Clinical Study Protocol
Sponsor:
GlaxoSmithKline Biologicals SA
Rue de l'Institut 89
1330 Rixensart, Belgium

Primary Study vaccine and number	Liquid formulation of GlaxoSmithKline (GSK) Biologicals' oral live attenuated human rotavirus (HRV) vaccine, *Rotarix* (444563)
Other study vaccine	Lyophilized formulation of GSK Biologicals' oral live attenuated HRV vaccine (444563)
eTrack study number and Abbreviated Title	116566 (ROTA-083)
EudraCT number	2012-001875-35
Date of protocol	Final Version 1: 11 April 2012
Date of protocol amendments	Amendment 1 Final: 24 July 2013 Amendment 2 Final: 19 September 2016 Amendment 3 Final: 31 October 2017 ***Amendment 4: Final: 30 October 2019***
Title	Immunogenicity and safety study of two different formulations of GlaxoSmithKline (GSK) Biologicals' oral live attenuated human rotavirus (HRV) vaccine, *Rotarix* in healthy infants.
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.
Co-ordinating author	PPD
Contributing authors (Amended: 30 October 2019)	• PPD, Clinical & Epidemiology Project Lead (CEPL) • PPD, PPD, Clinical Research and Development Leads (CRDL) • PPD, PPD, Statisticians • PPD, PPD, Lead Statisticians • PPD, PPD, PPD, PPD, Study Delivery Leads

eTrack study number and Abbreviated Title	116566 (ROTA-083)
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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.
Contributing authors **(Amended: 30 October 2019) (Continued…)**	• PPD, PPD**,** Project Delivery Leads • PPD, PPD, PPD, PPD**,** Clinical Laboratory Sciences (CLS), Clinical Readout Team Leaders • PPD, PPD, Laboratory Study Managers • PPD, PPD, PPD, Clinical Safety representatives • PPD, PPD, Oversight Data Managers • PPD, PPD, PPD**,** Study Data Managers • PPD, Global Regulatory Affairs representative • PPD, PPD, Global Patents representatives • PPD, PPD, PPD, PPD, PPD, PPD, Clinical Trial Supply Managers • PPD, PPD**,** Local Medical Leads • PPD, PPD**,** Local Delivery Leads

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Protocol Amendment 4 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	116566 (ROTA-083)
EudraCT number	2012-001875-35
Date of protocol amendment	Amendment 4 Final: 30 October 2019
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.
Sponsor signatory	Paul Gillard, MD Clinical & Epidemiology Project Lead, Live Viral Vaccines, RDC Belgium GlaxoSmithKline Biologicals, SA.
Signature	
Date	

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Protocol Amendment 4 Rationale

Amendment number: Amendment 4

Rationale/background for changes:

As per Protocol Amendment 3 (31 October 2017), subjects seropositive before vaccination are to be eliminated from the Per-Protocol Set (PPS) for immunogenicity.

Laboratory testing of the pre-vaccination blood samples from the first 141 subjects enrolled in the study indicated a seropositivity rate of 47.5 % in these subjects. This suggests an overall high seropositivity rate at baseline in the total study population.

Therefore, an elimination rate exceeding the 35% rate of non-evaluable subjects predefined using historical data from Rota-044 study [Narang, 2009] is expected.

Because (1) the pre-vaccination seropositivity rate is expected to be high and (2) the effect of the vaccine can be measured in seropositive subjects, as previously demonstrated (see Table 1 below), the following changes have been made to the protocol:

- The PPS was modified to include subjects seropositive at baseline.
- The statistical method to derive the 95% confidence interval (CI) for the geometric mean concentration (GMC) group ratio was revised to be an ANCOVA.
- The seroconversion threshold was redefined to account for seropositive subjects at pre-vaccination:
  - for subjects with a pre-vaccination anti-rotavirus 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-rotavirus IgA antibody concentration ≥20 U/mL, seroconversion is achieved when the post-vaccination concentration is ≥2 times the pre-vaccination concentration.

Table 1: Distribution of fold increase in anti-rotavirus IgA antibody concentrations from pre-vaccination to one month post-dose 2 (Total vaccinated cohort for seropositive subjects at pre-vaccination, Rota-044) [Narang, 2009]

	HRV N = 43		Placebo N = 40	
Ratio	n	%	n	%
≥2	26	60.5	3	7.5
≥3	21	48.8	2	5.0
≥4	17	39.5	2	5.0

N = number of subjects seropositive at baseline for anti-rotavirus IgA antibodies and with one month post-dose 2 data available; n/% = number/percentage of subject in a given category.

Protocol Amendment 4 Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any future protocol amendments or protocol administrative changes, with the terms of the clinical trial agreement and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline (GSK) Biologicals.
- To assume responsibility for the proper conduct of the study at this site.
- That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the GSK Biologicals' study vaccines and other study-related duties and functions as described in the protocol.
- To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.
- To ensure that no clinical samples (including serum samples) are retained onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject and/or the subject's legally acceptable representative.
- To perform no other biological assays on the clinical samples except those described in the protocol or its amendment(s).
- To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.
- That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the sponsor or the investigational vaccines, and more generally about his/her financial ties with the sponsor. GSK Biologicals will use and disclose the information solely for the purpose of complying with regulatory requirements.

Hence I:

- Agree to supply GSK Biologicals with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children).
- Agree to promptly update this information if any relevant changes occur during the course of the study and for one year following completion of the study.
- Agree that GSK Biologicals may disclose any information it has about such ownership interests and financial ties to regulatory authorities.
- Agree to provide GSK Biologicals with an updated Curriculum Vitae and other documents required by regulatory agencies for this study.

eTrack study number and Abbreviated Title	116566 (ROTA-083)
EudraCT number	2012-001875-35
Date of protocol amendment	Amendment 4 Final: 30 October 2019
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.
Investigator name	_____
Signature	_____
Date	_____

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Sponsor Information

1. Sponsor

GlaxoSmithKline Biologicals SA
Rue de l'Institute 89, 1330 Rixensart, Belgium.

2. Sponsor Medical Expert for the Study

Refer to the local study contact information document.

3. Sponsor Study Monitor

Refer to the local study contact information document.

4. Sponsor Study Contact for Reporting of a Serious Adverse Event

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to protocol Section 8.3.2.

SYNOPSIS

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.
Indication	Active immunization of infants against gastroenteritis (GE) due to rotavirus (RV).
Rationale for the study and study design	The lyophilized formulation of the HRV vaccine was licensed in India in February 2008**.** Following the request of the Regulatory Authority in India, this study will be conducted to generate additional clinical data for the liquid formulation of the HRV vaccine in India, as recommended by New Drug Advisory Committee on Vaccines (NDAC-Vaccines) of Drug Controller General of India (DCGI). This study will be conducted to evaluate the immunogenicity, reactogenicity and safety of GSK Biologicals' HRV liquid vaccine compared to GSK Biologicals' HRV lyophilized vaccine. The study is planned to be conducted in healthy infants aged 6-10 weeks at the time of the first dose. Two oral doses of the study vaccine will be administered to the subjects, according to a 0, 1 month schedule. The blood samples for immunogenicity assessment will be collected from all subjects before the first dose and at one month post dose 2.
Objectives **(Amended: 30 October 2019)**	**Primary** 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.*

Secondary

- 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.

  ***Definition**:

  - ***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.

Study design

- 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. Please refer to GLOSSARY OF TERMS for the definition of EoS.
- Study groups:

The study groups and epoch foreseen in the study are provided in Synopsis Table 1.

The study groups and treatment foreseen in the study are provided in Synopsis Table 2.

Synopsis 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	●

Synopsis 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 (HRV lyophilized vaccine)
- **Vaccination schedule**:
  - Two oral doses of the HRV vaccine are 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. The 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 allocation using GSK Biologicals' Randomization System on Internet (SBIR).
- **Blinding**: open

The blinding of study epoch is provided in Synopsis Table 3.

Synopsis Table 3 Blinding of study epoch

Study Epoch	Blinding
Epoch 001	open

- **Sampling schedule**: Details of the samples to be collected are as follows:
  - Blood samples 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

Number of subjects

The target will be to enrol 450 subjects who will be randomly assigned to one of the two study groups in a 1:1 ratio, to obtain at least 292 evaluable subjects (approximately 146 subjects in each group).

Endpoints

(Amended: 30 October 2019)

Primary

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

Secondary

- Anti-RV IgA antibody concentrations
  - Anti-RV IgA antibody seroconversion* rate, one month post dose 2 of HRV vaccine.

Definition:

- ***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.***

- 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 lyophilized vaccine and HRV liquid vaccine.
- Unsolicited adverse events
  - Occurrence of unsolicited AEs within 31 days (Day 1- Day 31) after any dose of HRV lyophilized vaccine and HRV liquid vaccine according to Medical Dictionary for Regulatory Activities (MedDRA) classification.

- Serious adverse events
  - Occurrence of SAEs from dose 1 of HRV lyophilized vaccine and HRV liquid vaccine up to study end.

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LIST OF ABBREVIATIONS

AE:	Adverse Event
ATP:	According-To-Protocol
$CCID_{50}$:	Median Cell Culture Infective Dose (quantity of virus causing infection in 50% of exposed cells)
CDISC:	Clinical Data Interchange Standards Consortium
CI:	Confidence Interval
CLS:	Clinical Laboratory Sciences
cm:	centimeter
DCGI:	Drug Controller General of India
DMEM:	Dulbecco's Modified Eagle Medium
eCRF:	electronic Case Report Form
ELISA:	Enzyme Linked Immunosorbent Assay
EoS:	End of Study
ES:	Exposed Set
eTDF:	electronic Temperature Excursion Decision Form
GCP:	Good Clinical Practice
GE:	Gastroenteritis
GMC:	Geometric Mean Concentration
GSK:	GlaxoSmithKline
HRV:	Human Rotavirus
IB:	Investigator Brochure
ICF:	Informed Consent Form
ICH:	International Conference on Harmonization
IEC:	Independent Ethics Committee
IgA:	Immunoglobulin A

IMP:	Investigational Medicinal Product
IRB:	Institutional Review Board
IS:	Intussusception
Kg:	Kilograms
LAR:	Legally Acceptable Representative
LSLV:	Last Subject Last Visit
m:	meter
MATEX:	MATerial EXcellence
MedDRA:	Medical Dictionary for Regulatory Activities
mg:	Milligrams
mL:	Milliliter
NDAC:	New Drug Advisory Committee
PCD:	Primary Completion Date
PPS:	Per-Protocol Set
RCC:	Reverse Cumulative Curve
RT-PCR:	Reverse Transcription Polymerase Chain reaction
RV:	Rotavirus
SAE:	Serious Adverse Event
SAS:	Statistical Analysis System
SBIR:	Randomization System on Internet
SCID:	Severe Combined Immunodeficiency
SmPC:	Summary of Product Characteristics
SPM:	Study Procedures Manual
U:	Unit
WHO:	World Health Organization

GLOSSARY OF TERMS

Adverse event:	Any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. An Adverse Event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e., lack of efficacy), abuse or misuse.
Blinding:	A procedure in which one or more parties to the trial are kept unaware of the treatment assignment in order to reduce the risk of biased study outcomes. The level of blinding is maintained throughout the conduct of the trial, and only when the data are cleaned to an acceptable level of quality will appropriate personnel be unblinded or when required in case of a Serious Adverse Event (SAE). In an open-label study, no blind is used. Both the investigator and the subject know the identity of the treatment assigned.
Child in care:	A child who has been placed under the control or protection of an agency, organization, institution or entity by the courts, the government or a government body, acting in accordance with powers conferred on them by law or regulation. The definition of a child in care can include a child cared for by foster parents or living in a care home or institution, provided that the arrangement falls within the definition above. The definition of a child in care does not include a child who is adopted or has an appointed legal guardian.
Diarrhea:	Passage of three or more looser than normal stools within a day.
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.

End of Study (EoS): **(Synonym of End of Trial)**	For studies without collection of human biologicals samples or imaging data EoS is the Last Subject Last Visit (LSLV). For studies with collection of Human Biologicals Samples or imaging data, EoS is defined as the date of the last testing/reading released of the Human Biological Samples or imaging data, related to primary and secondary endpoints. EoS must be achieved no later than 8 months after LSLV.
Epoch:	An epoch is a set of consecutive time points or a single time point from a single protocol. Epochs are defined to support a main purpose which is either to draw conclusions on subject participation or to draw a complete conclusion to define or precise the targeted label of the product. Supporting means that data collected at the time points included in an epoch must be sufficient to fulfil the purpose of the epoch. Typical examples of epochs are screening, primary vaccinations, boosters, yearly immunogenicity follow-ups, and surveillance periods for efficacy or safety.
eTrack:	GSK's tracking tool for clinical trials.
Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, and, therefore, included in the per-protocol analysis (see Sections 6.7.2 and 10.4 for details on criteria for evaluability).
Gastroenteritis:	Diarrhea with or without vomiting.
Immunological correlate of protection:	The defined immune response above which there is a high likelihood of protection in the absence of any host factors that might increase susceptibility to the infectious agent.
Investigational vaccine: **(Synonym of Investigational Medicinal Product)**	A pharmaceutical form of an active ingredient being tested in a clinical trial, including a product with a marketing authorisation when used in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.

Legally acceptable representative (LAR): **(The terms legal representative or legally authorized representative are used in some settings.)**	An individual or juridical or other body authorized under applicable law to consent, on behalf of a prospective subject, to the subject's participation in the clinical trial.
Primary completion date:	The date that the final subject was examined or received an intervention for the purpose of final collection of data for all primary outcomes, whether the clinical trial was concluded according to the pre-specified protocol or was terminated.
Protocol amendment:	The International Conference on Harmonization (ICH) defines a protocol amendment as: 'A written description of a change(s) to or formal clarification of a protocol.' GSK Biologicals further details this to include a change to an approved protocol that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study.
Randomization:	Process of random attribution of treatment to subjects in order to reduce bias of selection.
Self-contained study:	Study with objectives not linked to the data of another study.
Site monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of clinical studies at one or more investigational sites.
Seroconversion: (Amended: 30 October 2019)	***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.***
Seronegative:	A seronegative subject for anti-rotavirus IgA antibody is defined as a subject who has an antibody concentration below the clinically meaningful threshold of 20 U/ml.

Seropositive:	A seropositive subject is defined as a subject who has an antibody concentration greater than or equal to the clinically meaningful threshold of 20 U/ml.
Solicited adverse event:	AEs to be recorded as endpoints in the clinical study. The presence/occurrence/intensity of these events is actively solicited from the subject or an observer during a specified post-vaccination follow-up period.
Study vaccine:	Any investigational vaccine/product being tested and/or any authorized use of a vaccine/product/placebo as a reference or administered concomitantly, in a clinical trial that evaluates the use of an investigational vaccine/product.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the clinical study, either as a recipient of the vaccine(s)/product(s) or as a control.
Subject number	A unique number identifying a subject, assigned to each subject consenting to participate in the study.
Treatment:	Term used throughout the clinical study to denote a set of investigational product(s) or marketed product(s) or placebo intended to be administered to a subject.
Treatment number:	A number identifying a treatment to a subject, according to the treatment allocation.
Unsolicited adverse event:	Any AE reported in addition to those solicited during the clinical study. Also any 'solicited' symptom with onset outside the specified period of follow-up for solicited symptoms will be reported as an unsolicited AE.
Vomiting:	One or more episodes of forceful emptying of partially digested stomach contents ≥1 hour after feeding within a day.

TRADEMARK

The following trademark is used in the present protocol.

Note: In the body of the protocol (including the synopsis), the name of the vaccine will be written without the superscript symbol ™ or ® and in *italics*.

Trademarks of the GSK group of companies	Generic description
Rotarix	Human rotavirus vaccine

1. INTRODUCTION

1.1. Background (Amended: 30 October 2019)

Rotavirus (RV) infection is the leading cause of acute Gastroenteritis (GE) and severe diarrhea in infants and young children <5 years of age [Atherly, 2009]. It has been estimated that in 2013, approximately 215,000 deaths were caused due to RV. India, Nigeria, Pakistan, and Democratic Republic of Congo accounted for approximately half (49%) of all the estimated RV deaths in 2013 [Tate, 2016].

Hospital-based surveillance studies in Asia have shown that 20% to 50% of the hospitalizations for diarrhea among children <5 years of age, are associated with RV infection [Kang, 2006]. In 2013, an estimated 47,100 RV deaths occurred in India, which was 22% of all deaths that occurred globally due to RV in the same year [Tate, 2016].

In 2013, WHO recommended inclusion of RV vaccination in the national immunization programs of all countries, particularly in countries where RV GE associated fatality rates are high among children aged <5 years (e.g., south and south-eastern Asia and sub-Saharan Africa) [WHO position paper, 2013].

Rotarix is ***currently*** registered in ***over*** 130 countries and about ***552 446 066*** doses of the vaccine (lyophilized and liquid formulations) are estimated to have been distributed worldwide since its launch until July ***2019***.

GSK Biologicals' lyophilized Human Rotavirus (HRV) vaccine has been extensively tested in clinical studies in infants and the vaccine efficacy has been demonstrated in several clinical studies conducted in Europe, North America, Latin America and the Caribbean, Asia and Africa [Vesikari, 2007; Linhares, 2008; Salinas, 2005; Phua, 2009; Madhi, 2010]

Please refer to the current Investigator Brochure (IB) for information regarding the summary of potential risks and benefits of the *Rotarix* vaccine.

1.2. Rationale for the study and study design

The lyophilized formulation of the HRV vaccine was licensed in India in February 2008. This study will be conducted to generate additional clinical data for the liquid formulation of the HRV vaccine in India, as recommended by New Drug Advisory Committee on Vaccines (NDAC-Vaccines) of Drug Controller General of India (DCGI).

This study will be conducted to evaluate the immunogenicity, reactogenicity and safety of the GSK Biologicals' HRV liquid vaccine compared to the GSK Biologicals' HRV lyophilized vaccine. The study is planned to be conducted in healthy infants aged 6-10 weeks at the time of the first dose. Two oral doses of the study vaccine will be administered to the subjects, according to a 0, 1 month schedule. The blood samples for immunogenicity assessment will be collected from all subjects before the first dose and at one month after the second dose of the vaccine.

1.3. Benefit: Risk Assessment

Please refer to the current IB and prescribing information (lyophilized vaccine) for information regarding the summary of potential risks and benefits of the *Rotarix* vaccine.

The following section outlines the risk assessment and mitigation strategy for this study protocol:

1.3.1. Risk Assessment

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Investigational study vaccine (*Rotarix*)		
Intussusception (IS)	Spontaneous data	• Subjects will be followed up to the end of the study after the receipt of the vaccine to check for any safety signal. • Parents/ Legally acceptable representatives (LARs) of subjects should report any untoward symptoms experienced after receiving the vaccine immediately to the investigator. • All Serious Adverse Events (SAEs) should be reported by the investigator immediately to GSK. • Subjects with SCID will be excluded from participating in this study (Refer to Section 4.3 and Section 6 for more details).
Hematochezia	Spontaneous data	
Gastroenteritis with vaccine viral shedding in infants with severe combined immunodeficiency (SCID)	Spontaneous data	
Kawasaki disease	Based on signal observed for *RotaTeq* vaccine	
Study Procedures		
Allergic reaction to the vaccine	Spontaneous data	Subjects will be observed for at least 30 minutes after the vaccine administration, with medical attention available in case of anaphylactic reactions.

1.3.2. Benefit Assessment

By receiving the HRV vaccine, the subject may become protected against the RV disease. In addition, the subject's participation will benefit other children in the future, since information collected during this study will help in the evaluation of the safety, reactogenicity and immunogenicity of the HRV liquid vaccine.

In addition, the subject will undergo a physical examination at the first study visit. In case the study doctor discovers any medical condition, the subject will be referred to the local healthcare system.

1.3.3. Overall Benefit: Risk Conclusion

Considering the measures taken to minimize the risk to subjects participating in this study, the potential or identified risks in association with [HRV vaccine] are justified by the potential benefits (prevention/treatment) that may be afforded to subjects receiving the vaccine for immunization against RV.

2. OBJECTIVES

2.1. 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 10.1 for the definition of the primary endpoint.

2.2. Secondary objectives (Amended: 30 October 2019)

- 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**.**

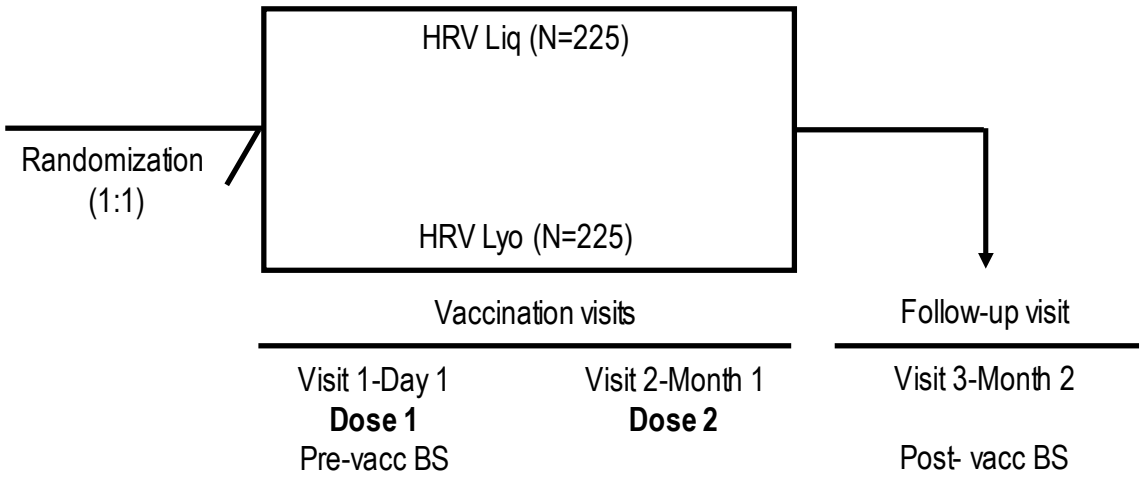
  ***Definition**:

  - ***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 10.2 for the definition of the secondary endpoints.

3. STUDY DESIGN OVERVIEW



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), 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.

  Refer to glossary of terms for the definition of PCD.
- **End of Study (EoS)**: Last testing results released for samples collected at Visit 3.

  Refer to glossary of terms for the definition of EoS.
- **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.

4. STUDY COHORT

4.1. Number of subjects/centers

The target enrolment will be 450 subjects (225 subjects in each study group) to obtain at least 292 evaluable subjects (146 subjects in each study group). Refer to Section 10.3 for the detailed description of the criteria used in the estimation of the sample size.

Overview of recruitment plan:

- The study will be conducted in multiple centers across India.
- Enrolment will be terminated when 450 subjects have been enrolled.
- The recruitment and randomization will be monitored by SBIR.

4.2. Inclusion criteria for enrolment

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

All subjects must satisfy ALL the following criteria at study entry:

- Subjects' parent(s)/LAR(s) who, in the opinion of the investigator, can and will comply, with the requirements of the protocol (e.g. completion of the diary cards, return for follow-up visits).
- Written informed consent obtained from the parent(s)/LAR(s) of the subject prior to performing any study specific procedure.
- A male or female between, and including, 6 and 10 weeks of age at the time of the first vaccination.
- Healthy subjects as established by medical history and clinical examination before entering into the study.
- Birth weight >2000 grams.

4.3. Exclusion criteria for enrolment

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the subject must not be included in the study:

- Child in care
  Please refer to the glossary of terms for the definition of child in care.
- Use of any investigational or non-registered product (drug or vaccine) other than the study vaccines during the period starting 30 days before the first dose of study vaccines) (Day-29 to Day 1), or planned use during the study period.
- Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs since birth. For corticosteroids, this will mean prednisone 0.5 mg/kg/day, or equivalent. Inhaled and topical steroids are allowed.
- Administration of any chronic drug therapy to be continued during the study period.
- Planned administration/administration of a vaccine not foreseen by the study protocol in the period starting 30 days before the first dose of vaccine administration 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, according to the local immunization practice.
- Concurrently participating in another clinical study, at any time during the study period, in which the subject has been or will be exposed to an investigational or a non-investigational product (pharmaceutical product or device).
- History of confirmed RV GE.
- Previous vaccination against RV.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, (including Severe Combined Immunodeficiency [SCID] disorder) based on medical history and physical examination (no laboratory testing required).
- Uncorrected congenital malformation (such as Meckel's diverticulum) of the gastrointestinal tract that would predispose for Intussusception (IS).
- History of IS.
- Very prematurely born infants (born ≤28 weeks of gestation).
- Hypersensitivity to latex.
- Family history of congenital or hereditary immunodeficiency.
- History of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccine.
- Major congenital defects or serious chronic illness.
- History of any neurological disorders or seizures.

- Acute disease and/or fever at the time of enrolment. This warrants deferral of vaccination.
  - 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 or the rectum.
  - Subjects with a minor illness (such as mild diarrhea, mild upper respiratory infection) without fever, may be enrolled at the discretion of the investigator.
- Acute or chronic, clinically significant pulmonary, cardiovascular, hepatic or renal functional abnormality, as determined by physical examination or medical history.
- Administration of immunoglobulins and/or any blood products since birth or planned administration during the study period.
- Administration of long-acting immune-modifying drugs at any time during the study period (e.g., infliximab).
- GE within 7 days preceding the study vaccine administration (warrants deferral of the vaccination).

5. CONDUCT OF THE STUDY

5.1. Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with the International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (GCP), all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

The study has been designed and will be conducted in accordance with the ICH Harmonized Tripartite Guideline for clinical investigation of medicinal products in the paediatric population (ICH E11) and all other applicable ethical guidelines.

GSK will obtain favourable opinion/approval to conduct the study from the appropriate regulatory agency, in accordance with applicable regulatory requirements, prior to a site initiating the study in that country.

Conduct of the study includes, but is not limited to, the following:

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favourable opinion/approval of study protocol and any subsequent amendments.
- Subject's parent(s)/LAR(s) informed consent.
- Investigator reporting requirements as stated in the protocol.

GSK will provide full details of the above procedures to the investigator, either verbally, in writing, or both.

Freely given and written or witnessed/thumb printed informed consent must be obtained from each subject's parent(s)/LAR(s) as appropriate, prior to participation in the study.

GSK Biologicals will prepare a model Informed Consent Form (ICF) which will embody the ICH GCP and GSK Biologicals required elements. While it is strongly recommended that this model ICF is to be followed as closely as possible, the informed consent requirements given in this document are not intended to pre-empt any local regulations which require additional information to be disclosed for informed consent to be legally effective. Clinical judgement, local regulations and requirements should guide the final structure and content of the local version of the ICF.

5.2. Subject identification and randomization

5.2.1. Subject identification

Subject identification numbers will be assigned sequentially to the subjects whose parent(s)/LAR(s) have consented to their participation in the study, according to the range of subject identification numbers allocated to each study center.

5.2.2. Randomization of treatment

5.2.2.1. Randomization of supplies

The numbering of supplies will be performed at GSK Biologicals, using a block scheme randomization in MATerial EXcellence (MATEX), a program developed for use in Statistical Analysis System (SAS®) (Cary, NC, US) by GSK Biologicals. Entire blocks will be shipped to the study center(s)/warehouse(s).

To allow GSK Biologicals to take advantage of greater rates of recruitment than anticipated at individual centers in this multi-center study and to thus reduce the overall study recruitment period, additional supplies will be prepared.

5.2.2.2. Treatment allocation to the subject

The treatment numbers will be allocated by dose.

5.2.2.2.1. Study group and treatment number allocation

The target will be to enrol 450 subjects who will be randomly assigned to one of the two study groups in a (1:1) ratio (225 subjects in each group).

Allocation of the subject to a study group at the investigator site will be performed using SBIR. The randomization algorithm will use a minimization procedure accounting for center and the study as minimization factors. Minimization factors will have equal weight in the minimization algorithm.

After obtaining the signed and dated ICF from the subject's parent/LAR and having checked the eligibility of the subject, the site staff in charge of the vaccine administration will access SBIR. Upon providing the subject identification number, SBIR will provide the treatment number to be used for the first dose.

The number of each administered treatment must be recorded in the eCRF on the Vaccine Administration screen.

When SBIR is not available, please refer to the SBIR user guide or the Study Procedures Manual (SPM) for specific instructions.

5.2.2.2.2. Treatment number allocation for subsequent doses

For the second dose, the study staff in charge of the vaccine administration will access SBIR, provide the subject identification number, and the system will provide a treatment number consistent with the allocated study group.

The number of each administered treatment must be recorded in the eCRF on the Vaccine Administration screen.

5.3. Method of blinding

This is an open-label study.

The laboratory in charge of the laboratory testing will be blinded to the treatment, and codes will be used to link the subject and study (without any link to the treatment attributed to the subject) to each sample.

5.4. General study aspects

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying SPM. The SPM provides the investigator and the site personnel with administrative and detailed technical information that does not impact the safety of the subjects.

5.5. Outline of study procedures

The list of study procedures is detailed in Table 4.

Table 4 List of study procedures

Age at enrolment	6-10 weeks		
Epoch	Epoch 001		
Type of contact	Visit 1	Visit 2	Visit 3
Time points	Day 1	Month 1	Month 2
Sampling time points	Pre-Vacc		Post-Vacc
Informed consent	●		
Check inclusion/exclusion criteria	●		
Collect demographic data	●		
Check contraindications and warnings and precautions	●	●	
Medical history	●		
Physical examination	●		
History of previous vaccination from birth	●		
Pre-vaccination body temperature	●	●	
Vaccines			
Study group and treatment number allocation	O		
Treatment number allocation for subsequent doses		O	
Recording of administered treatment number	●	●	
HRV vaccine administration	●	●	
Record regurgitation	●	●	
HRV vaccine replacement dose administration in case of regurgitation *	●	●	
Laboratory Assays			
Blood sampling for antibody determination (~2 ml)	●		●
Safety assessments			
Distribution of diary cards	O	O	
Recording of solicited adverse events within 8 days after each dose of HRV vaccine (Day 1–Day 8)	●	●	
Recording of non-serious adverse events within 31 days (Day 1–Day 31) post-vaccination	●	●	●
Record any concomitant medication/vaccination	●	●	●
Record any intercurrent medical conditions	●	●	●
Recording of AEs/SAEs leading to withdrawal from study	●	●	●
Recording of SAEs	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●	●
Return of diary cards		O	O
Diary card transcription by investigator		●	●
Study Conclusion			●

● is used to indicate a study procedure that requires documentation in the individual eCRF.
○ is used to indicate a study procedure that does not require documentation in the individual eCRF.
LAR = Legally Acceptable Representative; AE = Adverse Events; SAE = Serious Adverse Events
* If regurgitation or vomiting occurs after vaccination, a single replacement dose may be given at the same vaccination visit at the discretion of the Investigator.
Note: All subjects are allowed to receive routine childhood vaccinations according to the local immunization practice.

Whenever possible the investigator should arrange the study visits within the interval provided in Table 5.

Table 5 Intervals between study visits

Interval	Optimal length of interval [1]	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

[1] Whenever possible the investigator should arrange the study visits within this interval.
Note: The date of the previous visit serves as the reference date for the intervals between the study visits.

5.6. Detailed description of study procedures

5.6.1. Informed consent

The signed/witnessed/thumb printed informed consent of the subject's parent(s)/LAR(s) must be obtained before study participation. Refer to Section 5.1 for the requirements on how to obtain informed consent.

5.6.2. Check inclusion and exclusion criteria

Check all inclusion and exclusion criteria as described in Sections 4.2 and 4.3 before enrolment.

5.6.3. Collect demographic data

Record demographic data such as age, sex and geographical ancestry in the subject's eCRF.

5.6.4. Medical and vaccination history

Obtain the subject's medical and vaccination history by interview and/or review of the subject's medical records and record any pre-existing conditions or signs and/or symptoms present in a subject, prior to the first study vaccination in the eCRF.

5.6.5. Physical examination

Perform a physical examination of the subject, including assessment of oral/axillary/rectal body temperature, height and weight. Collected information needs to be recorded in the eCRF.

Physical examination at each study visit subsequent to the first vaccination visit will be performed only if the subjects' parent(s)/LAR(s) indicates during questioning that there might be some underlying pathology(ies) or if deemed necessary by the Investigator or delegate.

If the investigator determines that the subject's health on the day of vaccination temporarily precludes vaccination, the visit will be rescheduled.

Treatment of any abnormality observed during physical examination has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

5.6.6. Check contraindications, warnings and precautions to vaccination

Contraindications, warnings and precautions to vaccination must be checked at the beginning of each vaccination visit. Refer to Sections 6.5 and 6.6 for more details.

5.6.7. Assess pre-vaccination body temperature

The oral/axillary/rectal body temperature of each subject needs to be measured prior to any study vaccine administration. If the subject has fever on the day of vaccination, the vaccination visit will be rescheduled within the allowed interval for this visit (see Table 5).

5.6.8. Study group and treatment number allocation

Study group and treatment number allocation will be performed as described in Section 5.2.2. The number of each administered treatment must be recorded in the eCRF.

5.6.9. Sampling

Refer to the Module on Biospecimen Management in the SPM for detailed instructions for the collection, handling and processing of the samples.

5.6.9.1. Blood sampling for immune response assessment

Blood samples will be taken at Visit 1 and Visit 3 as specified in Section 5.5, outline of study procedures.

- A volume of approximately 2 mL of whole blood (to provide approximately 0.7 mL of serum) should be drawn from all subjects for anti-RV antibody determination at Visit 1 and Visit 3. After centrifugation, serum samples should be kept at –20°C/–4°Fahrenheit or below until shipment. Refer to the SPM for more details on sample storage conditions.

5.6.10. Study Vaccine administration

- After completing all prerequisite procedures prior to vaccination, two oral doses of the study vaccine will be administered at an approximate one-month interval to subjects, according to the immunization schedule for HRV vaccine administration (refer to Section 6.3 for detailed description of the vaccines administration procedure). If the investigator or delegate determines that the subject's health on the day of administration temporarily precludes vaccines administration, the visit will be rescheduled within the allowed interval for this visit (refer to Table 5).

- The subjects will be observed closely for at least 30 minutes following the administration of the vaccine, with appropriate medical treatment readily available in case of anaphylaxis.

5.6.11. Record regurgitation

If regurgitation or vomiting occurs after vaccination, a single replacement dose may be given at the same vaccination visit at the discretion of the Investigator. This information should be recorded in the eCRF. The subject may continue to participate in the study.

5.6.12. Check and record concomitant medication/vaccination and intercurrent medical conditions

Concomitant medication/vaccination must be checked and recorded in the eCRF as described in Section 6.7.

Intercurrent medical conditions must be checked and recorded in the eCRF as described in Section 6.8.

5.6.13. Recording of AEs and SAEs

- Refer to Section 8.2 for procedures for the investigator to record AEs and SAEs. Refer to Section 8.3 for guidelines and how to report SAE reports to GSK Biologicals.
- The subjects' parent(s)/LAR(s) will be instructed to contact the investigator immediately should the subjects manifest any signs or symptoms they perceive as serious.
- At each vaccination visit, diary cards will be provided to the subject's parent(s)/LAR(s). The subject's parent(s)/LAR(s) will be instructed to measure and record the oral, axillary or rectal body temperature, and any solicited general AEs (i.e., on the day of vaccination and during the next seven days) or any unsolicited AEs (i.e., on the day of vaccination and during the next 30 days after vaccination). The subject's parent(s)/LAR(s) will be instructed to return the completed diary card to the investigator at the next study visit.
- Collect and verify completed diary cards during discussion with the subject's parent(s)/LAR(s).
- Any unreturned diary cards will be sought from the subject's parent(s)/LAR(s) through telephone call(s) or any other convenient procedure.
- The investigator will transcribe the collected information into the eCRF in English.

5.6.14. Study conclusion

The investigator will:

- review data collected to ensure accuracy and completeness, and
- complete the study conclusion screen in eCRF.

5.7. Biological sample handling and analysis

Please refer to the SPM for details on biospecimen management (handling, storage and shipment).

Samples will not be labelled with information that directly identifies the subject but will be coded with the identification number for the subject (subject number).

- Collected samples will be used for protocol mandated research and purposes related to the improvement, development and quality assurance of the laboratory tests described in this protocol. This may include the management of the quality of these tests, the maintenance or improvement of these tests, the development of new test methods, as well as making sure that new tests are comparable to previous methods and work reliably.
- It is also possible that future findings may make it desirable to use the samples acquired in this study for future research, not described in this protocol. Therefore, all subjects will be asked to give a specific consent to allow GSK or a contracted partner to use the samples for future research. Future research will be subject to the laws and regulations in India and will only be performed once an IEC or Review Board has approved this research.

Information on further investigations and their rationale can be obtained from GSK Biologicals.

Any sample testing will be done in line with the consent of the individual subject's parent(s)/LAR(s).

Refer also to the Investigator Agreement, where it is noted that the investigator cannot perform any other biological assays except those described in the protocol or its amendment(s).

Collected samples will be stored for a maximum of 20 years (counting from when the last subject performed the last study visit), unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subject consent. These extra requirements need to be communicated formally to and discussed and agreed with GSK Biologicals.

5.7.1. Use of specified study materials

When materials are provided by GSK Biologicals, it is MANDATORY that all clinical samples (serum samples) be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the per-protocol analysis (See Section 10.4 for the definition of cohorts to be analyzed). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals does not provide material for collecting and storing clinical samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM.

5.7.2. Biological sample

The biological samples to be collected from subjects are described in Table 6.

Table 6 Biological samples

Sample type	Quantity	Unit	Time points
Blood	Approximately 2	ml	Visit 1 and Visit 3

ml-milliliter

5.7.3. Laboratory assays

Please refer to APPENDIX A for a detailed description of the assays performed in the study. Please refer to APPENDIX B for the address of the clinical laboratories used for sample analysis and/or sample management.

All serological assays for the determination of antibodies against RV will be performed by ELISA at GSK Biologicals' laboratory or in a laboratory designated by GSK Biologicals using standardized and validated procedures (refer to Table 7).

Table 7 Humoral Immunity (Antibody determination)

System	Component	Method	Kit/Manufacturer	Unit	Cut-off	Laboratory
SER	Rotavirus Ab.IgA	ELI	NA	U/ml	13*	GSK Biologicals**

SER = Serum
IgA = Immunoglobulin A
ELI = ELISA (Enzyme Linked Immunosorbent Assay)
U = Units; ml = milliliters
*This corresponds to the technical cut-off of the revalidated laboratory assay.
**GSK Biologicals laboratory refers to the Clinical Laboratory Sciences (CLS) in Rixensart, Belgium; Wavre, Belgium and Marburg, Germany

Additional exploratory testing on the vaccine and/or on the disease under study may be performed within the framework of the study if deemed necessary for accurate interpretation of the data or should such assay(s) become available at GSK. These assays may not be represented in the objectives/endpoints of the study protocol.

The GSK Biologicals' clinical laboratories have established a Quality System supported by procedures. The activities of GSK Biologicals' clinical laboratories are audited regularly for quality assessment by an internal (sponsor-dependent) but laboratory-independent Quality Department.

5.7.4. Biological samples evaluation

5.7.4.1. Immunological read-outs

The immunological read-outs are presented in Table 8.

Table 8 Immunological read-outs

Blood sampling time point				
Type of contact and time point	**Sampling time point**	**No. subjects**	**Component**	**Components priority rank**
Visit 1 (Day 1)	Pre-Vacc	All	Rota Ab.IgA	None
Visit 3 (Month 2)	Post-Vacc	All	Rota Ab.IgA	None

Pre-Vacc: Pre-vaccination; Post-Vacc: Post Vaccination; Ab: Antibody IgA: Immunoglobulin A

5.7.5. Immunological correlates of protection

No immunological correlate of protection has been demonstrated so far for the antigen used as part of the HRV vaccine. However, a study by Cheuvart et al., in 2014 indicated that post-vaccination anti-RV IgA seropositivity (antibody concentration ≥ 20 U/mL) may serve as a useful correlate of vaccine efficacy in clinical trials of *Rotarix* [Cheuvart, 2014].

The investigator is encouraged to share the immunological assay results for non-responders with the study subjects' parent(s)/LAR(s).

6. STUDY VACCINES AND ADMINISTRATION

6.1. Description of study vaccines

The study vaccines to be used have been developed and manufactured by GSK Biologicals.

The Quality Control Standards and Requirements for each study vaccines are described in separate Quality Assurance documents (e.g., release protocols, certificate of analysis) and the required approvals have been obtained.

The vaccines are labelled and packed according to applicable regulatory requirements.

Commercial vaccines are assumed to comply with the specifications given in the manufacturer's Summary of Product Characteristics (SmPC).

The study vaccines to be utilized in the study are detailed in Table 9.

Table 9 Study vaccine (Amended: 30 October 2019)

Treatment name	Vaccine name	Formulation	Presentation	Volume to be administered orally	Number of doses
HRV Liquid	HRV Liquid vaccine	**Active substance**: HRV ***RIX4414*** live attenuated >=$10^{6.0}$$CCID_{50}$ **Excipients:** Sucrose=1.073g; Di-sodium Adipate=132.74mg; DMEM=2.26mg; water for injection=1.5ml	Liquid vaccine in a pre-filled oral applicator	1.5 ml	2
HRV Lyophilized	HRV Lyophilized vaccine	HRV ***RIX4414*** live attenuated >=$10^{6.0}$$CCID_{50}$	Lyophilized vaccine in a monodose glass vial. Diluent (calcium carbonate buffer) supplied separately	1 ml	2
	HRV Diluent	$CaCO_3$=60mg	Diluent for lyophilized vaccine (calcium carbonate liquid antacid) supplied separately in a prefilled oral applicator		

HRV=Human Rotavirus; $CCID_{50}$ = Median Cell Culture Infective Dose (quantity of virus causing infection in 50% of exposed cells); DMEM = Dulbecco's Modified Eagle Medium; ml = milliliter;

6.2. Storage and handling of study vaccine

The study vaccines must be stored at the label storage temperature conditions in a safe and locked place. Access to the storage space should be limited to authorized study personnel. The storage conditions will be assessed during pre-study activities under the responsibility of the sponsor study contact. The storage temperature should be continuously monitored with calibrated (if not validated) temperature monitoring device(s) and recorded. Refer to the Module on Clinical Trial Supplies in the SPM for more details on storage of the study vaccines.

Temperature excursions must be reported in degree Celsius.

Any temperature excursion outside the range of 0.0 to +8.0°Celsius (for +2 to +8°C/+36 to +46°Fahrenheit label storage condition) impacting investigational medicinal products (IMPs) must be reported in the appropriate (electronic) temperature excursion decision form ([e]TDF). The impacted IMPs must not be used and must be stored in quarantine at label temperature conditions until usage approval has been obtained from the sponsor.

In case of temperature excursion below +2.0°Celsius down to 0.0°Celsius impacting IMP(s) there is no need to report in (e)TDF, but adequate actions must be taken to restore the +2 to +8°Celsius/+36 to +46°Fahrenheit label storage temperature conditions. The impacted IMP(s) may still be administered, but the site should avoid re-occurrence of such temperature excursion. Refer to the Module on Clinical Trial Supplies in the SPM for more details on actions to take.

Refer to the Module on Clinical Trial Supplies in the SPM for details and instructions on the temperature excursion reporting and usage decision process, packaging and accountability of the study vaccines.

6.3. Dosage and administration of study vaccine

Liquid formulation of HRV vaccine

The pre-filled oral applicator should be shaken well before use. The vaccine (approximately 1.5 mL) should then be administered orally as a single dose.

Lyophilized formulation of HRV vaccine

To prepare GSK Biologicals' HRV lyophilized vaccine for administration, the entire content of the supplied diluent (calcium carbonate buffer) should be transferred from the oral applicator into the vial of the lyophilized product via the intermediate device. The vial should be shaken well to re-suspend the vaccine. The entire volume of the re-suspended product (approximately 1 mL) should be withdrawn into the same oral applicator and the re-suspended product should then be administered promptly as a single oral dose.

Administration of the vaccines

In order to allow the swallowing of the entire volume of the single oral dose, the administration should occur in a quiet environment. The child should be seated in a reclining position. Administer orally (i.e., into the child's mouth towards the inner cheek) the entire content of oral applicator. Sufficient time should be allowed for the baby to swallow the vaccine solution, to avoid regurgitation or vomiting. Should however the subject regurgitate or vomit after study vaccine administration, a single replacement dose may be given at the same vaccination visit at the discretion of the Investigator. This information should be recorded in the eCRF. The subject may continue to participate in the study.

The vaccinees will be observed closely for at least 30 minutes, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines.

The vaccination regimen is summarized in Table 10.

Table 10 Dosage and administration

Type of contact and time point	Study group	Treatment name	Volume to be administered orally	Route	Site		
					Location	Directionality	Laterality
Visit 1 (Day 1), Visit 2 (Month 1)	HRV Liq	HRV Liquid	1.5 ml	O	Not applicable	Not applicable	Not applicable
Visit 1 (Day 1), Visit 2 (Month 1)	HRV Lyo	HRV Lyophilized	1 ml	O	Not applicable	Not applicable	Not applicable

O= Oral; ml-millilitre; HRV- Human Rota Virus

6.4. Replacement of unusable vaccine doses

In addition to the vaccine doses provided for the planned number of subjects (including over-randomization when applicable), at least 30% additional vaccine doses will be supplied to replace those that are unusable.

6.5. Contraindications to subsequent vaccination

The following events constitute absolute contraindications to further administration of HRV vaccine. If any of these events occur during the study, the subject must not receive additional doses of vaccines but may continue other study procedures at the discretion of the investigator (see Section 8.4).

- Anaphylaxis following the administration of vaccines.
- Hypersensitivity reaction following the administration of the HRV vaccine.
- Any uncorrected congenital malformation of the gastrointestinal tract (such as Meckel's diverticulum) that would predispose for IS.
- Any history of IS.
- SCID.

The following events constitute contraindications to administration of the study vaccines at that point in time; if any of these events occur at the time scheduled for vaccination, the subject may be vaccinated at a later date, within the time window specified in the protocol (see Section 5.5), or the subject may be withdrawn at the discretion of the investigator (see Section 8.4).

- Acute disease and/or fever at the time of vaccination.
  - 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.
  - Subjects with a minor illness (such as mild diarrhoea, mild upper respiratory infection) without fever may be vaccinated at the discretion of the investigator).
- GE within seven days preceding the study vaccine administration.

6.6. Warnings and precautions

The *Rotarix* vaccine should under no circumstances be injected.

There is no data on the safety and efficacy of *Rotarix* in infants with gastrointestinal illnesses. Administration of *Rotarix* may be considered with caution in such infants when, in the opinion of the physician, withholding the vaccine entails a greater risk.

Post-marketing safety data indicate a transient increased risk of IS after vaccination, mostly within 7 days following the administration of the first dose of *Rotarix* and, to a lesser extent, the second dose. The overall incidence of IS remains rare. Whether *Rotarix* affects the overall risk of IS, has not been established.

Therefore, parents/LARs should be advised to promptly report any symptoms indicative of IS (severe abdominal pain, persistent vomiting, bloody stools, abdominal bloating and/or high fever).

Excretion of the vaccine virus in the stools is known to occur after vaccination and lasts for 10 days on average with peak excretion around the seventh day. In clinical trials, cases of transmission of excreted vaccine virus to seronegative contacts of vaccinees have been observed without causing any clinical symptoms. *Rotarix* should be administered with caution to individuals with immunodeficient close contacts, such as individuals with malignancies, or who are otherwise immunocompromised or receiving immunosuppressive therapy. Contacts of recent vaccinees should be advised to observe careful hygiene (including washing their hands) when changing children's diapers.

The tip caps of the prefilled oral applicators of diluent may contain natural rubber latex which may cause allergic reactions in individuals who are sensitive to latex.

Refer to the approved product label/package insert or IB for more details.

6.7. Concomitant medications/products and concomitant vaccination

At each study visit, the investigator or delegate should question the subject's parent(s)/LAR(s) about any medications/products taken and vaccinations received by the subject.

6.7.1. Recording of concomitant medications/products and concomitant vaccinations

The following concomitant medication(s)/product(s)/vaccine(s) must be recorded in the eCRF.

- All concomitant medications/products, except vitamins and dietary supplements, administered during the period starting 30 days before and following each dose of study vaccine.

- Any concomitant vaccination administered in the period from first study vaccination (Visit 1) and ending at study Visit 3.
- Prophylactic medication (i.e., medication administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination).

  E.g., an anti-pyretic is considered to be prophylactic when it is given in the absence of fever and any other symptom, to prevent fever from occurring.
- Any concomitant medications/products/vaccines listed in Section 6.7.2.
- Any concomitant medications/products/vaccines relevant to a SAE to be reported as per protocol or administered during the study period for the treatment of a SAE. Concomitant medications relevant to SAEs need to be recorded on the expedited Adverse Event report.
- Any antipyretic administered in the period starting 6 hours before vaccination and ending 12 hours after vaccination.

6.7.2. Concomitant medications/products/vaccines that may lead to the elimination of a subject from per-protocol analyses

The use of the following concomitant medications/products/vaccines will not require withdrawal of the subject from the study but may determine a subject's evaluability in the per-protocol analysis. See Section 10.4 for cohorts to be analyzed.

- Any investigational or non-registered product (drug or vaccine) other than the study vaccines, used during the study period.
- Immunosuppressants or other immune-modifying drugs administered chronically (i.e., more than 14 days) during the study period between blood sampling at Visit 1 to blood sampling at 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.
- Long-acting immune-modifying drugs administered at any time during the study period (e.g., infliximab).
- A vaccine not foreseen by the study protocol administered during the period starting from 30 days before the first dose of HRV vaccine* administration and ending at Visit 3** blood sampling, with the exception of the inactivated influenza vaccine, and other licensed routine childhood vaccinations which are allowed at any time during the study.

  *Administration of any HRV vaccine other than the study vaccine will lead to elimination (Refer to Section 4.3).

  **In case an emergency mass vaccination for an unforeseen public health threat (e.g.: a pandemic) is organized by the public health authorities, outside the routine immunization program, the time period described above can be reduced if necessary for that vaccine provided it is licensed and used according to its Prescribing

Information and according to the local governmental recommendations and provided a written approval of the Sponsor is obtained.

A detailed, comprehensive list of reasons for elimination from PPS analyses will be established at the time of data cleaning.

6.8. Intercurrent medical conditions that may lead to elimination of a subject from per-protocol analyses

At each study visit subsequent to the first vaccination visit, it must be verified if the subject has experienced or is experiencing any intercurrent medical condition. If it is the case, the condition(s) must be recorded in the eCRF.

Subjects may be eliminated from the PPS for analysis of immunogenicity if, during the study, they incur a condition that has the capability of altering their immune response or are confirmed to have an alteration of their initial immune status.

7. HEALTH ECONOMICS

Not applicable.

8. SAFETY

The investigator or site staff is/are responsible for the detection, documentation and reporting of events meeting the criteria and definition of an AE or SAE as provided in this protocol.

Each subject's parent(s)/LAR(s) will be instructed to contact the investigator immediately should the subject manifest any signs or symptoms they perceive as serious.

8.1. Safety definitions

8.1.1. Definition of an adverse event

An AE is any untoward medical occurrence in a clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e., lack of efficacy), abuse or misuse.

Examples of an AE include:

- Significant or unexpected worsening or exacerbation of the condition/indication under study.

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study vaccines administration even though they may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study vaccines or a concurrent medication (overdose per se should not be reported as an AE/SAE).
- Signs, symptoms temporally associated with study vaccines administration.
- Significant failure of expected pharmacological or biological action.
- Pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e., invasive procedures, modification of subject's previous therapeutic regimen).

AEs to be recorded as endpoints (solicited AEs) are described in Section 8.1.3. All other AEs will be recorded as UNSOLICITED AEs.

Examples of an AE DO NOT include:

- Medical or surgical procedures (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE/SAE.
- Situations where an untoward medical occurrence did not occur (e.g., social and/or convenience admission to a hospital, admission for routine examination).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- Pre-existing conditions or signs and/or symptoms present in a subject prior to the study vaccination. These events will be recorded in the medical history section of the eCRF.

8.1.2. Definition of a serious adverse event

A SAE is any untoward medical occurrence that:

a. Results in death,

b. Is life-threatening,

   Note: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, had it been more severe.

c. Requires hospitalization or prolongation of existing hospitalization,

   Note: In general, hospitalization signifies that the subject has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or in an out-patient setting. Complications that occur during hospitalization are also considered AEs. If a complication prolongs

hospitalization or fulfils any other serious criteria, the event will also be considered serious. When in doubt as to whether 'hospitalization' occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition (known or diagnosed prior to informed consent signature) that did not worsen from baseline is NOT considered an AE.

d. Results in disability/incapacity, OR

Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza like illness, and accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

Medical or scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardise the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization.

8.1.3. Solicited adverse events

8.1.3.1. Solicited general adverse events

The following general AEs will be solicited (Table 11):

Table 11 Solicited general adverse events

Fever
Irritability/Fussiness
Diarrhea
Vomiting
Loss of appetite
Cough/runny nose

Note: Parent(s)/LAR(s) will be instructed to measure and record the oral, axillary or rectal body temperature in the evening. Should additional temperature measurements be performed at other times of day, parent(s)/LAR(s) will be instructed to record the highest temperature in the diary card.

An overview of the protocol-required reporting periods for solicited general AEs is given in Table 12.

8.1.4. Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events

In absence of diagnosis, abnormal laboratory findings (e.g., clinical chemistry, haematology, urinalysis) or other abnormal assessments (e.g., vital signs etc) that are judged by the investigator to be clinically significant will be recorded as AE or SAE if they meet the definition of an AE or SAE (refer to Sections 8.1.1 and 8.1.2). Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the study and do not worsen, will not be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgement in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

8.2. Detecting and recording adverse events and serious adverse events

8.2.1. Time period for detecting and recording adverse events and serious adverse events

All AEs starting within 31 days following administration of each dose of study vaccines (Day 1 to Day 31) must be recorded into the appropriate section of the eCRF, irrespective of intensity or whether or not they are considered vaccination-related.

The time period for collecting and recording SAEs will begin at the receipt of study vaccine and will end 30 days following administration of the last dose of study vaccine for each subject. See Section 8.3 for instructions on reporting of SAEs.

All AEs/SAEs leading to withdrawal from the study will be collected and recorded from the time of the first receipt of study vaccines.

In addition to the above-mentioned reporting requirements and in order to fulfil international reporting obligations, SAEs that are related to study participation (i.e., protocol-mandated procedures, invasive tests, a change from existing therapy) or are related to a concurrent GSK medication/vaccine will be collected and recorded from the time the subject consents to participate in the study until she/he is discharged from the study.

An overview of the protocol-required reporting periods for AEs and SAEs is given in Table 12.

Table 12 Reporting periods for collecting safety information

Event	Pre-V1*	V1 D1	7 days post vacc D 8	30 days post vacc D 31		V2 M1	7 days post vacc	30 days post vacc		V3 M2
										Study Conclusion
Solicited general AEs										
Unsolicited AEs										
AEs/SAEs leading to withdrawal from the study										
SAEs										
SAEs related to study participation or concurrent GSK medication/vaccine										

* Consent obtained. Pre-V: pre-vaccination; Post-Vacc: post-vaccination; V: Visit; D: Day, M: Month;
AEs = Adverse Events; SAEs = Serious Adverse Events

8.2.2. Post-Study adverse events and serious adverse events

A post-study AE/SAE is defined as any event that occurs outside of the AE/SAE reporting period defined in Table 12. Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study vaccines, the investigator will promptly notify the Study Contact for Reporting SAEs.

8.2.3. Evaluation of adverse events and serious adverse events

8.2.3.1. Active questioning to detect adverse events and serious adverse events

As a consistent method of collecting AEs, the subject's parent(s)/LAR(s) should be asked a non-leading question such as:

'*Has your child acted differently or felt different in any way since receiving the vaccines or since the last visit?'*

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE in the eCRF. The investigator is not allowed to send photocopies of the subject's medical records to GSK Biologicals instead of appropriately completing the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK Biologicals. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to GSK Biologicals.

The investigator will attempt to establish a diagnosis pertaining to the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

8.2.3.2. Assessment of adverse events

8.2.3.2.1. Assessment of intensity

The intensity of the following solicited AEs will be assessed as described in Table 13:

Table 13 Intensity scales for solicited symptoms in infants

Infants		
Adverse Event	**Intensity grade**	**Parameter**
Fever*		Record temperature in °C/°F
	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
Irritability/Fussiness	0	Behavior as usual
	1	Mild: Crying more than usual/no effect on normal activity
	2	Moderate: Crying more than usual/interferes with normal activity
	3	Severe: Crying that cannot be comforted/prevents normal activity
Diarrhea[$]		Record the number of looser than normal stools/day
	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[§]		Record the number of vomiting episodes/day
	0	Normal (no emesis)
	1	1 episode of vomiting/day
	2	2 episodes of vomiting/day
	3	≥3 episodes of vomiting/day
Loss of appetite	0	Appetite as usual
	1	Mild: Eating less than usual/no effect on normal activity
	2	Moderate: Eating less than usual/interferes with normal activity
	3	Severe: Not eating at all
Cough/runny nose	0	Normal
	1	Mild: Cough/runny nose which is easily tolerated
	2	Moderate: Cough/runny nose which interferes with daily activity
	3	Severe: 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.

The intensity grade for diarrhea, vomiting and fever as shown in Table 13 will be scored at GSK Biologicals.

The investigator will assess the maximum intensity that occurred over the duration of the event for all unsolicited AEs (including SAEs) recorded during the study. The assessment will be based on the investigator's clinical judgement.

The intensity should be assigned to one of the following categories:

1 (mild)	=	An AE which is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
2 (moderate)	=	An AE which is sufficiently discomforting to interfere with normal everyday activities.
3 (severe)	=	An AE which prevents normal, everyday activities (in a young child, such an AE would, for example, prevent attendance at a day-care center and would cause the parent(s)/LAR(s) to seek medical advice.)

An AE that is assessed as Grade 3 (severe) should not be confused with a SAE. Grade 3 is a category used for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 3. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 8.1.2.

8.2.3.2.2. Assessment of causality

The investigator is obligated to assess the relationship between study vaccines and the occurrence of each AE/SAE using clinical judgement. In case of concomitant administration of multiple vaccines/products, if possible, the investigator should specify if the AE could be causally related to a specific vaccine/product administered (i.e., investigational, control/placebo or co-administered vaccine). When causal relationship to a specific vaccine(s)cannot be determined the investigator should indicate the AE to be related to all products.

Alternative plausible causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study vaccines will be considered and investigated. The investigator will also consult the IB and/or SmPC and/or Prescribing Information for marketed products to determine his/her assessment.

There may be situations when a SAE has occurred and the investigator has minimal information to include in the initial report to GSK Biologicals. However, it is very important that the investigator always makes an assessment of causality for every event prior to submission of the Expedited Adverse Events Report to GSK Biologicals. The investigator may change his/her opinion of causality in light of follow-up information and update the SAE information accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Causality of all AEs should be assessed by the investigator using the following question:

Is there a reasonable possibility that the AE may have been caused by the study vaccine?

YES	:	There is a reasonable possibility that the study vaccines contributed to the AE.
NO	:	There is no reasonable possibility that the AE is causally related to the administration of the study vaccines. There are other, more likely causes and administration of the study vaccines is not suspected to have contributed to the AE.

If an event meets the criteria to be determined as 'serious' (see Section 8.1.2), additional examinations/tests will be performed by the investigator in order to determine ALL possible contributing factors for each SAE.

Possible contributing factors include:

- Medical history.
- Other medication.
- Protocol required procedure.
- Other procedure not required by the protocol.
- Lack of efficacy of the vaccines, if applicable.
- Erroneous administration.
- Other cause (specify).

8.2.3.3. Assessment of outcomes

The investigator will assess the outcome of all unsolicited AEs (including SAEs) recorded during the study as:

- Recovered/resolved.
- Recovering/resolving.
- Not recovered/not resolved.
- Recovered with sequelae/resolved with sequelae.
- Fatal (SAEs only).

8.2.3.4. Medically attended visits

For each solicited and unsolicited symptom the subject experiences, the subject's parent(s)/LAR(s) will be asked if the subject received medical attention defined as hospitalization, or an otherwise unscheduled visit to or from medical personnel for any reason, including emergency room visits. This information will be recorded in the eCRF.

8.3. Reporting of serious adverse events

8.3.1. Prompt reporting of serious adverse events to GSK Biologicals

SAEs that occur in the time period defined in Section 8.2 will be reported promptly to GSK within the timeframes described in Table 14, once the investigator determines that the event meets the protocol definition of a SAE.

Table 14 Timeframes for submitting serious adverse event and other events reports to GSK Biologicals

Type of Event	Initial Reports		Follow-up of Relevant Information on a Previous Report	
	Timeframe	Documents	Timeframe	Documents
SAEs	24 hours*‡	electronic Expedited Adverse Events Report	24 hours*	electronic Expedited Adverse Events Report

* Timeframe allowed after receipt or awareness of the information.
‡ The investigator will be required to confirm review of the SAE causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE.

8.3.2. Contact information for reporting serious adverse events

Study Contact for Reporting SAEs
Refer to the local study contact information document.
Back-up Study Contact for Reporting SAEs
24/24 hour and 7/7 day availability: **GSK Biologicals Clinical Safety & Pharmacovigilance** Outside US & Canada sites: Fax: PPD or PPD Email address: PPD

8.3.3. Completion and transmission of SAE reports to GSK Biologicals

Once an investigator becomes aware that a SAE has occurred in a study subject, the investigator (or designate) must complete the information in the electronic Expedited Adverse Events Report WITHIN 24 HOURS. The report will always be completed as thoroughly as possible with all available details of the event. Even if the investigator does not have all information regarding a SAE, the report should still be completed within 24 hours. Once additional relevant information is received, the report should be updated WITHIN 24 HOURS.

The investigator will always provide an assessment of causality at the time of the initial report. The investigator will be required to confirm the review of the SAE causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE.

8.3.3.1. Back-up system in case the electronic reporting system does not work

If the electronic reporting system does not work, the investigator (or designate) must complete, then date and sign a paper Expedited Adverse Events Report and fax it to the Study Contact for Reporting SAEs (refer to the Sponsor Information) or to GSK Biologicals Clinical Safety and Pharmacovigilance department within 24 hours.

This back-up system should only be used if the electronic reporting system is not working and NOT if the system is slow. As soon as the electronic reporting system is working again, the investigator (or designate) must complete the electronic Expedited Adverse Events Report within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic SAE reporting system.

8.3.4. Updating of SAE information after removal of write access to the subject's eCRF

When additional SAE information is received after removal of the write access to the subject's eCRF, new or updated information should be recorded on the appropriate paper report, with all changes signed and dated by the investigator. The updated report should be faxed to the Study Contact for Reporting SAEs (refer to the Sponsor Information) or to GSK Biologicals Clinical Safety and Pharmacovigilance department within the designated reporting time frames specified in Table 14.

8.3.5. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to GSK in accordance with the procedures detailed in Section 8.3.1. GSK Biologicals has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

Investigator safety reports are prepared according to the current GSK policy and are forwarded to investigators as necessary. An investigator safety report is prepared for a SAE(s) that is both attributable to the study vaccines and unexpected. The purpose of the report is to fulfil specific regulatory and GCP requirements, regarding the product under investigation.

8.4. Follow-up of adverse events and serious adverse events

8.4.1. Follow-up during the study

After the initial AE/SAE report, the investigator is required to proactively follow each subject and provide additional relevant information on the subject's condition to GSK Biologicals (within 24 hours for SAEs; refer to Table 14).

All SAEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until the last visit of the subject.

All AEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until 30 days after the last vaccination.

8.4.2. Follow-up after the subject is discharged from the study

The investigator will follow subjects:

- With SAEs, or subjects withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilised, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up.

If the investigator receives additional relevant information on a previously reported SAE, he/she will provide this information to GSK Biologicals using an electronic Expedited Adverse Events Report and/or pregnancy report as applicable.

GSK Biologicals may request that the investigator performs or arranges the conduct of additional clinical examinations/tests and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obliged to assist. If a subject dies during participation in the study or during a recognised follow-up period, GSK Biologicals will be provided with any available post-mortem findings, including histopathology.

8.5. Treatment of adverse events

Treatment of any AE is at the sole discretion of the investigator and according to current good medical practice. Any medication administered for the treatment of a SAE should be recorded in the subject's eCRF (refer to Section 6.7).

8.6. Subject card

Study subjects' parent(s)/LAR(s) must be provided with the address and telephone number of the main contact for information about the clinical study.

The investigator (or designate) must therefore provide a "subject card" to each subject's parent(s)/LAR(s). In an emergency situation, this card serves to inform the responsible attending physician that the subject is in a clinical study and that relevant information may be obtained by contacting the investigator.

Subjects' parent(s)/LAR(s) must be instructed to keep subject cards in their possession at all times during the study duration.

9. SUBJECT COMPLETION AND WITHDRAWAL

9.1. Subject completion

A subject who returns to the concluding visit foreseen in the protocol is considered to have completed the study.

9.2. Subject withdrawal

Withdrawals will not be replaced.

9.2.1. Subject withdrawal from the study

From an analysis perspective, a 'withdrawal' from the study refers to any subject who was did not come back for the concluding visit foreseen in the protocol.

All data collected until the date of withdrawal/last contact of the subject will be used for the analysis.

A subject is considered a 'withdrawal' from the study when no study procedure has occurred, no follow-up has been performed and no further information has been collected for this subject from the date of withdrawal/last contact.

Investigators will make an attempt to contact those subjects who do not return for scheduled visits or follow-up.

Information relative to the withdrawal will be documented in the eCRF. The investigator will document whether the decision to withdraw a subject from the study was made by the subject's parent(s)/LAR(s), or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- SAE
- Unsolicited non-serious AE
- Solicited AE
- Protocol violation (specify)
- Consent withdrawal, not due to an AE*
- Moved from the study area
- Lost to follow-up
- Other (specify)

*In case a subject is withdrawn from the study because the subject's parent(s)/LAR(s) has withdrawn consent, the investigator will document the reason for withdrawal of consent, if specified by the subject's parent(s)/LAR(s), in the eCRF.

Subjects who are withdrawn from the study because of SAEs/AEs must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn from the study as result of a SAE/AE until resolution of the event (see Section 8.4.2).

9.2.2. Subject withdrawal from study vaccine

A 'withdrawal' from the study vaccine refers to any subject who does not receive the complete treatment, i.e., when no further planned dose is administered from the date of withdrawal. A subject withdrawn from the study vaccine may not necessarily be withdrawn from the study as further study procedures or follow-up may be performed (safety or immunogenicity) if planned in the study protocol.

Information relative to premature discontinuation of the study vaccines will be documented on the Vaccine Administration screen of the eCRF. The investigator will document whether the decision to discontinue further vaccination/treatment was made by the subject's parent(s)/LAR(s), or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- SAE
- Unsolicited non-serious AE
- Solicited AE
- Not willing to be vaccinated
- Other (specify)

10. STATISTICAL METHODS

10.1. Primary Endpoint

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

10.2. Secondary Endpoints (Amended: 30 October 2019)

- Anti-RV IgA antibody concentrations
  - Anti-RV IgA antibody seroconversion* rate, one month post dose 2 of HRV vaccine.

Definition:

- ***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.***

- 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.

10.3. Determination of sample size (Amended: 30 October 2019)

Considering the total sample of 450 subjects (225 subjects per arm), ***Table 15 provides the power to demonstrate non-inferiority in terms of GMCs according to the percentage of non-evaluable subjects***.

Based on historical data from ROTA-044 study [Narang, 2009], 13.8% of subjects were non-evaluable for reasons other than 'initially positive or unknown for serum anti-RV IgA antibodies at Dose 1'. The power to reach the primary objective in a scenario of 13% non-evaluable subjects is 96% (Table 15). Considering a scenario of 35% non-evaluable subjects, the power to reach the primary objective is 90%. As shown in Table 15, the power decreases when the % of non-evaluable subjects increases.

Table 15 ***Power according to different scenarios for the % of non-evaluable subjects, with respect to a total sample size of 450 subjects* (Amended: 30 October 2019)***

Standard deviation [Log_{10} (concentration)]	Alpha	% of non-evaluable subjects	Power
0.790**	0.025	13%	96%
		15%	96%
		25%	94%
		30%	92%
		35%	90%
		40%	88%
		45%	85%
		50%	81%
		55%	77%

PASS one-sided non-inferiority for 2 independent means with common variance for log10(0.5) as non-inferiority margin, for the alternative hypothesis that the means are equal and for a type I error=2.5 %
****Reference used for power computation***

10.4. Cohorts for Analyses

10.4.1. Exposed Set

The 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.

10.4.2. Per-Protocol Set for analysis of immunogenicity (Amended: 30 October 2019)

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 5,
- 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, as per Table 5,
- 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.

10.5. Derived and transformed data (Amended: 30 October 2019)

Demography

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

Safety/Reactogenicity

Subjects who missed reporting symptoms (solicited/unsolicited or concomitant medications) will be treated as subjects without symptoms (solicited/unsolicited or concomitant medications, respectively).

Immunogenicity (Amended: 30 October 2019)

- A seronegative subject is a subject whose anti-RV IgA antibody concentration is below the clinically 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 clinically 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.
- For the immunogenicity measurement, missing or non-evaluable measurements will not be replaced.

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

10.6. Analysis of demographics

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.

10.7. Analysis of immunogenicity

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.

10.7.1. Within group assessment (Amended: 30 October 2019)

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).

10.7.2. Between groups assessment (Amended: 30 October 2019)

- 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 ratios 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)**.**

  Refer to the Section 2.1 for the success criteria of the primary objective.

10.8. Analysis of safety

The ES will be used for the analysis of safety.

10.8.1. 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 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.

10.9. Interpretation of analyses

Except for analyses addressing the primary objectives referred as confirmatory analyses, all the analyses will be descriptive/exploratory in nature. These descriptive analyses should be interpreted with caution

10.10. Statistical Methods (Amended: 30 October 2019)

- The exact CIs for a proportion within a group will be calculated using SAS [Clopper, 1934].
- The CI for GMCs will be obtained within each group separately. The CI for the mean of log-transformed concentration will be first obtained assuming that log-transformed values were normally distributed with unknown variance. The CI for the GMCs will then be obtained by exponential-transformation of the CI for the mean of log-transformed concentration.
- ***The standardized asymptomatic CI for the group difference in proportion will be calculated using SAS. The method used within GSK Biologicals is Method 6 [Newcombe, 1998].***

10.11. Conduct of analysis

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the final study report.

10.11.1. Sequence of analyses

A final analysis of all data collected up to Visit 3 will be conducted. This will include final analysis of immunogenicity, reactogenicity and safety. A clinical report will be written at this stage.

10.11.2. Statistical considerations for interim analyses

No interim analysis is planned for this study.

11. ADMINISTRATIVE MATTERS

To comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality, public disclosure requirements and publications must be fulfilled.

11.1. electronic Case Report Form instructions

A validated GSK defined electronic data collection tool will be used as the method for data collection.

In all cases, subject initials will not be collected nor transmitted to GSK. Subject data necessary for analysis and reporting will be entered/transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable GSK standards and data cleaning procedures.

While completed eCRFs are reviewed by a GSK Biologicals' Site Monitor at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate

clarification or correction by the investigator or appropriately qualified designee. In all cases, the investigator remains accountable for the study data.

The investigator will be provided with a CD-ROM of the final version of the data generated at the investigational site once the database is archived and the study report is complete and approved by all parties.

11.2. Study Monitoring by GSK Biologicals

GSK will monitor the study to verify that, amongst other items, the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol, any other study agreements, GCP and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

The investigator must ensure provision of reasonable time, space and qualified personnel for monitoring visits.

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform an eCRF review and Source Document Verification (SDV). By SDV we understand verifying eCRF entries by comparing them with the source data that will be made available by the investigator for this purpose.

The Source Documentation Agreement Form describes the source data for the different data in the eCRF. This document should be completed and signed by the site monitor and investigator and should be filed in the investigator's study file. Any data item for which the eCRF will serve as the source must be identified, agreed and documented in the source documentation agreement form.

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, GCP, and GSK procedures.

11.3. Record retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (e.g., audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must ensure that all reproductions

are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP, any institutional requirements, applicable laws or regulations, or GSK standards/procedures, otherwise, the minimum retention period will default to 25 years after completion of the study report.

The investigator/institution must notify GSK of any changes in the archival arrangements, including, but not limited to archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

11.4. Quality assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

11.5. Posting of information on publicly available clinical trial registers and publication policy

GSK assures that the key design elements of this protocol will be posted on the GSK website and in publicly accessible database(s) such as clinicaltrials.gov, in compliance with the current regulations.

GSK also assures that results of this study will be posted on the GSK website and in publicly accessible regulatory registry(ies) within the required time-frame, in compliance with the current regulations. The minimal requirement is to have primary endpoint summary results disclosed at latest 12 months post PCD and to have secondary endpoint disclosed at latest 12 months after the LSLV as described in the protocol.

GSK also aims to publish the results of these studies in searchable, peer reviewed scientific literature and follows the guidance from the International Committee of Medical Journal Editors.

11.6. Provision of study results to investigators

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report. The investigator will be provided

reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK Biologicals will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

11.7. Data Sharing

Under the framework of the SHARE initiative, results of GSK studies may be combined with non-GSK studies, to investigate further about the study product(s) and other product(s), and/or the disease/condition under investigation and related diseases and conditions.

12. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

13. REFERENCES (AMENDED: 30 OCTOBER 2019)

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WHO position paper. Weekly epidemiological record. Rotavirus vaccines WHO position paper-January 2013.2013; 88: 49-64.

APPENDIX A LABORATORY ASSAYS

Rotavirus Ab IgA Determination

The anti-RV antibody concentrations are determined by a validated anti-RV IgA ELISA. Microtiter plates (96-well) are coated with an anti-RV monoclonal antibody. The wells are washed and incubated with (positive wells) or without (negative wells) RV. Following incubation, the plates are washed and serum, standard and control dilutions are incubated in both types of wells (positive and negative). Bound anti-RV IgA in the well are detected by incubation with peroxidase conjugated anti-human IgA polyclonal antibodies. Color development proportional to the quantity of bound anti-RV IgA occurs in the presence of a chromogen, TetraMethylBenzidine (TMB), and measured spectrophotometrically. Specific optical densities are calculated for each sample/control/standard dilution by measuring the difference between positive and negative wells, the use of negative wells allowing to assess non-specific IgA binding. The concentrations of the samples expressed in units per milliliter are calculated relative to the four-parameter logistic function generated from the standard curve.

APPENDIX B CLINICAL LABORATORIES

Table 16 GSK Biologicals' laboratories

Laboratory	Address
GSK Biological's Clinical Laboratory Sciences (CLS), Rixensart	Biospecimen Reception-B7/44 Rue de l'Institut, 89 B-1330 Rixensart Belgium
GSK Biological's CLS, Wavre-Nord Noir Epine	Avenue Fleming, 20 B-1300 Wavre Belgium
GSK Vaccines GmbH	Emil-von-Behring-Str. 76 35041 Marburg Deutschland/ Germany

Table 17 Outsourced laboratories

Laboratory	Address
Q² Solutions Clinical Trials (US)	27027 Tourney Road, Suite 2E Valencia, CA 91355 US
Q² Solutions Clinical Trials (UK)	1 Simpson Parkway The Alba Campus Rosebank Livingston EH54 7EG UK

APPENDIX C AMENDMENTS AND ADMINISTRATIVE CHANGES TO THE PROTOCOL

GlaxoSmithKline Biologicals SA Vaccines R &D Protocol Amendment 1	
eTrack study number and Abbreviated Title	116566 (ROTA-083)
EudraCT number	2012-001875-35
Amendment number:	Amendment 1
Amendment date:	24 July 2013
Co-ordinating author:	PPD
Rationale/background for changes: NDAC- Vaccines (New Drug Advisory Committee on Vaccines) of Drugs Controller General of India (DCGI) recommended to conduct this study as a Phase IV study instead of phase III as initially planned because *Rotarix* liquid file application is considered as a line extension of *Rotarix* Lyophilized formulation. The protocol was amended to adapt the change recommended by NDAC-Vaccines of DCGI.	
Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:	
Contributing authors • PPD, ***Clinical Regulatory Representative*** • PPD ***and*** PPD Clinical Safety	
Detailed Title A phase ~~III~~ ***IV***, 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* given as a two dose primary vaccination, in healthy infants with no previous history of RV illness or vaccination.	
Rationale for the study and study design The lyophilized formulation of the HRV vaccine was licensed in India in November 2007. Following the request of the Regulatory Authority in India, this study will be conducted to generate ***the additional*** clinical data ***for*** the liquid formulation of HRV vaccine in India ***as recommended by NDAC- Vaccines (New Drug Advisory Committee on Vaccines) of DCGI.***	

Exclusion criteria for enrolment

- Acute disease and/or fever at the time of enrolment.
  - Fever is defined as temperature ≥ 37.5°C /99.5°F on oral, axillary or tympanic setting, or ≥ 38.0°C /100.4°F on rectal setting. The preferred route for recording temperature in this study will be axillary.
  - Subjects with a minor illness (such as mild diarrhoea, mild upper respiratory infection) without fever may, be enrolled at the discretion of the investigator ***(warrants deferral of the vaccination).***

GlaxoSmithKline Biologicals SA	
Vaccines R &D	
Protocol Amendment 2	
eTrack study number and Abbreviated Title	116566 (ROTA-083)
EudraCT number	2012-001875-35
Amendment number:	Amendment 2
Amendment date:	19 September 2016
Co-ordinating author:	PPD

Rationale/background for changes The ROTA-083 (116566) protocol and protocol amendment 1 were written in the protocol template edition 13.2. The protocol amendment 2 is a rewriting of the protocol to comply with the latest version of the protocol template (edition 15.0, effective 1st April, 2016).

The study design has not been impacted by this amendment.

The main changes included in the current amendment are listed below:

- The addition of the definitions for End of Study (EoS) and Primary Completion Date (PCD),
- addition of the section for Benefit and Risk assessment,
- the definition of fever has been updated and
- other minor changes to align the protocol with the specific control terminology used in Clinical Data Interchange Standards Consortium (CDISC). This includes renaming of the study cohorts and numbering of day, so that Day 1 corresponds to the date of vaccination

GlaxoSmithKline Biologicals SA

Vaccines R &D
Protocol Amendment 3

eTrack study number and Abbreviated Title	116566 (ROTA-083)
EudraCT number	2012-001875-35
Amendment number:	Amendment 3
Amendment date:	31 October 2017
Co-ordinating author:	PPD

Rationale/background for changes: Based on the feedback from the regulatory authorities in India on the protocol amendment 2, the following changes have been made to the protocol:

- The phase of this clinical trial is revised from Phase IV to Phase III.
- The study objectives have been updated to include a confirmatory primary objective and the study has been powered accordingly.
- The statistical considerations have been revised to define the success criteria for primary objective.
- Collection and testing of stool samples from subjects who develop gastroenteritis (GE) during the study period is removed in order to simplify the study procedure and considering no subjects were eliminated from the according-to-protocol (ATP) cohort due to HRV GE in study ROTA-044. The ROTA-044 study was a Phase IIIB, randomised, multicentre, double blind, placebo controlled study conducted in India to assess the immunogenicity and safety of two doses of GSK Biologicals' lyophilized formulation of HRV vaccine.
- The study procedures have been updated accordingly.
- The list of assays and section on biological samples evaluation have been updated.
- The laboratory assay section has been updated to account for the characteristics of revalidated GSK assay.
- The list of contributing authors has been updated and some typographic errors have been corrected.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Detailed Title: A phase ***III***,~~IV~~ randomized, open study to assess the immunogenicity, reactogenicity and safety of two different formulation~~s~~ 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.

Contributing authors

- PPD, Statistician
- PPD, Lead Statistician
- PPD, Study Delivery Lead
- PPD, PPD Clinical Laboratory Sciences (CLS), Clinical Readout Team Leaders
- PPD, PPD, Clinical Safety representatives
- PPD, Oversight Data Manager
- PPD, Study Data Manager
- PPD, Global Patents representative
- PPD, PPD Clinical Trial Supply Managers

Synopsis and Section 1.2- Rationale for the study and study design

The lyophilized formulation of the HRV vaccine was licensed in India in ~~November 2007~~ ***February 2008.***

The study is planned to be conducted in healthy infants aged ~~≥6~~ ***6-10 weeks*** at the time of the first dose. Two oral doses of the study vaccine will be administered to the subjects, according to a 0, 1 month schedule. The blood samples for immunogenicity assessment will be collected from all subjects before the first dose and at one month post dose 2. ~~In addition, stool samples will be collected from the subjects if they develop GE, to detect the presence of wild type RV~~

Please refer to the current Investigator Brochure (IB) for information regarding the summary of potential risks and benefits of the Rotarix vaccine.

Section 1.3 Benefit: Risk Assessment

Please refer to the ***current IB*** and prescribing information ***(lyophilized vaccine)*** for information regarding the summary of potential risks and benefits of the *Rotarix* vaccine.

The following section outlines the risk assessment and mitigation strategy for this study protocol

Section 1.3.1- Risk assessment

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Investigational study vaccine (*Rotarix*)		
Intussusception (IS)	Spontaneous data	• ***Subjects will be followed up to the end of the study after the receipt of the vaccine to check for any safety signal.*** • ***Parents/legally acceptable representatives (LARs) of*** subjects should report any untoward symptoms experienced after receiving the vaccine immediately to the investigator. • All Serious Adverse Events (SAEs) should be reported by the investigator immediately to GSK. • ***Subjects with SCID will be excluded from participating in this study (Refer to Section 4.3 and Section 6 for more details)***
Hematochezia	Spontaneous data	
Gastroenteritis with vaccine viral shedding in infants with severe combined immunodeficiency (SCID)	Spontaneous data	
Kawasaki disease	Based on signal observed for *RotaTeq* vaccine	

Synopsis and Section 2- Objectives

Primary objective:

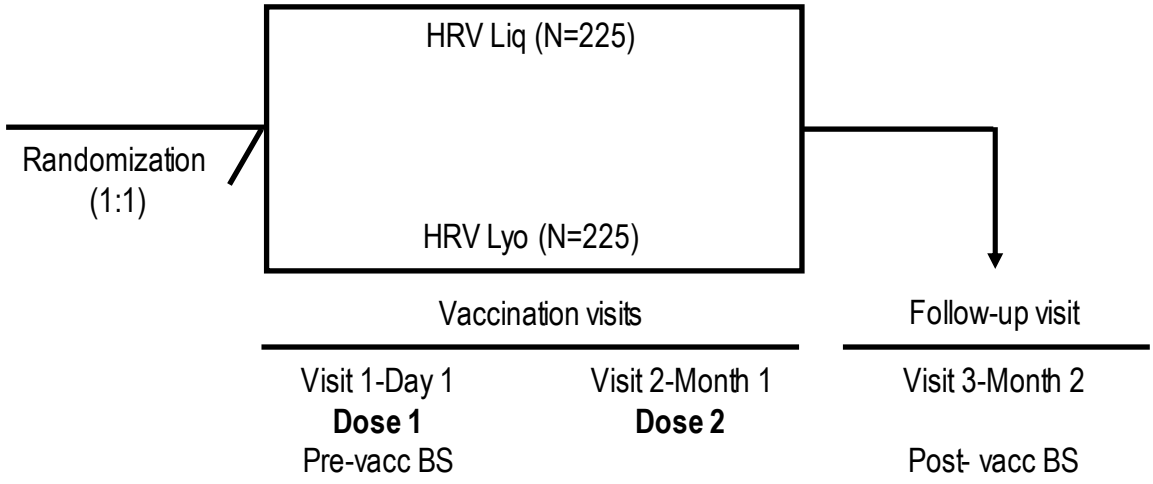
- ***To evaluate non-inferiority of GSK Biologicals' HRV liquid vaccine compared to GSK Biologicals' HRV lyophilized vaccine in terms of geometric mean concentrations (GMCs) for anti-RV antibodies, one month post dose 2 of HRV vaccine.***
  - ***Criterion: Non-inferiority will be stated if the lower 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 post dose 2 is greater than or equal to 0.5***
  - ~~To assess the immunogenicity of GSK Biologicals' HRV liquid vaccine and GSK Biologicals' HRV lyophilized vaccine, in terms of seroconversion rates, one month post dose 2 of HRV vaccine~~

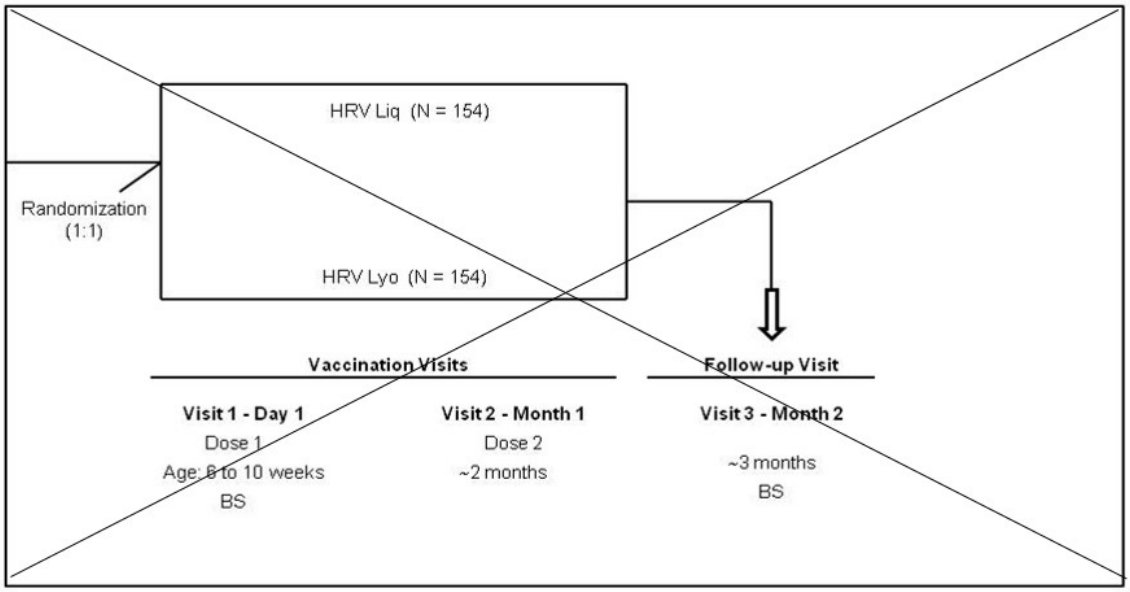
Secondary objectives

- ***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 immunogenicity of the two formulations of GSK Biologicals' HRV vaccine, in terms of Geometric Mean Concentration (GMC), one month post Dose 2 of HRV vaccine.~~
- ~~To assess the presence of wild type RV in GE stool samples collected from Dose 1 of the HRV vaccine up to Visit 3.~~

Section 3- Study Design Overview





HRV= Human Rota Virus; N = Number of subjects planned to be enrolled; Pre-Vacc= Pre-vaccination; Post-Vac= Post-vaccination; BS = Blood Sample

Study design: (Synopsis and Section 3)

- Experimental design: Phase ***III*** ~~IV~~ open-label, randomized ***(1:1)***, multi-centric, single-country study with two parallel groups.
- End of Study (EoS): Last testing results released for samples collected at Visit 3. ***Please refer to GLOSSARY OF TERMS for the definition of EoS***.

Synopsis Table 4 and 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*** ~~154~~	6 weeks-10 weeks	
HRV Lyo	***225*** ~~154~~	6 weeks-10 weeks	

- Sampling schedule: ~~Any GE episodes occurring from Dose 1 of the HRV vaccine up to Visit 3 will be recorded in the diary card. Parents/Legally Acceptable Representative(s) (LARs) will be instructed to collect the stool sample(s) if the subject develops GE during this period, from Dose 1 of HRV vaccine up to Visit 3. A stool sample will be collected as soon as possible, after illness begins, and preferably not later than 7 days after the start of GE symptoms, to identify the presence of wild-type RV exposure during vaccination and to eliminate such cases from Per-Protocol Set (PPS) analysis. Two occurrences of diarrhea will be classified as separate episodes if five or more diarrhea-free days occur between the episodes. Refer to glossary of terms for the definitions of GE and diarrhea~~

Section 4.1- Number of subjects/centers

The target will be to enrol ***450*** ~~308~~ ~~eligible~~ subjects ***(225 in each of the study groups)*** who will be randomly assigned to two study groups in a (1:1) ratio ~~(approximately 154 subjects in each group~~), to obtain ***at least 292*** ~~200~~ evaluable subjects (~~approximately~~ ***146***~~100~~ subjects in each group)

Overview of recruitment plan:

- Enrolment will be terminated when ~~308~~***450*** ~~eligible~~ subjects have been enrolled.

Section 4.3- Exclusion criteria

- Acute disease and/or fever at the time of enrolment. ***This warrants deferral of vaccination***.

Section 5.2.2.2- Study group and treatment number allocation to the subject:

The target will be to enrol ~~308~~ ***450*** ~~eligible~~ subjects who will be randomly assigned to two study groups in a (1:1) ratio (approximately ***225*** ~~154~~ subjects in each group~~), to obtain 200 evaluable subjects. (approximately 100 subjects in each group)~~.

Allocation of the subject to a study group at the investigator site will be performed using SBIR. The randomization algorithm will use a minimization procedure accounting for center ***and the study as minimization factors. Minimization factors will have equal weight in the minimization algorithm.***

After obtaining the signed and dated ICF from the subject's parent/LAR and having checked the eligibility of the subject, the site staff in charge of the vaccine administration will access SBIR. Upon providing the subject identification number, ***SBIR*** will provide the treatment number to be used for the first dose.

Table 4- List of study procedures

Age *at enrolment*	**6–10 weeks ~~~2 months~~ ~~~3 months~~**		
Epoch	***Epoch 001***		
Type of contact	**Visit 1**	**Visit 2**	**Visit 3**
Time points	**Day 1**	**Month 1**	**Month 2**
Sampling time points	**Pre-Vacc**		**Post-Vacc**
Informed consent	●		
Check inclusion/exclusion criteria	●		
Collect demographic data	●		
Check contraindications and warnings and precautions	●	●	
Medical history	●		
Physical examination	●		
History of previous vaccination from birth	●		
Pre-vaccination body temperature	●	●	
Vaccines			
Study group and treatment number allocation	O		
Treatment number allocation for subsequent doses		O	
Recording of administered treatment number	●	●	
HRV vaccine administration	●	●	
Record regurgitation	●	●	
HRV vaccine replacement dose administration in case of regurgitation *	●	●	
Laboratory Assays			
Blood sampling for antibody determination (2 ml)	●		●
~~Collection of stool sample post vaccination if the child develops GE~~			
Safety assessments			
Distribution of diary cards	O	O	
Recording of solicited adverse events within 8 days after each dose of HRV vaccine (Day 1–Day 8)	●	●	
Recording of non-serious adverse events within 31 days (Day 1–Day 31) post-vaccination	●	●	●
~~Recording of GE episodes for all subjects~~	~~●~~	~~●~~	~~●~~
Record any concomitant medication/vaccination	●	●	●
Record any intercurrent medical conditions	●	●	●
Recording of AEs/SAEs leading to withdrawal from study	●	●	●
Recording of SAEs	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●	●
Return of diary cards		O	O
Diary card transcription by investigator		●	●
Study Conclusion			●

● is used to indicate a study procedure that requires documentation in the individual eCRF.
○ is used to indicate a study procedure that does not require documentation in the individual eCRF.
LAR = Legally Acceptable Representative; ~~GE = Gastroenteritis~~; AE = Adverse Events; SAE = Serious Adverse Events
* If regurgitation or vomiting occurs after vaccination, a single replacement dose may be given at the same vaccination visit at the discretion of the Investigator.
Note: All subjects are allowed to receive routine childhood vaccinations according to the local immunization practice.

Table 5- Interval between study visits

Interval	Optimal length of interval [1]	Allowed interval[2]
Visit 1 → Visit 2	1 month	28-48 days after Dose 1
Visit 2 → Visit 3	1 month	~~21~~***31***-48 days after Dose 2

[1] Whenever possible the investigator should arrange the study visits within this interval.

[2] ~~Subjects will not be eligible for inclusion in the Per-Protocol Set (PPS) for analysis of immunogenicity if they make the study visit outside ***the 21-48 day*** interval.~~

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

Section 5.6.3: Collect demographic data

Record demographic data such as age, sex and ~~race~~ ***geographical ancestry*** in the subject's eCRF

Section 5.6.9.2- Other biological samples

~~**Collection of stool samples**~~

~~Parents/LARs of all subjects will be instructed to collect stool sample(s) from the subject if the subject develops GE from Dose 1 of the HRV vaccine up to Visit 3. A stool sample should be collected as soon as possible, after the illness begins, and preferably not later than 7 days after the start of GE symptoms. A second GE stool sample should be collected if the first sample is insufficient. A stool sample should be collected for each GE episode. Two occurrences of diarrhea will be classified as separate episodes five or more diarrhea-free days occur between the episodes.~~

~~Refer to the Module on Biospecimen Management in the SPM for detailed instructions for the collection, handling and processing of the samples~~

Table 6- Biological samples

Sample type	Quantity	Unit	Time points
Blood	Approximately 2	ml	Visit 1 and Visit 3
~~Stool~~	~~NA~~	~~NA~~	~~From Dose 1 of the HRV vaccine up to Visit 3~~

ml-milliliter~~; NA-Not Applicable~~

Section 5.6.10- Study vaccine administration

After completing all prerequisite procedures prior to vaccination, two oral doses of the study vaccine will be administered at an approximate one-month interval to subjects, according to the immunization schedule for HRV vaccine administration ~~in the participating country~~ (refer to Section 6.3 for detailed description of the vaccines administration procedure). If the investigator or delegate determines that the subject's health on the day of administration temporarily precludes vaccines administration, the visit will be rescheduled within the allowed interval for this visit (refer to Table 5)

Section 5.6.13; Recording of AEs and SAEs

At each vaccination visit, diary cards will be provided to the subject's parent(s)/LAR(s). The subject's parent(s)/LAR(s) will be instructed to measure and record the oral, axillary or rectal body temperature, and any solicited general AEs (i.e., on the day of vaccination and during the next 7 days) or any unsolicited AEs (i.e., on the day of vaccination and during the next 30 days occurring after vaccination) ~~or any GE episodes occurring from Dose 1 of the HRV vaccine up to Visit 3~~. The subject's parent(s)/LAR(s) will be instructed to return the completed diary card to the investigator at the next study visit.

Section 5.7.3- Laboratory assays

~~**GE Stool analysis**~~

~~All GE stool samples collected from Dose 1 of the HRV vaccine up to Visit 3 will be tested at GSK Biologicals' laboratory or in a validated laboratory designated by GSK Biologicals using standardized and validated procedures, for the purpose of identifying wild-type RV exposure during vaccination and to eliminate such cases from PPS immunogenicity analysis.~~

~~All GE stool samples will be analyzed by ELISA for detection of RV antigen. If a stool sample tests positive for RV antigen, the sample will be tested by Reverse Transcription Polymerase Chain reaction (RT-PCR) followed by sequencing to determine the G and P genotype.~~

Table 7- Humoral Immunity (Antibody determination)

System	Component	Method	Kit/Manufacturer	Unit	Cut-off	Laboratory
SER	Rotavirus Ab.IgA	ELI	N/A	U/ml	***13**** ~~20~~	GSK Biologicals**

SER = Serum
IgA = Immunoglobulin A
ELI =ELISA (Enzyme Linked Immunosorbent Assay)
U = Units; ml = milliliters
* ***This corresponds to the technical cut-off of the revalidated laboratory assay.***
GSK Biologicals laboratory refers to the Clinical Laboratory Sciences (CLS) in Rixensart, Belgium; Wavre, Belgium ***and Marburg, Germany*

Additional exploratory testing on the vaccine and/or on the disease under study may be performed within the framework of the study if deemed necessary for accurate interpretation of the data or should such assay(s) become available at GSK. These assays may not be represented in the objectives/endpoints of the study protocol

Table 8- Immunological read-outs

Blood sampling time point				
Type of contact and time point	**Sampling time point**	**No. subjects**	**Component**	**Components priority rank**
Visit 1 (Day 1)	Pre-Vacc	All	Rotavirus Ab.IgA	None
Visit 3 (Month 2)	Post-Vacc	All	Rotavirus Ab.IgA	None
		~~**GE analysis**~~[†]		
~~Visit 1 (Day 1) to Visit 3 (Month 2)~~	~~From Dose 1 of the HRV vaccine up to Visit 3~~	~~All subjects with GE~~	~~RV antigen~~	~~None~~

Pre-Vacc- Pre-vaccination; Post-Vacc- Post Vaccination; Ab- Antibody ~~RV = Rotavirus; IgA = Immunoglobulin A~~

~~† Stool analysis will be performed for subjects who experience GE episodes~~.

Section 5.7.5- Immunological correlates of protection

No immunological correlate of protection has been demonstrated so far for the antigen used as part of the HRV vaccine. ***However, a study by Cheuvart et al., in 2014 indicated that post-vaccination anti-RV IgA seropositivity (antibody concentration ≥ 20 U/mL) may serve as a useful correlate of vaccine efficacy in clinical trials of Rotarix [Cheuvart, 2014].***

The investigator is encouraged to share the immunological assay results for non-responders with the study subjects' parent(s)/LAR(s).

Table 12- Reporting periods for collecting safety information

Event	Pre-V1*	V1 D1	7 days post vacc D 8	30 days post vacc D 31		V2 M1	7 days post vacc	30 days post vacc		V3 M2
										Study Conclusion
Solicited general AEs										
Unsolicited AEs										
~~GE episodes**~~										
AEs/SAEs leading to withdrawal from the study										
SAEs										
SAEs related to study participation or concurrent GSK medication/vaccine										

* Consent obtained. Pre-V: pre-vaccination; Post-Vacc: post-vaccination; V: Visit; D: Day, M: Month;
~~GE = Gastroenteritis~~; AEs = Adverse Events; SAEs = Serious Adverse Events
~~** If GE appears during the solicited period, then it will be recorded on the solicited AEs screen of eCRF as diarrhea and if it appears outside the solicited period up to Visit 3, then it will be recorded as an unsolicited AE.~~

Table 13- Intensity scales for solicited symptoms in infants

Infants		
Adverse Event	**Intensity grade**	**Parameter**
Fever*		Record temperature in °C/°F
	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
Irritability/Fussiness	0	Behavior as usual
	1	Mild: Crying more than usual/no effect on normal activity
	2	Moderate: Crying more than usual/interferes with normal activity
	3	Severe: Crying that cannot be comforted/prevents normal activity
Diarrhea§		Record the number of looser than normal stools/day
	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§		Record the number of vomiting episodes/day
	0	Normal (no emesis)
	1	1 episode of vomiting/day
	2	2 episodes of vomiting/day
	3	≥3 episodes of vomiting/day
Loss of appetite	0	Appetite as usual
	1	Mild: Eating less than usual/no effect on normal activity
	2	Moderate: Eating less than usual/interferes with normal activity
	3	Severe: Not eating at all
Cough/runny nose	0	Normal
	1	Mild: Cough/runny nose which is easily tolerated
	2	Moderate: Cough/runny nose which interferes with daily activity
	3	Severe: 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.

The intensity grade for diarrhea, vomiting and fever as shown in Table 13 will be scored at GSK Biologicals.

Section 6.5: Contraindications to subsequent vaccination

- Subjects with a minor illness (such as mild diarrhoea, mild upper respiratory infection) without fever may be ***vaccinated*** ~~enrolled~~ at the discretion of the investigator**.**

Section 6.7.2: Concomitant medications/products/vaccines that may lead to the elimination of a subject from per-protocol analyses

- A vaccine not foreseen by the study protocol administered during the period starting from 30 days before the first dose of HRV vaccine* administration and ending at Visit 3** blood sampling, with the exception of the inactivated influenza vaccine, and other licensed routine childhood vaccinations which are allowed at any time during the study.

  ****Administration of any HRV vaccine other than the study vaccine will lead to elimination (refer to Section 4.3).***

  **In case an emergency mass vaccination for an unforeseen public health threat (e.g.: a pandemic) is organized by the public health authorities, outside the routine immunization program, the time period described above can be reduced if necessary for that vaccine provided it is licensed and used according to its Prescribing Information and according to the local governmental recommendations and provided a written approval of the Sponsor is obtained.

A detailed, comprehensive list of reasons for elimination from PPS analyses will be established at the time of data cleaning

Synopsis, Section 10.1 and Section 10.2- Endpoints

Primary Endpoint

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

Secondary

- Anti-RV antibody concentrations
  - ***Anti-RV IgA antibody seroconversion rate, one month post dose 2 of HRV vaccine***.
- ~~Presence of wild type RV in GE stool samples~~
  - ~~Presence of wild type RV in GE stools collected from Dose 1 of the HRV vaccine up to Visit 3~~

Section 10.3 Determination of sample size

A total sample of 450 subjects (225 subjects per arm) is based on a 90% power to detect non-inferiority on GMCs using PASS 2014, under the alternative hypothesis

of equal means and variances (Table 15). Based on historical data (ROTA- 044) it is assumed that up to 35% of the subjects will not be evaluable for the analysis of the primary endpoint, therefore the sample was increased accordingly.

Table 15: Probability that the lower limit of the 95% CI around the anti-RV IgA antibody GMC ratio (HRV Liq/HRV Lyo), one month after dose 2 of HRV vaccine, is ≥ 0.5

Endpoint	*Standard deviation [Log_{10} (titre)]*	*N evaluable (each HRV group)*	*Power*	*Alpha*
Anti-RV IgA antibody concentration	*0.790**	*146*	*90%*	*0.025*

****=Reference used for power computation***

~~Considering a target sample size of 308 enrolled subjects (1:1 randomization, 154 subjects in each group) and assuming that up to 35% of the subjects will not be available for the analysis of the primary endpoint (Reference: Rota-044), it can be considered that at least 200 subjects (100 in each group) will be available for the analysis.~~

- ~~Table 15 presents the exact two-sided 95% Confidence Interval (CI) with the sample size for different values of seroconversion rates, for subjects in HRV Liq group~~**.**

Table 15 Exact 95 percent CI with the sample size for different values of seroconversion rates, for subjects in HRV Liq group

Number of evaluable subjects (N)	Anti rotavirus IgA seroconversion rate with 95%CI			
	n	%*	Exact 95%CI	
			LL	UL
100	50	50	39.8	60.2
100	60	60	49.7	69.7
100	70	70	60.0	78.8
100	80	80	70.8	87.3
100	85	85	76.5	91.4
100	90	90	82.4	95.1

*Range of seroconversion rates in agreement with following reference studies:
Rota-044: 58.3% [95% CI: 48.7; 67.4], Rota-036: 83.9% [95% CI: 83.9; 88.8], Rota-061: 88.6% [86.1; 90.8]
n = Number of subjects,
% = (n/N)*100, LL = Lower Limit, UL = Upper Limit
95% CI = 95% Confidence Interval

Section 10.4.2- Per-Protocol Set for analysis of immunogenicity

- ***for whom the post-vaccination immunogenicity data are within the 21-48 days interval after the second dose***.
- who have no RV other than vaccine strain in GE stool samples collected up to Visit 3 blood sample,

Section 10.5- Derived and transformed data

Immunogenicity

- A seronegative subject is a subject whose anti-RV IgA antibody concentration is below the clinically meaningful threshold of <20 U/ml*.
- The GMCs calculations are performed by taking the anti-log of the mean of the log concentrations transformations. Antibody concentrations below the cut-off (<***13**** ~~20~~ 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 clinically meaningful threshold to define seroconversion while 13 U/ml corresponds to technical cut off of revalidated laboratory assay.

Section 10.6- Analysis of demographics.

The number of subjects enrolled into the study as well as the number of subjects excluded from PPS analyses will be tabulated.

The median, mean, range and standard deviation of age (in weeks) at each HRV liquid vaccine dose will be computed. The median, mean and standard deviation of height in centimeter (cm) and weight in kilograms (kg) at Visit 1 will be computed. ~~The Body Mass Index (BMI) at Visit 1 will also be computed as weight (in kg)/height² (in meters)~~. The ~~racial~~ ***geographical ancestry*** and sex composition will be presented.

Section 10.7.1- Within groups 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).
- ~~For each treatment group and for anti-rotavirus IgA antibody concentration,~~
  - ~~Seroconversion rates at Visit 3 and their exact 95% CI will be computed [Clopper, 1934].~~
  - ~~GMCs and their 95% CIs at Visit 3 will be computed.~~

Section 10.7.2- 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)***.

Refer to the Section 2.1 for the success criteria of the primary objective.

Section 10.8.1- 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 ~~[Clopper, 1934~~].The same calculations will be done for symptoms (solicited or unsolicited) rated as grade 3 in intensity, ~~and~~ 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 ~~[Clopper, 1934~~]. The same calculations will be done for each individual solicited general symptom rated as grade 3 in intensity ~~and~~ 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 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 [Clopper, 1934], will be tabulated by group, and by preferred term. Similar tabulation will be done for unsolicited AEs rated as grade 3, for unsolicited AEs with causal relationship to vaccination, ***for symptoms resulting in medically attended visit*** and for AEs or SAEs leading to drop out.

~~The percentage of subjects reporting GE episodes from Dose 1 of HRV lyophilized vaccine/HRV liquid vaccine up to Visit 3 will be tabulated by group~~.

~~The percentage of subjects with presence of wild-type RV in GE stool samples collected from Dose 1 of HRV lyophilized vaccine/HRV liquid vaccine up to Visit 3 will be tabulated by group.~~

~~The percentages of GE episodes with no available stool results from Dose 1 of HRV vaccine up to Visit 3 will be tabulated~~.

Section 10.7 Interpretation of analyses

Except for analyses addressing the primary objectives referred as confirmatory analyses, all the analyses will be descriptive/exploratory in nature. ~~All analyses will be descriptive with the aim to characterise the observed outcomes.~~ These descriptive analyses should be interpreted with caution.

Section 10.11- Conduct of analysis

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the final study report

Appendix A- Laboratory Assays

~~**Antigen Detection in Stool Samples**~~

~~The RV antigen in stool samples collected during GE episodes will be detected by ELISA at Central Lab (GSK or designated laboratory).~~

~~**RV strain genotyping**~~

~~The RV RNA will be isolated from ELISA RV positive stool samples. In order to determine the RV P and G type, VP4 and VP7 genes will be then amplified by Reverse Transcriptase Polymerase Reactions (RT-PCR) using two separate primer sets. VP4 and VP7 amplimers will be further analyzed by direct sequencing. The sequences will be interpreted using BLAST database searches in Genbank and phylogenetic analysis.~~

Appendix B Clinical Laboratories

Table 16 GSK Biologicals' laboratories

Laboratory	Address
GSK Biological's Clinical Laboratory Sciences (CLS), Rixensart	Biospecimen Reception-B7/44 Rue de l'Institut, 89 B-1330 Rixensart Belgium
GSK Biological's CLS, Wavre-Nord Noir Epine	Avenue Fleming, 20 B-1300 Wavre Belgium
GSK Vaccines GmbH	***Emil-von-Behring-Str. 76*** ***35041 Marburg*** ***Deutschland/ Germany***

Table 17 Outsourced laboratories

Laboratory	Address
Q² Solutions Clinical Trials (US)	27027 Tourney Road, Suite 2E Valencia, CA 91355 US
Q² Solutions Clinical Trials (UK)	1 Simpson Parkway The Alba Campus Rosebank Livingston EH54 7EG UK
~~DDL Diagnostic Laboratory~~	~~Visseringlaan 25~~ ~~2288 ER Rijswijk~~ ~~The Netherlands~~

GlaxoSmithKline Biologicals SA	
Vaccines R &D	
Protocol Amendment 4	
eTrack study number and Abbreviated Title	116566 (ROTA-083)
EudraCT number	2012-001875-35
Amendment number:	Amendment 4
Amendment date:	30 October 2019
Co-ordinating author:	PPD

Rationale/background for changes: As per Protocol Amendment 3 (31 October 2017), subjects seropositive before vaccination are to be eliminated from the Per-Protocol Set (PPS) for immunogenicity.

Laboratory testing of the pre-vaccination blood samples from the first 141 subjects enrolled in the study indicated a seropositivity rate of 47.5 % in these subjects. This suggests an overall high seropositivity rate at baseline in the total study population.

Therefore, an elimination rate exceeding the 35% rate of non-evaluable subjects predefined using historical data from Rota-044 study [Narang, 2009] is expected.

Because (1) the pre-vaccination seropositivity rate is expected to be high and (2) the effect of the vaccine can be measured in seropositive subjects, as previously demonstrated (see Table 1 below), the following changes have been made to the protocol:

- The PPS was modified to include subjects seropositive at baseline.
- The statistical method to derive the 95% confidence interval (CI) for the geometric mean concentration (GMC) group ratio was revised to be an ANCOVA.
- The seroconversion threshold was redefined to account for seropositive subjects at pre-vaccination:
  - for subjects with a pre-vaccination anti-rotavirus 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-rotavirus IgA antibody concentration ≥20 U/mL, seroconversion is achieved when the post-vaccination concentration is ≥2 times the pre-vaccination concentration.

Table 1: Distribution of fold increase in anti-rotavirus IgA antibody concentrations from pre-vaccination to one month post-dose 2 (Total vaccinated cohort for seropositive subjects at pre-vaccination, Rota-044) [Narang, 2009]

	HRV N = 43		Placebo N = 40	
Ratio	n	%	n	%
≥2	26	60.5	3	7.5
≥3	21	48.8	2	5.0
≥4	17	39.5	2	5.0

N = number of subjects seropositive at baseline for anti-rotavirus IgA antibodies and with one month post-dose 2 data available; n/% = number/percentage of subject in a given category.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Contributing authors:

PPD, Clinical Research and Development Lead (CRDL)

PPD, PPD, Study Delivery Leads

PPD Project Delivery Lead

PPD, Clinical Laboratory Sciences (CLS), Clinical Readout Team Leader

PPD, Laboratory Study Manager

PPD, Study Data Manager

PPD, PPD, Clinical Trial Supply Managers

PPD, Local Medical Lead

PPD, Local Delivery Lead

Synopsis and Section 2.2: 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.

Definition:

- ***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.***

~~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.~~

Synopsis and Section 10.2: Secondary endpoints

- Anti-RV IgA antibody concentrations
  - Anti-RV IgA antibody seroconversion***** rate, one month post dose 2 of HRV vaccine.

****Definition:***

- ***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***

Glossary of terms

Seroconversion:	***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.
	~~Appearance of anti-rotavirus Immunoglobulin A (IgA) antibody concentration ≥20 units (U)/milliliter (ml) in subjects initially (i.e., prior to the first dose of Human Rotavirus [HRV] vaccine) seronegative for anti-RV IgA antibody.~~

Section 1.1: Background

Rotarix is ***currently*** registered in ***over*** ~~at least~~ 130 countries and about ***552 446 066*** ~~405,524,425~~ doses of the vaccine (lyophilized and liquid formulations) are estimated to have been distributed worldwide since its launch until July ***2019*** ~~2017.~~

Table 9: Study vaccine

Treatment name	Vaccine name	Formulation	Presentation	Volume to be administered orally	Number of doses
HRV Liquid	HRV Liquid vaccine	**Active substance**: HRV ***RIX4414*** ~~RIX4144~~ live attenuated >=$10^{6.0}$$CCID_{50}$ **Excipients:** Sucrose=1.073g; Di-sodium Adipate=132.74mg; DMEM=2.26mg; water for injection=1.5ml	Liquid vaccine in a pre-filled oral applicator	1.5 ml	2
HRV Lyophilized	HRV Lyophilized vaccine	HRV ***RIX4414*** ~~RIX4144~~ live attenuated >=$10^{6.0}$$CCID_{50}$	Lyophilized vaccine in a monodose glass vial. Diluent (calcium carbonate buffer) supplied separately	1 ml	2
	HRV Diluent	$CaCO_3$=60mg	Diluent for lyophilized vaccine (calcium carbonate liquid antacid) supplied separately in a prefilled oral applicator		

HRV=Human Rotavirus; $CCID_{50}$ = Median Cell Culture Infective Dose (quantity of virus causing infection in 50% of exposed cells); DMEM = Dulbecco's Modified Eagle Medium; ml = milliliter;

Section 10.3: Determination of sample size

Considering the ~~a~~ total sample of 450 subjects (225 subjects per arm)~~is based on a 90% power to detect non-inferiority on GMCs using PASS 2014, under the alternative hypothesis of equal means and variances (Table 15). Based on historical data (study ROTA- 044) it is assumed that up to 35% of the subjects will not be evaluable for the analysis of the primary endpoint, therefore the sample was increased accordingly.~~, ***Table 15 provides the power to demonstrate non-inferiority in terms of GMCs according to the percentage of non-evaluable subjects***.

Based on historical data from ROTA-044 study [Narang, 2009], 13.8% of subjects were non-evaluable for reasons other than 'initially positive or unknown for serum anti-RV IgA antibodies at Dose 1'. The power to reach the primary objective in a scenario of 13% non-evaluable subjects is 96% (Table 15). Considering a scenario of 35% non-evaluable subjects, the power to reach the primary objective is 90%. As shown in Table 15, the power decreases when the % of non-evaluable subjects increases.

Table 15: Power according to different scenarios for the % of non-evaluable subjects, with respect to a total sample size of 450 subjects*

Standard deviation [Log_{10} (concentration)]	*Alpha*	*% of non-evaluable subjects*	*Power*
*0.790***	*0.025*	*13%*	*96%*
		15%	*96%*
		25%	*94%*
		30%	*92%*
		35%	*90%*
		40%	*88%*
		45%	*85%*
		50%	*81%*
		55%	*77%*

PASS one-sided non-inferiority for 2 independent means with common variance for log10(0.5) as non-inferiority margin, for the alternative hypothesis that the means are equal and for a type I error=2.5 %
****Reference used for power computation***

~~Table 15: Probability that the lower limit of the 95% CI around the anti-RV IgA antibody GMC ratio (HRV Liq/HRV Lyo), one month after dose 2 of HRV vaccine, is ≥ 0.5. (Amended: 31 October 2017)~~

~~Endpoint~~	~~Standard deviation [Log_{10} (titre)]~~	~~N evaluable (each HRV group)~~	~~Power~~	~~Alpha~~
~~Anti-RV IgA antibody concentration~~	~~0.790*~~	~~146~~	~~90%~~	~~0.025~~

~~*=Reference used for power computation~~

Section 10.4.2: Per-Protocol Set for analysis of immunogenicity

- ~~who are seronegative for serum anti-RV IgA antibodies on the day of dose 1,~~

Section 10.5: Derived and transformed data

Immunogenicity

- ~~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.~~

- ***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.***

Section 10.7.1: Within group assessment

- The distribution of anti-RV IgA antibody concentrations at ***Visit 1 and*** Visit 3 will be displayed using Reverse Cumulative Curves (RCCs).

Section 10.7.2: 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 ratios and their 95% CI will be derived by exponential transformation of the corresponding group contrast in the model*** (primary objective)***.***

Section 10.10: Statistical Methods

- ***The standardized asymptomatic CI for the group difference in proportion will be calculated using SAS. The method used within GSK Biologicals is Method 6 [Newcombe, 1998].***

Section 13: References

Narang A, Bose A, Pandit AN, Dutta P, Kang G, Bhattacharya SK, Datta SK, Suryakiran PV, Delem A, Han HH, Bock HL. Immunogenicity, reactogenicity and safety of human rotavirus vaccine (RIX4414) in Indian infants. Hum Vaccin. 2009 Jun;5(6):414-9.