3 F – ≤ 39.5°C/103.1 F
	3	temperature > 39.5°C/103.1 F

$ Diarrhea is defined as passage of three or more looser than normal stools within a day.
§ Vomiting is defined as one or more episodes of forceful emptying of partially digested stomach contents ≥1 hour after feeding within a day.

- Note that for all tables described in this section, the way the percentage of subjects will be derived will depend on the event analysed (see table below for details). As a result, the N value will differ from one table to another.

Event	N used for deriving % per subject for Vaccination phase	N used for deriving % per dose for Vaccination phase
Concomitant vaccination	All subjects with study vaccine administered	All study visits with study vaccine administered
Solicited general symptom	Primary analysis: all subjects with study vaccine administered Sensitivity analysis as applicable, all subjects with at least one solicited general symptom documented as either present or absent (i.e., symptom screen completed)	Primary analysis: all study visits with study vaccine administered Sensitivity analysis: all study visits with study vaccine administered and with at least one solicited general symptom documented as either present or absent (i.e., symptom screen completed)
Unsolicited symptom	All subjects with study vaccine administered	All study visits with study vaccine administered
Concomitant medication	All subjects with study vaccine administered	All study visits with study vaccine administered

For summaries by MedDRA primary preferred term combining solicited and unsolicited adverse events, solicited adverse events will be coded as per the following MedDRA codes.

Solicited symptom	Lower level term code	Corresponding Lower level term decode
Fever	10016558	Fever
Irritability/Fussiness	10022998	Irritability
Diarrhoea	10012727	Diarrhoea
Vomiting	10047700	Vomiting
Loss of appetite	10003028	Appetite lost
Cough/runny nose	10011224	Cough

11.2.6. Data presentation description

The following decimal description from the decision rules will be used for the demography, immunogenicity and safety/reactogenicity.

Display Table	Parameters	Number of decimal digits
All summaries	% of count, including LL & UL of CI	1
Demographic characteristics	Mean, median, SD	1
Immunogenicity	% of difference, including LL & UL of CI	2
Immunogenicity	GMC group ratio, including LL & UL of CI	2
Immunogenicity	Anti-RV IgA GMC	1

GlaxoSmithKline	**Statistical Analysis Plan**
Detailed Title:	A phase III, randomized, open study to assess the immunogenicity, reactogenicity and safety of two different formulations of GlaxoSmithKline (GSK) Biologicals' oral live attenuated human rotavirus (HRV) vaccine, Rotarix, when given as a two-dose primary vaccination, in healthy infants with no previous history of rotavirus illness or vaccination.
eTrack study number and Abbreviated Title	l116566 (ROTA-083)
Scope:	All data pertaining to the above study.
Date of Statistical Analysis Plan	Amendment 1 Final 11 November 2019
Co-ordinating author:	PPD (study statistician); PPD (Lead statistician)
Reviewed by:	PPD (Clinical and Epidemiology Project Lead) PPD (Clinical Research and Development Lead) PPD (Lead statistician) PPD (Peer reviewer statistician) PPD (Lead statistical analyst) PPD (Lead Scientific writer) PPD (Regulatory Affair) PPD (Clinical Safety representative) PPD (Public disclosure representative)
Approved by:	PPD (Clinical and Epidemiology Project Lead) PPD (Clinical Research and Development Lead) PPD (Lead statistician) PPD (Lead Scientific writer) delegating to PPD (Scientific writer) PPD (Lead statistical analyst)

APP 9000058193 Statistical Analysis Plan Template (Effective date: 14 April 2017)

TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

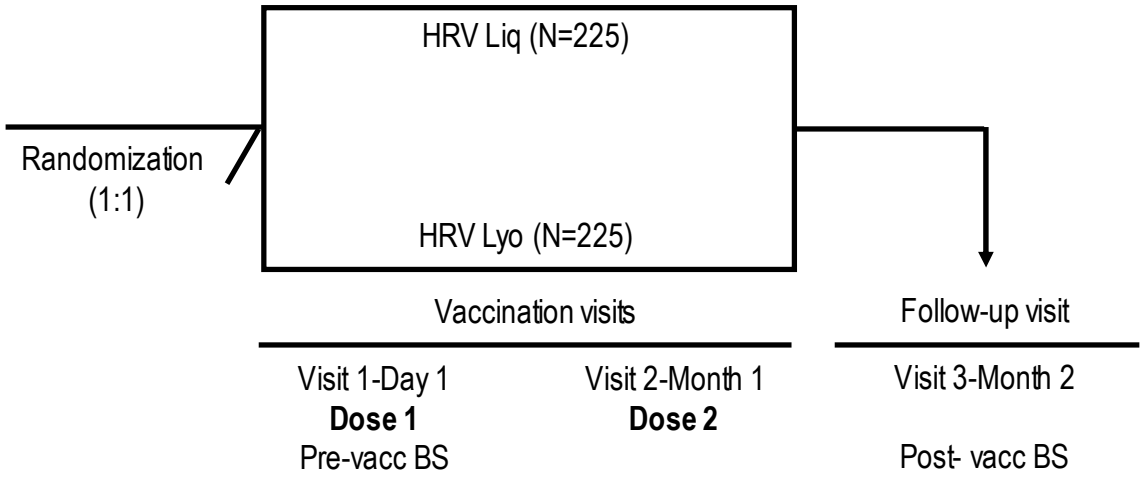
LIST OF ABBREVIATIONS

AE	Adverse event
CDISC	Clinical Data Interchange Standards Consortium
CI	Confidence Interval
CRF	Case Report Form
CTRS	Clinical Trial Registry Summary
eCRF	electronic Case Report Form
EoS	End of Study
ES	Exposed Set
GMC	Geometric mean antibody concentration
GMT	Geometric mean antibody titer
GSK	GlaxoSmithKline
HRV	Human Rotavirus
IgA	Immunoglobulin A
kg	Kilograms
LL	Lower Limit of the confidence interval
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligrams
mL	Milliliter
PPS	Per Protocol Set
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SBIR	GSK Biological's Internet Randomization System
SD	Standard Deviation
SR	Study Report
TFL	Tables Figures and Listings
TOC	Table of Content
UL	Upper Limit of the confidence interval

1. DOCUMENT HISTORY

Date	Description	Protocol Version
29-AUG-2018	Final version	Amendment 3 – 31-OCT-2017
11-NOV-2019	Amendment 1: The SAP was amended to be aligned with the protocol amendment. – Seroconversion was redefined in section 3.2 of Secondary Objectives – Between group assessment of immunogenicity was changed to an ANCOVA model – Code 2020 was redefined in section 5.2.2.1 – Within group assessment will include a antibody concentrations fold increase calculation in section 6.3.1.1	Amendment 4 – 30-OCT-2019

2. STUDY DESIGN



N= number of subjects planned to be enrolled; HRV= Human Rotavirus; Pre-Vacc= Pre-vaccination; Post-Vacc=Post-Vaccination; BS=Blood sample

Protocol waivers or exemptions are not allowed unless necessary for the management of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures (Section 5.5 in the protocol), are essential and required for study conduct.

- **Experimental design**: Phase **III**, open-label, randomized **(1:1)**, multi-centric, single-country study with two parallel groups.
- **Duration of the study**: The intended duration of the study, per subject, is approximately two months.
  - Epoch 001: Primary starting at Visit 1 (Day 1) and ending at Visit 3 (Month 2).
- **Primary Completion Date (PCD)**: Last subject attending Visit 3.

- **End of Study (EoS)**: Last testing results released for samples collected at Visit 3.
- **Study groups**: The study groups and epoch foreseen in the study are provided in Table 1.

Table 1 Study groups and epoch foreseen in the study

Study groups	Number of subjects	Age at Dose 1 (Min/Max)	Epoch 001
HRV Liq	***225***	6 weeks-10 weeks	●
HRV Lyo	***225***	6 weeks- 10 weeks	●

- **Treatment groups**: Table 2 presents the study groups and the vaccine to be administered in the study.
  - HRV liquid vaccine group (also referred to as HRV Liq).
  - HRV lyophilized vaccine group (also referred to as HRV Lyo).

Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/Product name	Study Groups	
		HRV Liq	HRV Lyo
HRV liquid	GSK Biologicals' HRV liquid vaccine	x	
HRV lyophilized	GSK Biologicals' HRV lyophilized vaccine		x
	GSK Biologicals' calcium carbonate buffer		x

- **Control**: active control (Lyophilized HRV vaccine)
- **Vaccination schedule**:
  - Two oral doses of the HRV vaccine to be given according to a 0, 1 month schedule.
  - All subjects are allowed to receive routine childhood vaccinations according to the local immunization practice. Administration of all routine childhood vaccinations given since birth will be recorded in the electronic Case Report Form (eCRF).
- **Treatment allocation**: Randomized (1:1). Treatment number will be allocated using GSK Biologicals' Randomization System on Internet (SBIR).
- **Blinding:** open

The blinding of study epoch is provided in Table 3.

Table 3 Blinding of the study epoch

Study Epoch	Blinding
Epoch 001	open

- **Sampling schedule**: Details of the samples to be collected are as follows:
  - Blood samples (approximately 2 ml) will be collected from all subjects at Visit 1 and Visit 3 to measure serum anti-RV Immunoglobulin A (IgA) antibody concentrations using Enzyme Linked Immunosorbent Assay (ELISA).

- **Type of study**: self-contained
- **Data collection**: eCRF.
- **Intervals between study visits:** Intervals between study visits to define vaccination and blood sample schedule intervals for Per Protocol Set (PPS) are as follows:

Table 4 Intervals between study visits

Interval	Optimal length of interval	Allowed interval
Visit 1 → Visit 2	1 month	28-48 days after Dose 1
Visit 2 → Visit 3	1 month	***31***-48 days after Dose 2

Note: The date of the previous visit serves as the reference date for the intervals between the study visits.

3. OBJECTIVES

3.1. Primary objective

- To evaluate non-inferiority of GSK Biologicals' HRV liquid vaccine compared to GSK Biologicals' HRV lyophilized vaccine in terms of geometric mean concentrations (GMCs) for anti-RV antibodies, one month post dose 2 of HRV liquid vaccine and HRV lyophilized vaccine.
  - Criterion: Non-inferiority will be stated if the lower limit of the two-sided 95% confidence interval (CI) for the ratio of anti RV IgA antibody GMCs between HRV liquid vaccine over the HRV lyophilized vaccine, one month after dose 2 is greater than or equal to 0.5.

Refer to Section 4.1 for the definition of the primary endpoint.

3.2. Secondary objectives

- To assess the immunogenicity of the HRV liquid vaccine and HRV lyophilized vaccine, in terms of seroconversion* rates, one month post dose 2 of HRV vaccine.

  **** Seroconversion is defined as:***

  - ***for subjects with a pre-vaccination anti-RV IgA antibody concentration <20 U/mL, seroconversion is achieved when the post-vaccination concentration is ≥20 U/mL.***
  - ***for subjects with a pre-vaccination anti-RV IgA antibody concentration ≥20 U/mL, seroconversion is achieved when the post-vaccination concentration is ≥2 times the pre-vaccination concentration.***
- To assess the reactogenicity of the HRV liquid vaccine and the HRV lyophilized vaccine in terms of solicited adverse events (AEs), during the 8-day (Day 1–Day 8) follow-up period after each vaccination.
- To assess the safety of the HRV liquid vaccine and the HRV lyophilized vaccine in terms of unsolicited AEs, during the 31-day (Day 1–Day 31) follow-up period after each vaccination and serious adverse events (SAEs), during the entire study period.

Refer to Section 4.2 for the definition of the secondary endpoints.

4. ENDPOINTS

4.1. Primary Endpoint

- Anti-RV IgA antibody concentrations
  - Serum anti-RV IgA antibody concentrations, expressed as GMCs, one month post dose 2 of HRV vaccine.

4.2. Secondary Endpoints

- Anti-RV IgA antibody concentrations
  - Anti-RV IgA antibody seroconversion rate, one month post dose 2 of HRV vaccine.
- Solicited general symptoms
  - Occurrence of each type of solicited general symptom within the 8-day (Day1-Day 8) solicited follow-up period, after each dose of HRV vaccine.
- Unsolicited adverse events
  - Occurrence of unsolicited AEs within 31 days (Day 1-Day 31) after any dose of HRV vaccine according to Medical Dictionary for Regulatory Activities (MedDRA) classification.
- Serious adverse events
  - Occurrence of SAEs from dose 1 of HRV vaccine up to study end.

5. ANALYSIS SETS

5.1. Definition

5.1.1. Exposed Set

The Exposed Set (ES) will include all subjects with at least one study vaccine administration documented.

- A safety analysis based on the ES will include all vaccinated subjects.
- An immunogenicity analysis based on the ES will include all vaccinated subjects for whom immunogenicity data is available.

5.1.2. Per-Protocol Set for analysis of immunogenicity

The PPS for immunogenicity will include all eligible subjects from the ES:

- who have received both doses of study vaccine according to their random assignment.
- for whom the HRV vaccine, liquid or lyophilized formulation, is administered according to protocol. Note that the subjects who regurgitate after vaccination and receive a replacement dose are to be retained in the PPS,
- who comply with the vaccination schedule for HRV vaccine (liquid or lyophilized formulation), as per Table 4,
- who have not received a vaccine prohibited by the protocol up to Visit 3 blood sample,
- who have not received medication prohibited by the protocol up to Visit 3 blood sample,
- whose underlying medical condition(s) was (were) not prohibited by the protocol up to Visit 3 blood sample,
- with no protocol violation of demographics (unknown age at study entry or outside protocol defined age-interval),
- who comply with blood sampling schedule,
- for whom immunogenicity data are available at pre- and post-vaccination sampling time points,
- for whom the post-vaccination immunogenicity data are within the 21-48 days interval after the second dose,
- who have no other concomitant infection up to Visit 3 blood sample, which may influence the immune response.

5.2. Criteria for eliminating data from Analysis Sets

Elimination codes are used to identify subjects to be eliminated from analysis. Detail is provided below for each sets.

5.2.1. Elimination from Exposed Set (ES)

Code 1030 (Study vaccine not administered at all) and code 900 (invalid informed consent or fraud data) will be used for identifying subjects eliminated from ES.

5.2.2. Elimination from Per-protocol analysis Set (PPS)

5.2.2.1. Excluded subjects

A subject will be excluded from the PPS analysis under the following conditions

Code	Decode => Condition under which the code is used
900	Questionable subject => Invalid informed consent or fraud data
1030	Study vaccine dose not administrated but subject number allocated => subjects enrolled but not vaccinated
1040	Administration of vaccine(s) forbidden in the protocol => • Administration of a vaccine not foreseen by the study protocol and administered during the period starting from 30 days before the first vaccination and ending at Visit 3, with the exception of the inactivated influenza vaccine, which is allowed at any time during the study, and other licensed routine childhood vaccinations.
1070	Study vaccine dose not administered according to protocol => • Subjects for whom the second administered dose is not aligned with the first dose (e.g. lyo as second dose after a first dose with liquid) • Subject who did not receive the second dose • Subjects orally vaccinated with the correct vaccine but who regurgitated during the same visit, without replacement dose • Route of vaccination which is not oral for HRV study vaccines • Wrong reconstitution of administered vaccine
1080	Vaccine temperature deviation => vaccine administered despite a Good Manufacturing Practices (GMP) no-go temperature deviation
1090	Expired vaccine administered => Subjects who received an expired vaccine
2010	Protocol violation (inclusion/exclusion criteria) => ineligible subject: • A male or female infant is not between and including 6 and 10 weeks (42-70 days) of age at the time of the first study vaccination • Infant born less than or equal to 28 weeks of gestation • Previous vaccination against HRV • Other considerations as stated in section 4.2 – 4.3 in the protocol
2020	Initially unknown antibody status = > Unknown antibody status at pre-dose 1 blood sample.

2040	Administration of any medication forbidden by the protocol => • Any investigational or non-registered product (drug or vaccine) other than the study vaccines used during the study period between the first vaccination at Visit 1 to the blood sampling at Visit 3. • Immunosuppressants or other immune-modifying drugs administered chronically (i.e., more than 14 days) during the study period between Visit 1 to Visit 3. For corticosteroids, this will mean prednisone ≥ 0.5 mg/kg/day, or equivalent. Inhaled and topical steroids are allowed. • Immunoglobulins and/or any blood products administered during the study period between the first vaccination at Visit 1 and the blood sampling at Visit 3. • Administration of long-acting immune-modifying drugs between the first vaccination at Visit 1 to the blood sampling at Visit 3 (e.g., infliximab)
2060	Concomitant infection related to the vaccine which may influence immune response => Subjects with non-vaccine type RV detected in GE stool samples that may impact immunogenicity at Visit 3.
2070	Concomitant infection not related to the vaccine which may influence immune response = > Condition that has the capability of altering their immune response at visit 3 such autoimmune disease.
2080	Non-compliance with vaccination schedule (including wrong and unknown dates) => Subjects who did not comply with the interval for dose 2 (Dose 2 should be between 28-48 days after Dose 1).
2090	Non-compliance with blood sampling schedule (including wrong and unknown dates => Subjects who did not comply with the blood sample interval (blood sample post dose 2 should be between 21-48 days after Dose 2).
2100	Essential serological data missing => Anti-HRV results not available post-vaccination.
2120	Obvious incoherence or abnormality or error in data => E.g. Sample mismanagement impacting results.

5.2.2.2. Right censored Data

Not applicable.

5.2.2.3. Visit-specific censored Data

Not applicable.

6. STATISTICAL ANALYSES

Note that standard data derivation rule and stat methods are described in Section 11 and will not be repeated below.

6.1. Demography

6.1.1. Analysis of demographics/baseline characteristics planned in the protocol

The distribution of subjects enrolled among the study centers will be tabulated as a whole and for each group.

The number of subjects who withdraw from the study will be tabulated by group according to the reason for drop-out.

The number of subjects enrolled into the study as well as the number of subjects excluded from PPS analyses will be tabulated as a whole and for each group.

The deviations from specifications for age and intervals between study visits will be tabulated by group.

The median, mean, range and standard deviation of age (in weeks) at each HRV vaccine dose will be computed by group. The median, mean and standard deviation of height in centimeter (cm) and weight in kilograms (kg) at Visit 1 will be computed by group. The geographical ancestry and sex composition will be presented.

Summary of co-administered vaccinations (i.e., vaccinations given on the day of each HRV vaccine dose) and intercurrent vaccinations (i.e., vaccinations other than the HRV lyophilized and HRV liquid vaccine administered from birth up to Visit 3, excluding vaccination given on the day of HRV vaccine doses) will be summarized by group for the ES. Summary of vaccines from birth up to visit 3 will be summarized.

For a given subject and a given demographic variable, missing measurements will not be replaced.

6.1.2. Additional considerations

All demography summaries will be generated for the ES. The summary of age, height, weight, race and sex will also be provided for the PPS. Number and reason for elimination from PPS will be tabulated by group.

6.2. Exposure

6.2.1. Analysis of exposure planned in the protocol

Not applicable.

6.2.2. Additional considerations

The number of doses administered will be tabulated for each group.

6.3. Immunogenicity

6.3.1. Analysis of immunogenicity planned in the protocol

The primary analysis will be based on the PPS for analysis of immunogenicity. If, in any vaccine group, the percentage of vaccinated subjects with serological results excluded from the PPS for analysis of immunogenicity is 5% or more, a second analysis based on the ES will be performed to complement the PPS analysis.

6.3.1.1. Within group assessment

The following calculations will be performed for each group

- For each group, at each time point that anti-rotavirus IgA is measured,
  - GMCs and their 95% CIs will be computed.
  - Seropositivity/seroconversion rates and their exact 95% CI will be computed,
  - The distribution of anti-RV IgA antibody concentrations at Visit 1 and Visit 3 will be displayed using Reverse Cumulative Curves (RCCs).

6.3.1.2. Between groups assessment

- The 95% CI for the ratio of anti-RV IgA antibody GMCs at Visit 3 between the HRV liquid vaccine over the HRV lyophilized vaccine will be computed using an ANCOVA model on the logarithm-transformed concentrations. This model will include the vaccine group and the logarithm of the baseline concentration as covariables. The GMC ratio and their 95% CI will be derived by exponential transformation of the corresponding group contrast in the model (primary objective).
- The asymptotic standardized 95% CI for the difference in seroconversion rate at Visit 3 between the HRV liquid vaccine and HRV lyophilized vaccine will be computed (secondary objective) as described in Section 11.1.

Refer to the Section 3.1 for the success criteria of the primary objective.

6.3.1.2.1. Definitions related with between groups assessment

- A seronegative subject is a subject whose anti-RV IgA antibody concentration is below the clinical meaningful threshold of <20 U/ml*.
- A seropositive subject is a subject whose anti-RV IgA antibody concentration is greater than or equal to the clinical meaningful threshold of 20 U/ml.

- ***Seroconversion is defined as:***
  - ***for subjects with a pre-vaccination anti-RV IgA antibody concentration <20 U/mL, seroconversion is achieved when the post-vaccination concentration is ≥20 U/mL.***
  - ***for subjects with a pre-vaccination anti-RV IgA antibody concentration ≥20 U/mL, seroconversion is achieved when the post-vaccination concentration is ≥2 times the pre-vaccination concentration.***
- The GMCs calculations are performed by taking the anti-log of the mean of the log concentrations transformations. Antibody concentrations below the technical cut-off (<13 U/ml*) of the assay will be given an arbitrary value of half the cut-off for the purpose of GMC calculation.

*Note: 20 U/ml corresponds to the clinical meaningful threshold to define seroconversion rate, while 13 U/ml corresponds to technical cut-off of revalidated laboratory assay.

An immunogenicity analysis based on the ES will include all vaccinated subjects for whom immunogenicity data is available. For the immunogenicity measurement, missing or non-evaluable measurements will not be replaced.

6.3.2. Additional considerations

Complementary to the within group assessment of immunogenicity; fold increase from pre-vaccination to one month post-dose 2 of anti-RV antibody concentrations will be computed for each group.

6.4. Analysis of safety

The ES will be used for the analysis of safety.

6.4.1. Analysis of safety planned in the protocol

6.4.1.1. Within groups assessment

The following calculations will be performed for each group:

- The percentage of doses and of subjects reporting at least one symptom (solicited or unsolicited) during the 8-day (Day 1-Day 8) solicited follow-up period will be computed, along with exact 95% CI. The same calculations will be done for symptoms (solicited or unsolicited) rated as grade 3 in intensity, for symptoms (solicited or unsolicited) assessed as causally related to vaccination and for symptoms resulting in medically attended visit.
- The percentage of doses and of subjects reporting each individual solicited general symptom will be computed, over the 8-day (Day 1-Day 8) solicited follow-up period, post vaccination, along with exact 95% CI. The same calculations will be done for each individual solicited general symptom rated as grade 3 in intensity, for each individual solicited general symptom assessed as causally related to vaccination and for symptoms resulting in medically attended visit.

- The verbatim reports of unsolicited AEs will be reviewed by a physician and the signs and symptoms will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). Every verbatim term will be matched with the appropriate Preferred Term. The percentage of subjects with unsolicited AEs occurring within 31-day (Day 1-Day 31) follow-up period after any dose with its exact 95% CI will be tabulated by preferred term. Similar tabulation will be done for unsolicited AEs rated as grade 3, for unsolicited AEs with causal relationship to vaccination, for unsolicited AE resulting in medically attended visit and for AEs or SAEs leading to drop out.
- The percentages of subjects who started taking at least one concomitant medication, by type, from Day 1 to Day 8 after vaccinations will be tabulated with exact 95% CI. The percentages of subjects who started taking at least one concomitant medication, by type, during the study period will also be tabulated with exact 95% CI.

SAEs reported during the study period will be described in detail.

6.4.2. Additional considerations

The percentage of doses with unsolicited AEs occurring within 31-day (Day 1-Day 31) follow-up period after any dose with its exact 95% CI will be tabulated by preferred term. Similar tabulation will be done for unsolicited AEs rated as grade 3, for unsolicited AEs with causal relationship to vaccination, for unsolicited AE resulting in medically attended visit.

Safety analysis will be done per administered dose. Subjects who missed reporting symptoms (solicited/unsolicited or concomitant medications) will be treated as subjects without symptoms (solicited/unsolicited or concomitant medications, respectively). A sensitivity analysis will be performed on documented doses i.e. accounting only for doses where solicited symptoms are indicated as being absent/present in case more than 5% of the doses are not documented.

6.4.2.1. Solicited Adverse Events

Summary of temperature will be provided by 0.5° increment (i.e. ≥ 38.0°C, >38.5; > 39, > 39.5, > 40). Fever, defined as a body temperature of ≥38°C irrespective of route of measurement, will be integrated to the summaries as a general symptom.

7. ANALYSIS INTERPRETATION

Except for analyses addressing criteria specified in the primary objective, all the analyses will be descriptive/exploratory in nature. The use of these descriptive/exploratory analyses should be limited to support the confirmatory analyses or to generate hypothesis.

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

Description	Analysis ID	Disclosure Purpose (CTRS=public posting, SR=study report, internal)	Dry run review needed (Y/N)	*Study Headline Summary (SHS)*requiring expedited communication to upper management (Yes/No)	Reference for TFL
Final	E1_01	SR	Yes	Yes	All tables from TFL TOC

8.2. Statistical considerations for interim analyses

Not applicable.

9. CHANGES FROM PLANNED ANALYSES

Not applicable.

10. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES (TFL)

The TFL Table Of Content (TOC) is as separate document. It provides the list of tables/listings and figures needed for the study report, and It also identifies the tables eligible for each analysis and their role (synopsis, in-text, post-text, SHS, CTRS).

The following group names will be used in the TFLs, to be in line with the T-domains:

Group order in tables	Group label in tables	Group definition for footnote
1	HRV Liq	HRV liquid vaccine
2	HRV Lyo	HRV lyophilised vaccine

11. ANNEX 1 STANDARD DATA DERIVATION RULE AND STATISTICAL METHODS

11.1. Statistical Method References

The exact two-sided 95% CIs for a proportion within a group will be the Clopper-Pearson exact CI [Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of binomial. *Biometrika*. [1934;26:404-413].

The standardised asymptotic two-sided 95% CI for the group difference in proportions is based on the method described in the following paper: Robert G. Newcombe, interval estimation for the difference between independent proportions: comparison of eleven methods, *Statist Med.* 1998; 17, 873-890]. The standardised asymptotic method used is the method six.

The GMC group ratio will be obtained using an ANCOVA model on the logarithm-transformed concentrations. This model will include the vaccine group and the logarithm of the baseline concentration as covariables. The ANCOVA model will include the vaccine group. The GMC ratio and their 95% CI will be derived by exponential transformation of the corresponding group contrast in the model.

11.2. Standard data derivation

11.2.1. Date derivation

- SAS date derived from a character date: in case day is missing, 15 is used. In case day & month are missing, 30June is used.
- Onset day for an event (AE, medication, vaccination, …): the onset day is the number of days between the last study vaccination & the onset/start date of the event. This is 1 for an event starting on the same day as a vaccination. See SAS date derived in case the start date of the event is incomplete.
- Duration: Duration of an event is expressed in days. It is the number of days between the start & the stop dates + 1. Therefore duration is 1 day for an event starting & ending on the same day.

11.2.2. Dose number

- The study dose number is defined in reference to the number of study visits at which vaccination occurred. More specifically dose 1 refers to all vaccines administered at the first vaccination visit while dose 2 corresponds to all vaccinations administered at the second vaccination visit even if this is the first time a product is administered to the subject.
- Relative dose: the relative dose for an event (AE, medication, vaccination) is the most recent study dose given before an event. In case the event takes place on the day a study dose is given, the related dose will be that of the study dose, even if the

event actually took place before vaccination. For instance, if an adverse event begins on the day of the study vaccination but prior to administration of the vaccine, it will be assigned to this dose. In case a study dose is not administered and an event occurs after the subsequent study dose (e.g. 3rd study dose), the relative dose of the event will be study dose associated to the subsequent study dose (e.g. dose 3).

- The number of doses for a product is the number of time the product was administered to a subject.
- The incidence per dose is the number of vaccination visits at which an event was reported among all vaccination visits.

11.2.3. Demography

- Age: Age at the reference activity, computed as the number of complete weeks between the date of birth and the reference activity.

11.2.4. Immunogenicity

- For a given subject and given immunogenicity measurement, missing or non-evaluable measurements will not be replaced.

  The GMCs calculations are performed by taking the anti-log of the mean of the log concentration transformations. Antibody concentrations below the cut-off of the assay will be given an arbitrary value of half the cut-off of the assay for the purpose of GMC calculation.

- In general, the assay cut-off is the value under which there is no quantifiable result available. For an assay with a specific 'assay cut_off', numerical immuno result is derived from a character field (rawres):
  - If rawres is 'NEG' or '-' or '(-)', numeric result= assay cut_off/2,
  - if rawres is 'POS' or '+' or '(+)', numeric result = assay cut_off,
  - if rawres is '< value' and value<=assay cut_off, numeric result =assay cut_off/2,
  - if rawres is '< value' and value>assay cut_off, numeric result =value,
  - if rawres is '> value' and value<assay cut_off, numeric result =assay cut_off/2,
  - if rawres is '> value' and value>=assay cut_off, numeric result =value,
  - if rawres is '<= value' or '>= value' and value<assay cut_off, numeric result =assay cut_off/2,
  - if rawres is '<= value' or '>= value' and value>=assay cut_off, numeric result =value,
  - if rawres is a value < assay cut_off, numeric result = assay cut_off/2,
  - if rawres is a value >= assay cut_off, numeric result = rawres,
  - else numeric result is left blank.

11.2.5. Safety

- For analysis of solicited, unsolicited adverse events (such as serious adverse events or adverse events by primary MedDRA term) and for the analysis of concomitant medications, all vaccinated subjects will be considered. Subjects who did not report the event or the concomitant medication will be considered as subjects without the event or the concomitant medication respectively.
- In case there will be more than 5% of subjects without documented dose for solicited symptoms (i.e., symptom screen not completed), sensitivity analysis will include only vaccinated subjects for doses with documented safety data (i.e., symptom screen completed).
- The following rules will be used for the analysis of solicited symptoms:
  - Subject who didn't document the presence or absence of a solicited symptom after one dose will be considered not having that symptom after that dose in the analysis done on "administrated dose".
  - Subjects who documented the absence of a solicited symptom after one dose will be considered not having that symptom after that dose.
  - Subjects who documented the presence of a solicited symptom and fully or partially recorded daily measurement over the solicited period will be included in the summaries at that dose and classified according to their maximum observed daily recording over the solicited period.
  - Subjects who documented the presence of a solicited symptom after one dose without having recorded any daily measurement will be considered as having that symptom after that dose).
- Intensity of the following solicited AEs will be assessed as described in Table 5 and Table 6.

Table 5 Intensity scales to be used by the parent(s)/LAR(s) for solicited symptoms during the solicited follow-up period

Adverse Event	Intensity grade	Parameter
Fever*		Record temperature in °C/°F using any age-appropriate route.
Irritability/Fussiness	0	Behavior as usual
	1	Crying more than usual/no effect on normal activity
	2	Crying more than usual/interferes with normal activity
	3	Crying that cannot be comforted/prevents normal activity
Diarrhea$		Record the number of looser than normal stools/day
Vomiting§		Record the number of vomiting episodes/day
Loss of appetite	0	Appetite as usual
	1	Eating less than usual/no effect on normal activity
	2	Eating less than usual/interferes with normal activity
	3	Not eating at all
Cough/runny nose	0	Normal
	1	Cough/runny nose which is easily tolerated
	2	Cough/runny nose which interferes with daily activity
	3	Cough/runny nose which prevents daily activity

*Fever is defined as temperature ≥ 38.0°C/100.4°F. The preferred location for measuring temperature in this study will be the oral cavity, the axilla and the rectum.

$ Diarrhea is defined as passage of three or more looser than normal stools within a day.

§ Vomiting is defined as one or more episodes of forceful emptying of partially digested stomach contents ≥1 hour after feeding within a day.

Table 6 Intensity scales for diarrhea, vomiting and fever occurring during the solicited period

Adverse Event	Intensity grade	Parameter
Diarrhea [$]	0	Normal (0-2 looser than normal stools/day)
	1	3 looser than normal stools/day
	2	4-5 looser than normal stools/day
	3	≥ 6 looser than normal stools/day
Vomiting [§]	0	Normal (no emesis)
	1	1 episode of vomiting/day
	2	2 episodes of vomiting/day
	3	≥ 3 episodes of vomiting/day
Fever	0	temperature < 38.0°C/100.4° F
	1	temperature ≥ 38.0°C/100.4° F – ≤ 38.5°C/101.3 F
	2	temperature > 38.5°C/101.3 F – ≤ 39.5°C/103.1 F
	3	temperature > 39.5°C/103.1 F

[$] Diarrhea is defined as passage of three or more looser than normal stools within a day.
[§] Vomiting is defined as one or more episodes of forceful emptying of partially digested stomach contents ≥1 hour after feeding within a day.

- Note that for all tables described in this section, the way the percentage of subjects will be derived will depend on the event analysed (see table below for details). As a result, the N value will differ from one table to another.

Event	N used for deriving % per subject for Vaccination phase	N used for deriving % per dose for Vaccination phase
Concomitant vaccination	All subjects with study vaccine administered	All study visits with study vaccine administered
Solicited general symptom	Primary analysis: all subjects with study vaccine administered Sensitivity analysis as applicable, all subjects with at least one solicited general symptom documented as either present or absent (i.e., symptom screen completed)	Primary analysis: all study visits with study vaccine administered Sensitivity analysis: all study visits with study vaccine administered and with at least one solicited general symptom documented as either present or absent (i.e., symptom screen completed)
Unsolicited symptom	All subjects with study vaccine administered	All study visits with study vaccine administered
Concomitant medication	All subjects with study vaccine administered	All study visits with study vaccine administered

For summaries by MedDRA primary preferred term combining solicited and unsolicited adverse events, solicited adverse events will be coded as per the following MedDRA codes.

Solicited symptom	Lower level term code	Corresponding Lower level term decode
Fever	10016558	Fever
Irritability/Fussiness	10022998	Irritability
Diarrhoea	10012727	Diarrhoea
Vomiting	10047700	Vomiting
Loss of appetite	10003028	Appetite lost
Cough/runny nose	10011224	Cough

11.2.6. Data presentation description

The following decimal description from the decision rules will be used for the demography, immunogenicity and safety/reactogenicity.

Display Table	Parameters	Number of decimal digits
All summaries	% of count, including LL & UL of CI	1
Demographic characteristics	Mean, median, SD	1
Immunogenicity	% of difference, including LL & UL of CI	2
Immunogenicity	GMC group ratio, including LL & UL of CI	2
Immunogenicity	Anti-RV IgA GMC	1

GlaxoSmithKline	**Statistical Analysis Plan**
Detailed Title:	A phase III, randomized, open study to assess the immunogenicity, reactogenicity and safety of two different formulations of GlaxoSmithKline (GSK) Biologicals' oral live attenuated human rotavirus (HRV) vaccine, Rotarix, when given as a two-dose primary vaccination, in healthy infants with no previous history of rotavirus illness or vaccination.
eTrack study number and Abbreviated Title	l16566 (ROTA-083)
Scope:	All data pertaining to the above study.
Date of Statistical Analysis Plan	Final 29-August-2018
Co-ordinating author:	PPD (study statistician); PPD (Lead statistician)
Reviewed by:	PPD (Clinical and Epidemiology Project Lead) PPD (Clinical Research and Development Lead) PPD (Lead statistician) PPD (Peer reviewer statistician) PPD (Lead statistical analyst) PPD (Lead Scientific writer) PPD (Regulatory Affair) PPD (Clinical Safety representative) PPD (Public disclosure representative)
Approved by:	PPD (Clinical and Epidemiology Project Lead) PPD (Clinical Research and Development Lead) PPD (Lead statistician) PPD (Lead Scientific writer) delegating to PPD (Scientific writer) PPD (Lead statistical analyst)

APP 9000058193 Statistical Analysis Plan Template (Effective date: 14 April 2017)

TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

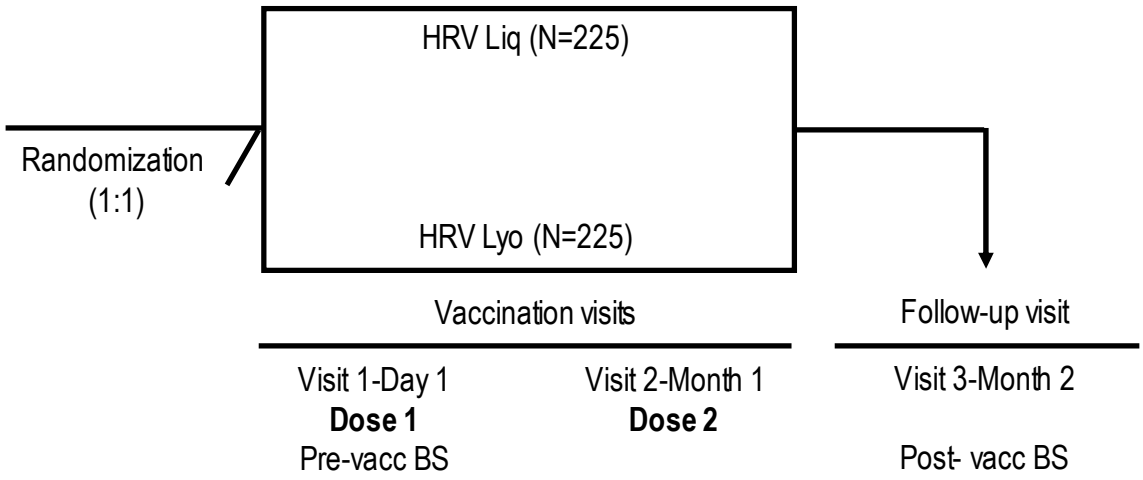
LIST OF ABBREVIATIONS

AE	Adverse event
CDISC	Clinical Data Interchange Standards Consortium
CI	Confidence Interval
CRF	Case Report Form
CTRS	Clinical Trial Registry Summary
eCRF	electronic Case Report Form
EoS	End of Study
ES	Exposed Set
GMC	Geometric mean antibody concentration
GMT	Geometric mean antibody titer
GSK	GlaxoSmithKline
HRV	Human Rotavirus
IgA	Immunoglobulin A
kg	Kilograms
LL	Lower Limit of the confidence interval
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligrams
mL	Milliliter
PPS	Per Protocol Set
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SBIR	GSK Biological's Internet Randomization System
SD	Standard Deviation
SR	Study Report
TFL	Tables Figures and Listings
TOC	Table of Content
UL	Upper Limit of the confidence interval

1. DOCUMENT HISTORY

Date	Description	Protocol Version
29-Aug-2018	Final version	Protocol Amendment 3 – 31-OCT-2017

2. STUDY DESIGN



N= number of subjects planned to be enrolled; HRV= Human Rotavirus; Pre-Vacc= Pre-vaccination; Post-Vacc=Post-Vaccination; BS=Blood sample

Protocol waivers or exemptions are not allowed unless necessary for the management of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures (Section 5.5 in the protocol), are essential and required for study conduct.

- **Experimental design**: Phase **III**, open-label, randomized **(1:1)**, multi-centric, single-country study with two parallel groups.
- **Duration of the study**: The intended duration of the study, per subject, is approximately two months.
  - Epoch 001: Primary starting at Visit 1 (Day 1) and ending at Visit 3 (Month 2).
- **Primary Completion Date (PCD)**: Last subject attending Visit 3.
- **End of Study (EoS)**: Last testing results released for samples collected at Visit 3.
- **Study groups**: The study groups and epoch foreseen in the study are provided in Table 1.

Table 1 Study groups and epoch foreseen in the study

Study groups	Number of subjects	Age at Dose 1 (Min/Max)	Epoch 001
HRV Liq	***225***	6 weeks-10 weeks	●
HRV Lyo	***225***	6 weeks- 10 weeks	●

- **Treatment groups**: Table 2 presents the study groups and the vaccine to be administered in the study.
  - HRV liquid vaccine group (also referred to as HRV Liq)
  - HRV lyophilized vaccine group (also referred to as HRV Lyo).

Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/Product name	Study Groups	
		HRV Liq	HRV Lyo
HRV liquid	GSK Biologicals' HRV liquid vaccine	x	
HRV lyophilized	GSK Biologicals' HRV lyophilized vaccine		x
	GSK Biologicals' calcium carbonate buffer		x

- **Control**: active control (Lyophilized HRV vaccine)
- **Vaccination schedule**:
  - Two oral doses of the HRV vaccine to be given according to a 0, 1 month schedule.
  - All subjects are allowed to receive routine childhood vaccinations according to the local immunization practice. Administration of all routine childhood vaccinations given since birth will be recorded in the electronic Case Report Form (eCRF).
- **Treatment allocation**: Randomized (1:1). Treatment number will be allocated using GSK Biologicals' Randomization System on Internet (SBIR).
- **Blinding:** open

The blinding of study epoch is provided in Table 3.

Table 3 Blinding of the study epoch

Study Epoch	Blinding
Epoch 001	open

- **Sampling schedule**: Details of the samples to be collected are as follows:
  - Blood samples (approximately 2 ml) will be collected from all subjects at Visit 1 and Visit 3 to measure serum anti-RV Immunoglobulin A (IgA) antibody concentrations using Enzyme Linked Immunosorbent Assay (ELISA).
- **Type of study**: self-contained

- **Data collection**: eCRF.
- **Intervals between study visits:** Intervals between study visits to define vaccination and blood sample schedule intervals for Per Protocol Set (PPS) are as follows:

Table 4 Intervals between study visits

Interval	Optimal length of interval	Allowed interval
Visit 1 → Visit 2	1 month	28-48 days after Dose 1
Visit 2 → Visit 3	1 month	***31***-48 days after Dose 2

Note: The date of the previous visit serves as the reference date for the intervals between the study visits.

3. OBJECTIVES

3.1. Primary objective

- To evaluate non-inferiority of GSK Biologicals' HRV liquid vaccine compared to GSK Biologicals' HRV lyophilized vaccine in terms of geometric mean concentrations (GMCs) for anti-RV antibodies, one month post dose 2 of HRV liquid vaccine and HRV lyophilized vaccine.
  - Criterion: Non-inferiority will be stated if the lower limit of the two-sided 95% confidence interval (CI) for the ratio of anti RV IgA antibody GMCs between HRV liquid vaccine over the HRV lyophilized vaccine, one month after dose 2 is greater than or equal to 0.5.

Refer to Section 4.1 for the definition of the primary endpoint.

3.2. Secondary objectives

- To assess the immunogenicity of the HRV liquid vaccine and HRV lyophilized vaccine, in terms of seroconversion rates, one month post dose 2 of HRV vaccine.

  *Seroconversion rate is defined as the percentage of subjects with anti-RV IgA antibody concentration ≥ 20 U/mL one-month post dose 2 among subjects with anti-RV IgA antibody concentration < 20 U/mL at pre-vaccination.
- To assess the reactogenicity of the HRV liquid vaccine and the HRV lyophilized vaccine in terms of solicited adverse events (AEs), during the 8-day (Day 1–Day 8) follow-up period after each vaccination.
- To assess the safety of the HRV liquid vaccine and the HRV lyophilized vaccine in terms of unsolicited AEs, during the 31-day (Day 1–Day 31) follow-up period after each vaccination and serious adverse events (SAEs), during the entire study period.

Refer to Section 4.2 for the definition of the secondary endpoints.

4. ENDPOINTS

4.1. Primary Endpoint

- Anti-RV IgA antibody concentrations
  - Serum anti-RV IgA antibody concentrations, expressed as GMCs, one month post dose 2 of HRV vaccine.

4.2. Secondary Endpoints

- Anti-RV IgA antibody concentrations
  - Anti-RV IgA antibody seroconversion rate, one month post dose 2 of HRV vaccine.
- Solicited general symptoms
  - Occurrence of each type of solicited general symptom within the 8-day (Day1-Day 8) solicited follow-up period, after each dose of HRV vaccine.
- Unsolicited adverse events
  - Occurrence of unsolicited AEs within 31 days (Day 1-Day 31) after any dose of HRV vaccine according to Medical Dictionary for Regulatory Activities (MedDRA) classification.
- Serious adverse events
  - Occurrence of SAEs from dose 1 of HRV vaccine up to study end.

5. ANALYSIS SETS

5.1. Definition

5.1.1. Exposed Set

The Exposed Set (ES) will include all subjects with at least one study vaccine administration documented.

- A safety analysis based on the ES will include all vaccinated subjects.
- An immunogenicity analysis based on the ES will include all vaccinated subjects for whom immunogenicity data is available.

5.1.2. Per-Protocol Set for analysis of immunogenicity

The PPS for immunogenicity will include all eligible subjects from the ES:

- who have received both doses of study vaccine according to their random assignment.
- for whom the HRV vaccine, liquid or lyophilized formulation, is administered according to protocol. Note that the subjects who regurgitate after vaccination and receive a replacement dose are to be retained in the PPS,
- who comply with the vaccination schedule for HRV vaccine (liquid or lyophilized formulation), as per Table 4,
- who have not received a vaccine prohibited by the protocol up to Visit 3 blood sample,
- who have not received medication prohibited by the protocol up to Visit 3 blood sample,
- who are seronegative for serum anti-RV IgA antibodies on the day of dose 1,
- whose underlying medical condition(s) was (were) not prohibited by the protocol up to Visit 3 blood sample,
- with no protocol violation of demographics (unknown age at study entry or outside protocol defined age-interval),
- who comply with blood sampling schedule,
- for whom immunogenicity data are available at pre- and post-vaccination sampling time points.
- for whom the post-vaccination immunogenicity data are within the 21-48 days interval after the second dose
- who have no other concomitant infection up to Visit 3 blood sample, which may influence the immune response.

5.2. Criteria for eliminating data from Analysis Sets

Elimination codes are used to identify subjects to be eliminated from analysis. Detail is provided below for each sets.

5.2.1. Elimination from Exposed Set (ES)

Code 1030 (Study vaccine not administered at all) and code 900 (invalid informed consent or fraud data) will be used for identifying subjects eliminated from ES

5.2.2. Elimination from Per-protocol analysis Set (PPS)

5.2.2.1. Excluded subjects

A subject will be excluded from the PPS analysis under the following conditions

Code	Decode => Condition under which the code is used
900	Questionable subject => Invalid informed consent or fraud data
1030	Study vaccine dose not administrated but subject number allocated => subjects enrolled but not vaccinated
1040	Administration of vaccine(s) forbidden in the protocol => • Administration of a vaccine not foreseen by the study protocol and administered during the period starting from 30 days before the first vaccination and ending at Visit 3, with the exception of the inactivated influenza vaccine, which is allowed at any time during the study, and other licensed routine childhood vaccinations.
1070	Study vaccine dose not administered according to protocol => • Subjects for whom the second administered dose is not aligned with the first dose (e.g. lyo as second dose after a first dose with liquid) • Subject who did not receive the second dose • Subjects orally vaccinated with the correct vaccine but who regurgitated during the same visit, without replacement dose • Route of vaccination which is not oral for HRV study vaccines • Wrong reconstitution of administered vaccine
1080	Vaccine temperature deviation => vaccine administered despite a Good Manufacturing Practices (GMP) no-go temperature deviation
1090	Expired vaccine administered=> Subjects who received an expired vaccine
2010	Protocol violation (inclusion/exclusion criteria) => ineligible subject: • A male or female infant is not between and including 6 and 10 weeks (42-70 days) of age at the time of the first study vaccination • Infant born less than or equal to 28 weeks of gestation • Previous vaccination against HRV • Other considerations as stated in section 4.2 – 4.3 in the protocol

2020	Initially seropositive or initially unknown antibody status = > Anti-HRV concentration ≥ 20 U/mL or unknown at pre-dose 1 blood sample
2040	Administration of any medication forbidden by the protocol:=> • Any investigational or non-registered product (drug or vaccine) other than the study vaccines used during the study period between the first vaccination at Visit 1 to the blood sampling at Visit 3. • Immunosuppressants or other immune-modifying drugs administered chronically (i.e., more than 14 days) during the study period between Visit 1 to Visit 3. For corticosteroids, this will mean prednisone ≥ 0.5 mg/kg/day, or equivalent. Inhaled and topical steroids are allowed. • Immunoglobulins and/or any blood products administered during the study period between the first vaccination at Visit 1 and the blood sampling at Visit 3. • Administration of long-acting immune-modifying drugs between the first vaccination at Visit 1 to the blood sampling at Visit 3 (e.g., infliximab)
2060	Concomitant infection related to the vaccine which may influence immune response => Subjects with non-vaccine type RV detected in GE stool samples that may impact immunogenicity at Visit 3.
2070	Concomitant infection not related to the vaccine which may influence immune response = > Condition that has the capability of altering their immune response at visit 3 such autoimmune disease.
2080	Non-compliance with vaccination schedule (including wrong and unknown dates) => Subjects who did not comply with the interval for dose 2 (Dose 2 should be between 28-48 days after Dose 1).
2090	Non-compliance with blood sampling schedule (including wrong and unknown dates => Subjects who did not comply with the blood sample interval (blood sample post dose 2 should be between 21-48 days after Dose 2).
2100	Essential serological data missing => Anti-HRV results not available post-vaccination.
2120	Obvious incoherence or abnormality or error in data => E.g. Sample mismanagement impacting results.

5.2.2.2. Right censored Data

Not applicable

5.2.2.3. Visit-specific censored Data

Not applicable

6. STATISTICAL ANALYSES

Note that standard data derivation rule and stat methods are described in Section 11 and will not be repeated below.

6.1. Demography

6.1.1. Analysis of demographics/baseline characteristics planned in the protocol

The distribution of subjects enrolled among the study centers will be tabulated as a whole and for each group.

The number of subjects who withdraw from the study will be tabulated by group according to the reason for drop-out.

The number of subjects enrolled into the study as well as the number of subjects excluded from PPS analyses will be tabulated as a whole and for each group.

The deviations from specifications for age and intervals between study visits will be tabulated by group.

The median, mean, range and standard deviation of age (in weeks) at each HRV vaccine dose will be computed by group. The median, mean and standard deviation of height in centimeter (cm) and weight in kilograms (kg) at Visit 1 will be computed by group. The geographical ancestry and sex composition will be presented.

Summary of co-administered vaccinations (i.e., vaccinations given on the day of each HRV vaccine dose) and intercurrent vaccinations (i.e., vaccinations other than the HRV lyophilized and HRV liquid vaccine administered from birth up to Visit 3, excluding vaccination given on the day of HRV vaccine doses) will be summarized by group for the ES. Summary of vaccines from birth up to visit 3 will be summarized.

For a given subject and a given demographic variable, missing measurements will not be replaced.

6.1.2. Additional considerations

All demography summaries will be generated for the ES. The summary of age, height, weight, race and sex will also be provided for the PPS. Number and reason for elimination from PPS will be tabulated by group.

6.2. Exposure

6.2.1. Analysis of exposure planned in the protocol

Not applicable

6.2.2. Additional considerations

The number of doses administered will be tabulated for each group.

6.3. Immunogenicity

6.3.1. Analysis of immunogenicity planned in the protocol

The primary analysis will be based on the PPS for analysis of immunogenicity. If, in any vaccine group, the percentage of vaccinated subjects with serological results excluded from the PPS for analysis of immunogenicity is 5% or more, a second analysis based on the ES will be performed to complement the PPS analysis.

6.3.1.1. Within group assessment

The following calculations will be performed for each group

- For each group, at each time point that anti-rotavirus IgA is measured,
  - GMCs and their 95% CIs will be computed.
  - Seropositivity/seroconversion rates and their exact 95% CI will be computed,
  - The distribution of anti-RV IgA antibody concentrations at Visit 3 will be displayed using Reverse Cumulative Curves (RCCs).

6.3.1.2. Between groups assessment

- The 95% CI for the ratio of anti-RV IgA antibody GMCs at Visit 3 between the HRV liquid vaccine over the HRV lyophilized vaccine will be computed (primary objective).
- The asymptotic standardized 95% CI for the difference in seroconversion rate at Visit 3 between the HRV liquid vaccine and HRV lyophilized vaccine will be computed (secondary objective) as described in Section 11.1.

  Refer to the Section 3.1 for the success criteria of the primary objective.

6.3.1.2.1. Definitions related with between groups assessment

- A seronegative subject is a subject whose anti-RV IgA antibody concentration is below the clinical meaningful threshold of <20 U/ml*.
- A seropositive subject is a subject whose anti-RV IgA antibody concentration is greater than or equal to the clinical meaningful threshold of 20 U/ml.
- Seroconversion rate is defined as the percentage of subjects who were initially seronegative (i.e., with anti-RV IgA antibody concentration < 20 U/mL prior the first dose of HRV vaccine) and developed anti-RV IgA antibody concentration ≥ 20 U/mL at Visit 3.
- The GMCs calculations are performed by taking the anti-log of the mean of the log concentrations transformations. Antibody concentrations below the technical cut-off (<13 U/ml*) of the assay will be given an arbitrary value of half the cut-off for the purpose of GMC calculation.

*Note: 20 U/ml corresponds to the clinical meaningful threshold to define seroconversion rate, while 13 U/ml corresponds to technical cut-off of revalidated laboratory assay.

An immunogenicity analysis based on the ES will include all vaccinated subjects for whom immunogenicity data is available. For the immunogenicity measurement, missing or non-evaluable measurements will not be replaced.

6.3.2. Additional considerations

Not applicable

6.4. Analysis of safety

The ES will be used for the analysis of safety.

6.4.1. Analysis of safety planned in the protocol

6.4.1.1. Within groups assessment

The following calculations will be performed for each group:

The percentage of doses and of subjects reporting at least one symptom (solicited or unsolicited) during the 8-day (Day 1-Day 8) solicited follow-up period will be computed, along with exact 95% CI. The same calculations will be done for symptoms (solicited or unsolicited) rated as grade 3 in intensity, for symptoms (solicited or unsolicited) assessed as causally related to vaccination and for symptoms resulting in medically attended visit.

The percentage of doses and of subjects reporting each individual solicited general symptom will be computed, over the 8-day (Day 1-Day 8) solicited follow-up period, post vaccination, along with exact 95% CI. The same calculations will be done for each individual solicited general symptom rated as grade 3 in intensity, for each individual solicited general symptom assessed as causally related to vaccination and for symptoms resulting in medically attended visit.

The verbatim reports of unsolicited AEs will be reviewed by a physician and the signs and symptoms will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). Every verbatim term will be matched with the appropriate Preferred Term. The percentage of subjects with unsolicited AEs occurring within 31-day (Day 1-Day 31) follow-up period after any dose with its exact 95% CI will be tabulated by preferred term. Similar tabulation will be done for unsolicited AEs rated as grade 3, for unsolicited AEs with causal relationship to vaccination, for unsolicited AE resulting in medically attended visit and for AEs or SAEs leading to drop out.

The percentages of subjects who started taking at least one concomitant medication, by type, from Day 1 to Day 8 after vaccinations will be tabulated with exact 95% CI. The percentages of subjects who started taking at least one concomitant medication, by type, during the study period will also be tabulated with exact 95% CI.

SAEs reported during the study period will be described in detail.

6.4.2. Additional considerations

The percentage of doses with unsolicited AEs occurring within 31-day (Day 1-Day 31) follow-up period after any dose with its exact 95% CI will be tabulated by preferred term. Similar tabulation will be done for unsolicited AEs rated as grade 3, for unsolicited AEs with causal relationship to vaccination, for unsolicited AE resulting in medically attended visit.

Safety analysis will be done per administered dose. Subjects who missed reporting symptoms (solicited/unsolicited or concomitant medications) will be treated as subjects without symptoms (solicited/unsolicited or concomitant medications, respectively). A sensitivity analysis will be performed on documented doses i.e. accounting only for doses where solicited symptoms are indicated as being absent/present in case more than 5% of the doses are not documented.

6.4.2.1. Solicited Adverse Events

Summary of temperature will be provided by 0.5° increment (i.e. ≥ 38.0°C, >38.5; > 39, > 39.5, > 40). Fever, defined as a body temperature of ≥38°C irrespective of route of measurement, will be integrated to the summaries as a general symptom.

7. ANALYSIS INTERPRETATION

Except for analyses addressing criteria specified in the primary objective, all the analyses will be descriptive/exploratory in nature. The use of these descriptive/exploratory analyses should be limited to support the confirmatory analyses or to generate hypothesis.

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

Description	Analysis ID	Disclosure Purpose (CTRS=public posting, SR=study report, internal)	Dry run review needed (Y/N)	*Study Headline Summary (SHS)*requiring expedited communication to upper management (Yes/No)	Reference for TFL
Final	E1_01	SR	Yes	Yes	All tables from TFL TOC

8.2. Statistical considerations for interim analyses

Not applicable

9. CHANGES FROM PLANNED ANALYSES

Not applicable

10. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES (TFL)

The TFL Table Of Content (TOC) is as separate document. It provides the list of tables/listings and figures needed for the study report, and It also identifies the tables eligible for each analysis and their role (synopsis, in-text, post-text, SHS, CTRS).

The following group names will be used in the TFLs, to be in line with the T-domains:

Group order in tables	Group label in tables	Group definition for footnote
1	HRV Liq	HRV liquid vaccine
2	HRV Lyo	HRV lyophilised vaccine

11. ANNEX 1 STANDARD DATA DERIVATION RULE AND STATISTICAL METHODS

11.1. Statistical Method References

The exact two-sided 95% CIs for a proportion within a group will be the Clopper-Pearson exact CI [Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of binomial. *Biometrika*. [1934;26:404-413].

The standardised asymptotic two-sided 95% CI for the group difference in proportions is based on the method described in the following paper:Robert G. Newcombe, interval estimation for the difference between independent proportions: comparison of eleven methods, *Statist Med.* 1998; 17, 873-890]. The standardised asymptotic method used is the method six.

The GMC group ratio will be obtained using an ANOVA model on the logarithm-transformed concentrations. The ANOVA model will include the vaccine group. The GMC ratio and their 95% CI will be derived by exponential transformation of the corresponding group contrast in the model.

11.2. Standard data derivation

11.2.1. Date derivation

- SAS date derived from a character date: in case day is missing, 15 is used. In case day & month are missing, 30June is used.
- Onset day for an event (AE, medication, vaccination, …): the onset day is the number of days between the last study vaccination & the onset/start date of the event. This is 1 for an event starting on the same day as a vaccination. See SAS date derived in case the start date of the event is incomplete.
- Duration: Duration of an event is expressed in days. It is the number of days between the start & the stop dates + 1. Therefore duration is 1 day for an event starting & ending on the same day.

11.2.2. Dose number

- The study dose number is defined in reference to the number of study visits at which vaccination occurred. More specifically dose 1 refers to all vaccines administered at the first vaccination visit while dose 2 corresponds to all vaccinations administered at the second vaccination visit even if this is the first time a product is administered to the subject.
- Relative dose: the relative dose for an event (AE, medication, vaccination) is the most recent study dose given before an event. In case the event takes place on the day a study dose is given, the related dose will be that of the study dose, even if the event actually took place before vaccination. For instance, if an adverse event begins on the day of the study vaccination but prior to administration of the vaccine, it will be assigned to this dose. In case a study dose is not administered and an event occurs after the subsequent study dose (eg 3rd study dose), the relative dose of the event will be study dose associated to the subsequent study dose (eg dose 3).
- The number of doses for a product is the number of time the product was administered to a subject.
- The incidence per dose is the number of vaccination visits at which an event was reported among all vaccination visits.

11.2.3. Demography

- Age: Age at the reference activity, computed as the number of complete weeks between the date of birth and the reference activity.

11.2.4. Immunogenicity

- For a given subject and given immunogenicity measurement, missing or non-evaluable measurements will not be replaced.

  The GMCs calculations are performed by taking the anti-log of the mean of the log concentration transformations. Antibody concentrations below the cut-off of the assay will be given an arbitrary value of half the cut-off of the assay for the purpose of GMC calculation.
- In general, the assay cut-off is the value under which there is no quantifiable result available. For an assay with a specific 'assay cut_off', numerical immuno result is derived from a character field (rawres):
  - If rawres is 'NEG' or '-' or '(-)', numeric result= assay cut_off/2,
  - if rawres is 'POS' or '+' or '(+)', numeric result = assay cut_off,
  - if rawres is '< value' and value<=assay cut_off, numeric result =assay cut_off/2,
  - if rawres is '< value' and value>assay cut_off, numeric result =value,
  - if rawres is '> value' and value<assay cut_off, numeric result =assay cut_off/2,
  - if rawres is '> value' and value>=assay cut_off, numeric result =value,
  - if rawres is '<= value' or '>= value' and value<assay cut_off, numeric result =assay cut_off/2,

- if rawres is '<= value' or '>= value' and value>=assay cut_off, numeric result =value,
- if rawres is a value < assay cut_off, numeric result = assay cut_off/2,
- if rawres is a value >= assay cut_off, numeric result = rawres,
- else numeric result is left blank.

11.2.5. Safety

- For analysis of solicited, unsolicited adverse events (such as serious adverse events or adverse events by primary MedDRA term) and for the analysis of concomitant medications, all vaccinated subjects will be considered. Subjects who did not report the event or the concomitant medication will be considered as subjects without the event or the concomitant medication respectively.
- In case there will be more than 5% of subjects without documented dose for solicited symptoms (i.e., symptom screen not completed), sensitivity analysis will include only vaccinated subjects for doses with documented safety data (i.e., symptom screen completed).
- The following rules will be used for the analysis of solicited symptoms:
  - Subject who didn't document the presence or absence of a solicited symptom after one dose will be considered not having that symptom after that dose in the analysis done on "administrated dose"
  - Subjects who documented the absence of a solicited symptom after one dose will be considered not having that symptom after that dose.
  - Subjects who documented the presence of a solicited symptom and fully or partially recorded daily measurement over the solicited period will be included in the summaries at that dose and classified according to their maximum observed daily recording over the solicited period.
  - Subjects who documented the presence of a solicited symptom after one dose without having recorded any daily measurement will be considered as having that symptom after that dose).

- Intensity of the following solicited AEs will be assessed as described in Table 5 and Table 6

Table 5 Intensity scales to be used by the parent(s)/LAR(s) for solicited symptoms during the solicited follow-up period

Adverse Event	Intensity grade	Parameter
Fever*		Record temperature in °C/°F using any age-appropriate route.
Irritability/Fussiness	0	Behavior as usual
	1	Crying more than usual/no effect on normal activity
	2	Crying more than usual/interferes with normal activity
	3	Crying that cannot be comforted/prevents normal activity
Diarrhea$		Record the number of looser than normal stools/day
Vomiting§		Record the number of vomiting episodes/day
Loss of appetite	0	Appetite as usual
	1	Eating less than usual/no effect on normal activity
	2	Eating less than usual/interferes with normal activity
	3	Not eating at all
Cough/runny nose	0	Normal
	1	Cough/runny nose which is easily tolerated
	2	Cough/runny nose which interferes with daily activity
	3	Cough/runny nose which prevents daily activity

*Fever is defined as temperature ≥ 38.0°C/100.4°F. The preferred location for measuring temperature in this study will be the oral cavity, the axilla and the rectum.
$ Diarrhea is defined as passage of three or more looser than normal stools within a day.
§ Vomiting is defined as one or more episodes of forceful emptying of partially digested stomach contents ≥1 hour after feeding within a day.

Table 6 Intensity scales for diarrhea, vomiting and fever occurring during the solicited period

Adverse Event	Intensity grade	Parameter
Diarrhea $	0	Normal (0-2 looser than normal stools/day)
	1	3 looser than normal stools/day
	2	4-5 looser than normal stools/day
	3	≥ 6 looser than normal stools/day
Vomiting §	0	Normal (no emesis)
	1	1 episode of vomiting/day
	2	2 episodes of vomiting/day
	3	≥ 3 episodes of vomiting/day
Fever	0	temperature < 38.0°C/100.4° F
	1	temperature ≥ 38.0°C/100.4° F – ≤ 38.5°C/101.3 F
	2	temperature > 38.5°C/101.3 F – ≤ 39.5°C/103.1 F
	3	temperature > 39.5°C/103.1 F

$ Diarrhea is defined as passage of three or more looser than normal stools within a day.
§ Vomiting is defined as one or more episodes of forceful emptying of partially digested stomach contents ≥1 hour after feeding within a day.

- Note that for all tables described in this section, the way the percentage of subjects will be derived will depend on the event analysed (see table below for details). As a result, the N value will differ from one table to another.

Event	N used for deriving % per subject for Vaccination phase	N used for deriving % per dose for Vaccination phase
Concomitant vaccination	All subjects with study vaccine administered	All study visits with study vaccine administered
Solicited general symptom	Primary analysis: all subjects with study vaccine administered Sensitivity analysis as applicable, all subjects with at least one solicited general symptom documented as either present or absent (i.e., symptom screen completed)	Primary analysis: all study visits with study vaccine administered Sensitivity analysis: all study visits with study vaccine administered and with at least one solicited general symptom documented as either present or absent (i.e., symptom screen completed)
Unsolicited symptom	All subjects with study vaccine administered	All study visits with study vaccine administered
Concomitant medication	All subjects with study vaccine administered	All study visits with study vaccine administered

For summaries by MedDRA primary preferred term combining solicited and unsolicited adverse events, solicited adverse events will be coded as per the following MedDRA codes

Solicited symptom	Lower level term code	Corresponding Lower level term decode
Fever	10016558	Fever
Irritability/Fussiness	10022998	Irritability
Diarrhoea	10012727	Diarrhoea
Vomiting	10047700	Vomiting
Loss of appetite	10003028	Appetite lost
Cough/runny nose	10011224	Cough

11.2.6. Data presentation description

The following decimal description from the decision rules will be used for the demography, immunogenicity and safety/reactogenicity.

Display Table	Parameters	Number of decimal digits
All summaries	% of count, including LL & UL of CI	1
Demographic characteristics	Mean, median, SD	1
Immunogenicity	% of difference, including LL & UL of CI	2
Immunogenicity	GMC group ratio, including LL & UL of CI	2
Immunogenicity	Anti-RV IgA GMC	1