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

Primary Study vaccine	*Porcine circovirus* (PCV)-free liquid formulation of GlaxoSmithKline (GSK) Biologicals' oral live attenuated human rotavirus (HRV) vaccine (444563)
Other Study vaccines	• Lyophilized formulation of the oral live attenuated HRV vaccine (Rotarix®, GSK Biologicals) • Diphtheria and tetanus toxoids and acellular pertussis adsorbed, hepatitis B (recombinant) and inactivated poliovirus vaccine (Pediarix®, GSK Biologicals) • *Haemophilus* b conjugate vaccine (tetanus toxoid conjugate) (Hiberix®, GSK Biologicals) • Pneumoccocal 13-valent conjugate vaccine (diphtheria CRM197 Protein) (Prevenar 13®, Manufactured by Wyeth Pharmaceuticals Inc. Marketed by Pfizer Inc.)
eTrack study number and Abbreviated Title	201663 (ROTA-090)
Investigational New Drug (IND) number	BB-IND-16992
EudraCT number	2016-003210-27
Date of protocol	Final Version 1: 27 October 2016
Title	Immunogenicity, reactogenicity and safety study of Pediarix®, Hiberix® and Prevenar 13® co-administered with two different formulations of GSK Biologicals' HRV vaccine (444563) in healthy infants 6-12 weeks of age.
Detailed Title	A phase IIIA, randomized, single-blind, multi-centric study to evaluate the immunogenicity, reactogenicity and safety of three doses of Pediarix®, Hiberix® and Prevenar 13® when co-administered with two doses of the PCV-free liquid formulation of GSK Biologicals' oral live attenuated HRV vaccine as compared to the currently licensed lyophilized formulation of the HRV vaccine in healthy infants 6-12 weeks of age.
Co-ordinating author	PPD, Scientific writer
Contributing authors	• PPD, Clinical & Epidemiology Project Lead (CEPL), • PPD, Clinical Research and Development Lead (CRDL) • PPD and PPD, Statisticians

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Contributing authors	• PPD, Lead Statistician • PPD and PPD, Study Delivery Leads • PPD, Project Delivery Lead • PPD, Clinical Read-Out Team Leader • PPD, Laboratory Study Manager • PPD, Clinical Safety representative • PPD and PPD, Oversight Data Managers • PPD, Study Data Manager • PPD, Global Regulatory Affairs representative • PPD, Global Patents representative • PPD, Clinical Trial Supply Manager

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Protocol Sponsor Signatory Approval

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Sponsor signatory	Paul Gillard, Clinical & Epidemiology Project Lead (CEPL), GlaxoSmithKline Biologicals, SA.

Signature _____

Date _____

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Protocol 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 vaccine, 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.

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Investigator name

_____

Signature

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Date

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Sponsor Information

1. Sponsor

GlaxoSmithKline Biologicals
Rue de l'Institut 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 8.4.2.

SYNOPSIS

Detailed Title	A phase IIIA, randomized, single-blind, multi-centric study to evaluate the immunogenicity, reactogenicity and safety of three doses of Pediarix®, Hiberix® and Prevenar 13® when co-administered with two doses of the PCV-free liquid formulation of GSK Biologicals' oral live attenuated HRV vaccine as compared to the currently licensed lyophilized formulation of the HRV vaccine in healthy infants 6-12 weeks of age.
Indication	Active immunization of infants against gastroenteritis (GE) due to rotavirus (RV).
Rationale for the study and study design	• Rationale for the study Using advanced technology in 2010, researchers from the University of California, San Francisco identified Deoxyribonucleic acid (DNA) fragments of Porcine circovirus type 1 (PCV-1) in *Rotarix*. Further investigations conducted by GSK and the United States (US) Food and Drug Administration (FDA) confirmed the presence of PCV-1 DNA fragments in *Rotarix* and its starting materials. Low levels of PCV-1 viral particles were also detected during the production process and in the final container. Retrospective laboratory investigations conducted by GSK on 40 human rotavirus (HRV) vaccine recipients showed that none of the subjects who received the HRV vaccine demonstrated seroconversion to PCV-1, however, PCV-1 from the vaccine was identified in the stool samples of 4 infants from the HRV group (at Day 3 and Day 7 post Dose 1). The detection of PCV-1 DNA only at the earliest time-points post-vaccination was consistent with transient passage of DNA through the infants' digestive tracts without replication. Lack of PCV-1 infection is further supported by the absence of anti-PCV-1 antibody in the HRV vaccine recipients, including the infants who had PCV-1 DNA detected in their stool samples [Dubin, 2013]. In a follow-up retrospective laboratory analysis of samples of 596 subjects, a post-vaccination anti-PCV-1 antibody seropositivity rate of 1% [90% Confidence Interval (CI): 0.3-2.6] in recipients of the human RV vaccine (3/299 samples) and 0.3% [90% CI: 0.0-1.6] in the placebo group (1/296 samples) was observed. The difference in the post-vaccination seropositivity and the seroconversion rates between the two study groups were -0.66 [90% CI: -2.16-0.60] and -0.33 [90% CI: -1.70–0.89], respectively. The 90% CI for both group differences included 0, indicating that there was no statistically significant increase as compared to the placebo

[Han, 2016]. Therefore, currently available data does not suggest occurrence of PCV-1 infection in infants who received *Rotarix* in clinical trials. These results are consistent with published literature which indicates that PCV-1 is not capable of causing infection in humans [Hattermann, 2004a; Hattermann, 2004b].

GSK has replaced the cell bank and virus seeds used as base production material for its HRV vaccine. In accordance with the regulators, the company continues to manufacture *Rotarix* to the existing standards of approved production and quality, to meet public health needs worldwide.

This study will assess if there is any immune interference between the PCV-free liquid HRV vaccine and routine infant vaccinations currently in use in the US, namely *Pediarix*, *Hiberix* and *Prevenar 13* as compared to the currently licensed lyophilized formulation of the HRV vaccine when co-administered with the same routine vaccinations.

- Rationale for the study design

The study is designed as a randomized, controlled, single-blind study with two parallel groups. This study will evaluate the immunogenicity, reactogenicity and safety of the vaccines *Pediarix*, *Hiberix* and *Prevenar 13* that are recommended for children in the US during the first year of life, when co-administered with the PCV-free liquid HRV vaccine as compared to the licensed lyophilized vaccine.

The currently licensed lyophilized *Rotarix* vaccine co-administered with routine infant vaccines in the US has been shown not to impair the immune response to any of the co-administered antigens [Dennehy 2008]. Therefore, the use of the currently licensed lyophilized vaccine as the control is considered appropriate. Furthermore, this study design will avoid logistical constraints related to the inclusion of co-administration and separate administration of US routine infant vaccines in the same study.

An Independent Data Monitoring Committee (IDMC), consisting of clinical experts and a biostatistician will review the safety data periodically to monitor the safety aspects of the PCV-free liquid HRV vaccine.

This study will have a single-blind design to ensure that the subject's parent(s)/Legally Acceptable Representative(s) (LARs) are not influenced by the knowledge of the *Rotarix* formulation when reporting safety events and when

complying with the study procedure.

Objectives

Co-Primary

- To demonstrate the non-inferiority of the immune responses to three doses of Pediarix, Hiberix and Prevenar 13 when co-administered with two doses of the PCV-free liquid HRV vaccine, as compared to when co-administered with the currently licensed lyophilized HRV vaccine, 1 month after Dose 3 of routine infant vaccines.

Criteria for non-inferiority:

  - *Lower limits of the two-sided standardized asymptotic 95% confidence intervals (CIs) on the differences between groups (HRV Liq group minus HRV Lyo group) in the percentages of subjects with seroprotective concentrations (≥0.1 IU/mL) for each of anti-diphtheria (anti-D) and anti-tetanus (anti-T) antibodies are ≥-10% (clinical limit for non-inferiority),*
  - *The lower limit of the two-sided standardized asymptotic 95% CI on the difference between groups (HRV Liq group minus HRV Lyo group) in the percentages of subjects with seroprotective concentration (≥10 mIU/mL) for antibodies against hepatitis B surface antigen (anti-HBs) is ≥-10% (clinical limit for non-inferiority),*
  - *Lower limits of the two-sided standardized asymptotic 95% CIs on the differences between groups (HRV Liq group minus HRV Lyo group) in the percentages of subjects with seroprotective titers (≥8 ED_{50}) for each of anti-poliovirus serotypes 1, 2 and 3 antibodies are ≥-5% (clinical limit for non-inferiority),*
  - *Lower limits of the two-sided 95% CIs on the geometric mean antibody concentrations (GMC) ratios (HRV Liq group over HRV Lyo group) for antibodies against each of the pertussis toxoid (PT), filamentous hemagglutinin (FHA) and pertactin (PRN) antigens [anti-PT, anti-FHA and anti-PRN] are ≥0.67 (clinical limit for non-inferiority),*
  - *Lower limits of the two-sided 95% CIs on the GMC ratios (HRV Liq group over HRV Lyo group) for each of the 13 Streptococcus pneumoniae (S. pneumoniae) serotypes are ≥0.5 (clinical limit for*

non-inferiority),

- *The lower limit of the two-sided standardized asymptotic 95% CI on the difference between groups (HRV Liq group minus HRV Lyo group) in the percentages of subjects with concentration of antibodies against polyribosyl-ribitol-phosphate antigen (anti-PRP) ≥0.15 μg/mL is ≥- 5%,*
- *The lower limit of the two-sided standardized asymptotic 95% CI on the difference between groups (HRV Liq group minus HRV Lyo group) in the percentages of subjects with concentration of antibodies against anti-PRP ≥1.0 μg/mL is ≥-10% (clinical limit for non-inferiority).*

- To rule out a 10% decrease in seroresponse to PT, FHA and PRN antigen when *Pediarix* is co-administered with PCV-free-liquid HRV vaccine compared to when *Pediarix* is co-administered with the currently licensed lyophilized HRV vaccine.
  - seroresponse is defined as the percentage of subjects showing an antibody concentration above a threshold that leads to 95% seroresponse in the control group (lyophilized HRV vaccine),
  - p-value on the difference in seroresponse between groups is < 2.5% for each PT, FHA and PRN antigen (p-value is computed by integrating on the p-value for the null hypothesis that the seroresponse rate in the liquid group is < 85% and the a-posteriori probability of the threshold in the lyophilized group).

Secondary

Immunogenicity:

- To assess the immunogenicity of the PCV-free liquid HRV vaccine and currently licensed lyophilized HRV vaccine in terms of serum anti-rotavirus immunoglobulin A (IgA) antibody seropositivity rate at Visit 4, 3 months after Dose 2 of the HRV vaccine.
- To assess the immunogenicity of routine infant vaccines *Pediarix*, *Hiberix* and *Prevenar 13* when co-administered with the PCV-free liquid HRV vaccine and currently licensed lyophilized HRV vaccine at Visit 4, 1 month after Dose 3 of routine infant vaccines.

Reactogenicity and safety:

- To assess the reactogenicity of the PCV-free liquid HRV vaccine and currently licensed lyophilized HRV vaccine in terms of general solicited adverse events (AEs) during the 8 day (Day 1-Day 8) follow-up period after each dose of HRV vaccine.
- To assess the safety of the study vaccines in terms of unsolicited AEs during the 31 day (Day 1-Day 31) follow-up period after each dose of HRV vaccine and serious adverse events (SAEs) during the entire study period.

Study design

- Experimental design: Phase IIIA, single-blind, randomized, controlled, multi-centric study with two parallel groups.
- Duration of the study: The total duration of the study, per subject, will be approximately 10 months including the 6 months of Extended Safety Follow-Up (ESFU) period after the last dose of study vaccine administered.
  - Epoch 001: Primary starting at Visit 1 (Day 1) and ending at the ESFU contact (Month 10).
- Primary completion Date (PCD): Last subject attending Visit 4.
- End of Study (EoS): Last testing results released for samples collected at Visit 4. If the last testing results are available before the Last Subject Last Visit (LSLV), i.e., before the ESFU, the EoS will then be the LSLV.
- Study groups:

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

The study groups and the 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	640	6 weeks-12 weeks	●
HRV Lyo	640	6 weeks-12 weeks	●

Synopsis Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine name	Study Groups	
		HRV Liq	HRV Lyo
HRV Liquid	HRV PCV-free	x	
HRV Lyophilized	HRV*		x
Pediarix	DTPa-HBV-IPV	x	x
Hiberix	*Hib*	x	x
Prevenar 13	*Prevenar 13*	x	x

*Licensed lyophilized HRV vaccine.

- Control: active control-GSK Biologicals' currently licensed lyophilized HRV vaccine.
- Vaccination schedule: Two doses of HRV vaccine to be administered according to a 0, 2 month schedule as per the immunization schedule for HRV vaccine administration in the US.

  Co-administration of routine childhood vaccines *Pediarix*, *Hiberix* and *Prevenar 13* will be performed as follows:

  – All the subjects will receive a dose each of *Pediarix*, *Hiberix* and *Prevenar 13* at Visit 1 (Day 1), Visit 2 (Month 2) and Visit 3 (Month 4).

  – The routine booster dose for the co-administered vaccines will not be administered to subjects as a part of this study. Subject's parent(s)/LARs will be reminded at Visit 4 to consult their primary health care provider regarding the booster dose of the vaccines for their child.

- Treatment allocation: Randomized 1:1 using GSK Biologicals' Randomization System on Internet (SBIR).
- Blinding: single-blind.

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

Synopsis Table 1 Blinding of study epoch

Study Epoch	Blinding
Epoch 001	single-blind

- Sampling schedule: Details of the samples to be collected are as follows:
  - Blood samples will be collected from all subjects at Visit 4 (Month 5) to measure serum anti-RV IgA antibody concentrations and antibody concentrations/titers against all the antigens in the co-administered vaccines.
- Type of study: self-contained.
- Data collection: Electronic Case Report Form (eCRF).
- Safety monitoring: An IDMC consisting of clinical experts and a biostatistician will review the safety data by treatment group periodically to monitor the safety aspects of the PCV-free liquid HRV vaccine.

Number of subjects The target will be to enrol 1280 eligible subjects who will be randomly assigned to two study groups in a (1:1) ratio (approximately 640 subjects in each group).

Endpoints

Primary

- Immunogenicity with respect to components of the routine infant vaccines, one month after Dose 3 of routine infant vaccines (Visit 4):
  - Anti-D antibody concentration ≥0.1 IU/mL,
  - Anti-T antibody concentration ≥0.1 IU/mL,
  - Anti-HBs antibody concentrations ≥10 mIU/mL,
  - Anti-poliovirus types 1, 2 and 3 antibody titers ≥8 ED_{50},
  - Anti-PT, anti-FHA and anti-PRN antibody concentrations expressed as GMCs,
  - Anti-pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) antibody concentrations expressed as GMCs,
  - Anti-PRP antibody concentrations ≥0.15 µg/mL,
  - Anti-PRP antibody concentrations ≥1.0 µg/mL.
- Difference in seroresponse with respect to PT, FHA and PRN antigen components one month after Dose 3 of routine infant vaccines (Visit 4):
  - Seroresponse to anti-PT, anti-FHA and anti-PRN.

Secondary

- Serum anti-rotavirus IgA antibody seropositivity 3 months after Dose 2 of HRV vaccine (Visit 4).
  - Serum anti-RV IgA antibody concentrations ≥20 U/mL and ≥90 U/mL 1-2 months after Dose 2.
- Immunogenicity with respect to components of the routine infant vaccines, one month after Dose 3 of routine infant vaccines (Visit 4):
  - PT, anti-FHA and anti-PRN antibody concentrations ≥2.693 IU/mL, ≥2.046 IU/mL and ≥2.187 IU/mL, respectively.
  - Anti-pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) antibody concentrations ≥0.35 µg/mL for the ELISA,
  - Anti-D, anti-T, anti-PRP and anti-HBs antibody concentrations expressed as GMCs and anti-poliovirus types 1, 2 and 3 antibody concentrations expressed as Geometric Mean Titers (GMTs).
- Occurrence of general solicited AEs during the 8 day (Day 1-Day 8) follow-up period after each dose of HRV vaccine.
- Occurrence of unsolicited AEs within 31 days (Day 1-Day 31) after any dose of HRV vaccine, according to the Medical Dictionary for Regulatory Activities (MedDRA) classification.
- Occurrence of SAEs from Dose 1 of study vaccines up to study end.

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LIST OF ABBREVIATIONS

ACIP	Advisory Committee on Immunization Practices
AE	Adverse Event
BMI	Body Mass Index
$CCID_{50}$	Median Cell Culture Infective Dose (quantity of virus causing infection in 50% of exposed cells)
CDC	Centers for Disease Control
CDISC	Clinical Data Interchange Standards Consortium
CI	Confidence Interval
CLIA	ChemiLuminescence ImmunoAssay
CLS	Clinical Laboratory Sciences
CSR	Clinical Study Report
D	Diphtheria
DNA	Deoxyribonucleic acid
DT	Diphtheria toxoid
ECL	ElectroChemiLuminescence
eCRF	electronic Case Report Form
ELISA	Enzyme Linked Immunosorbent Assay
EoS	End of Study
ES	Exposed Set
ESFU	Extended Safety Follow-Up
eTDF	Electronic Temperature excursion Decision Form
FDA	Food and Drug Administration, United States of America
FHA	Filamentous Hemagglutinin
GCP	Good Clinical Practice
GE	Gastroenteritis

GMC	Geometric Mean Concentration
GMT	Geometric Mean Titer
GSK	GlaxoSmithKline
HBs	Hepatitis B surface antigen
HHE	Hypotonic Hyporesponsive Episodes
Hib	*Haemophilus* type b
HRV	Human Rotavirus
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin G
IM	Intramuscular
IMP	Investigational Medicinal Product
IND	Investigational New Drug
IRB	Institutional Review Board
IS	Intussusception
Kg	Kilograms
LAR	Legally Acceptable Representative
LLOQ	Lower Limit Of Quantification
LSLV	Last Subject Last Visit
MATEX	MATerial Excellence

MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligrams
mL/ml	Milliliter
NEU	Neutralization Assay
PASS	Power Analysis and Sample Size
PCD	Primary Completion Date
PCV	Porcine circovirus
PPS	Per-Protocol Set
PRN	Pertactin
PRP	Polyribosyl-Ribitol-Phosphate: polysaccharide component of the Hib bacterium capsule
PT	Pertussis Toxoid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
RV	Rotavirus
SAS	Statistical Analysis System
SAE	Serious Adverse Event
SBIR	Randomization System on Internet
SCID	Severe Combined Immunodeficiency
SDV	Source Document Verification
SmPC	Summary of Product Characteristics
SPM	Study Procedures Manual
S. pneumoniae	*Streptococcus pneumoniae*
T	Tetanus
TT	Tetanus toxoid

TVC	Total vaccinated cohort
U	Unit
US	United States
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. In a single-blind study, the investigator and/or his staff are aware of the treatment assignment but the subject is not.
Child in care:	A child who has been placed under the control or protection of an agency, organisation, 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 enrollment 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 biological 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 [IMP])**	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):	An individual or juridical or other body authorized under applicable law to consent, on behalf of a prospective

(The terms legal representative or legally authorized representative are used in some settings.)	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.
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.
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/product:	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 vaccines 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 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.

TRADEMARKS

The following trademarks are used in the present protocol.

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

Trademarks of the GlaxoSmithKline group of companies	Generic description
Rotarix®	Human rotavirus vaccine
Pediarix®	Diphtheria and tetanus toxoids and acellular pertussis adsorbed, hepatitis B (recombinant) and inactivated poliovirus vaccine
Hiberix®	Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate)

Trademarks not owned by the GlaxoSmithKline group of companies	Generic description
Prevenar 13® (Wyeth Pharmaceuticals Inc.; Marketed by Pfizer Inc.)	Pneumoccocal 13-valent conjugate vaccine (diphtheria CRM_{197} protein)

1. INTRODUCTION

1.1. Background

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].

Although RV infection rarely causes death in Europe, North America and Australia, it remains the most common cause of hospitalization for GE in children [Desselberger, 2012]. In developed countries, RV infection remains the most common cause of hospitalization for GE in children and leads to major medical and societal costs. Over the last many years, the development of vaccines has been beneficial in the prevention of considerable morbidity and mortality due to RV. Two live oral HRV vaccines have been licensed in many countries; one is derived from an attenuated human strain of RV and the other combines five bovine-human reassortant strains [Glass, 2006]. Each of these vaccines have been proven highly effective in preventing severe RV diarrhea by substantially reducing the number and the associated costs of child hospitalizations and clinical visits for acute diarrhea in children. Moreover, these vaccines could reduce deaths from diarrhea and improve child survival through programs such as childhood immunizations and diarrheal disease control in developing countries. The World Health Organisation (WHO) recognises HRV vaccination as an effective measure to prevent RV infection and to reduce disease burden, and recommends its inclusion into all national infant immunization programs, 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].

GlaxoSmithKline (GSK) Biologicals' HRV vaccine (Rotarix™) is a vaccine for oral use, containing the live attenuated human rotavirus (HRV) RIX4414 strain. It has previously been observed that infants aged younger than three months who received the vaccine did not develop diarrhea, vomiting or fever during the trial [Vesikari, 2004(a)]. The initial trials that GSK conducted in Finland showed safety, immunogenicity and efficacy of the *Rotarix* vaccine [Vesikari, 2004(b)]. In Latin American and European studies, vaccine efficacy of oral live attenuated HRV vaccine *Rotarix* was high, ranging from 80.5% to 90.4% against severe RV GE, and 83.0% to 96.0% against hospitalization due to RV GE during the first two years of life.[Vesikari, 2007; Linhares, 2008]. Furthermore, results from a phase III clinical study undertaken in Singapore, Hong Kong, and Taiwan showed that during the first two years of life, two doses of *Rotarix* vaccine provided a high level of protection against severe RV GE (vaccine efficacy: 96.1%), and had a safety profile similar to the placebo [Phua, 2012]. Such safety and efficacy studies in Europe, Latin America and Asia have confirmed that the vaccine is safe, well-tolerated and efficacious (range: 80-96%) in preventing severe RV GE in the first two years of life [Cunliffe, 2014].

Rotarix is registered in at least 130 countries and more than 300 million doses of the vaccine (lyophilized and liquid formulations) are estimated to have been distributed worldwide since its launch until July 2016.

Please refer to the current Investigator Brochure (IB) for information regarding the pre-clinical and clinical studies and the epidemiological information of *Rotarix* vaccine.

1.2. Rationale for the study and study design

1.2.1. Rationale for the study

Using advanced technology in 2010, researchers from the University of California, San Francisco identified Deoxyribonucleic acid (DNA) fragments of Porcine circovirus type 1 (PCV-1) in *Rotarix*. Further investigations conducted by GSK and the United States (US) Food and Drug Administration (FDA) confirmed the presence of PCV-1 DNA fragments in *Rotarix* and its starting materials. Low levels of PCV-1 viral particles were also detected during the production process and in the final container. Retrospective laboratory investigations conducted by GSK on 40 HRV vaccine recipients showed that none of the subjects who received the HRV vaccine demonstrated seroconversion to PCV-1, however, PCV-1 from the vaccine was identified in the stool samples of 4 infants from the HRV group (at Day 3 and Day 7 post Dose 1). The detection of PCV-1 DNA only at the earliest time-points post-vaccination was consistent with transient passage of DNA through the infants' digestive tracts without replication. Lack of PCV-1 infection is further supported by the absence of anti-PCV-1 antibody in the HRV vaccine recipients, including the infants who had PCV-1 DNA detected in their stool samples [Dubin, 2013]. In a follow-up retrospective laboratory analysis of samples of 596 subjects, a post-vaccination anti-PCV-1 antibody seropositivity rate of 1% [90% Confidence Interval (CI): 0.3-2.6] in recipients of the human RV vaccine (3/299 samples) and 0.3% [90% CI: 0.0-1.6] in the placebo group (1/296 samples) was observed. The difference in the post-vaccination seropositivity and the seroconversion rates between the two study groups were -0.66 [90% CI: -2.16-0.60] and -0.33 [90% CI: -1.70–0.89], respectively. The 90% CI for both group differences included 0, indicating that there was no statistically significant increase as compared to the placebo [Han, 2016]. Therefore, currently available data does not suggest occurrence of PCV-1 infection in infants who received *Rotarix* in clinical trials. These results are consistent with published literature which indicates that PCV-1 is not capable of causing infection in humans [Hattermann, 2004a; Hattermann, 2004b].

GSK has replaced the cell bank and virus seeds used as base production material for its HRV vaccine. In accordance with the regulators, the company continues to manufacture *Rotarix* to the existing standards of approved production and quality, to meet public health needs worldwide.

This study will assess if there is any immune interference between the PCV-free liquid HRV vaccine and routine infant vaccinations currently in use in the US, namely *Pediarix*, *Hiberix* and *Prevenar 13* as compared to the currently licensed lyophilized formulation of the HRV vaccine when co-administered with the same routine vaccinations.

1.2.2. Rationale for the study design

The study is designed as a randomized, controlled, single-blind study with two parallel groups. This study will evaluate the immunogenicity, reactogenicity and safety of the vaccines *Pediarix*, *Hiberix* and *Prevenar 13* that are recommended for children in the US during the first year of life, when co-administered with the PCV-free liquid HRV vaccine as compared to the licensed lyophilized vaccine.

The currently licensed lyophilized *Rotarix* vaccine co-administered with routine infant vaccines in the US has been shown not to impair the immune response to any of the co-administered antigens [Dennehy, 2008]. Therefore, the use of the currently licensed lyophilized vaccine as the control is considered appropriate. Furthermore, this study design will avoid logistical constraints related to the inclusion of co-administration and separate administration of US routine infant vaccines in the same study.

An Independent Data Monitoring Committee (IDMC), consisting of clinical experts and a biostatistician will review the safety data periodically to monitor the safety aspects of the PCV-free liquid HRV vaccine.

This study will have a single-blind design to ensure that the subject's parent(s)/Legally Acceptable Representative(s) (LARs) are not influenced by the knowledge of the Rotarix formulation when reporting safety events and when complying with the study procedure.

1.3. Benefit: Risk Assessment

Please refer to the current IB and the Prescribing information for the summary of potential risks and benefits of the *Rotarix* vaccine.

Please refer to the Prescribing information for information regarding the summary potential risks and benefits of *Pediarix*, *Hiberix* and *Prevenar 13* vaccines.

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 6 months after receipt of the vaccine to check for any safety signal. • Parent(s)/LAR(s) should report any untoward symptoms their child experiences after receiving the vaccine immediately to the investigator. • All 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 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 *Rota Teq* vaccine	
GSK comparator (*Pediarix*)		
Apnoea (in premature infants)	Spontaneous data	Risk highest in infants born ≤28 weeks of gestation (Refer to Section 4.3 for more details).
Syncope	Spontaneous data	Subjects will be observed for at least 30 minutes after vaccine administration, with medical attention available in case of adverse event.
Hypotonic Hyporesponsive Episodes (HHE).	Spontaneous data	
Convulsion (with or without fever)	Spontaneous data	• Subjects will be followed up to 6 months after receipt of the vaccine to check for any safety signal. • Parent(s)/LAR(s) should report any untoward symptoms their child experiences after receiving the vaccine immediately to the investigator. • All SAEs should be reported by the investigator immediately to GSK.
Fever	Clinical trial data	
Encephalopathy	Spontaneous data	

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
GSK comparator (*Hiberix*)		
Apnoea (in premature infants)	Spontaneous data	Risk highest in infants born ≤28 weeks of gestation (Refer to Section 4.3 for more details).
Syncope	Spontaneous data	Subjects will be observed for at least 30 minutes after vaccine administration, with medical attention available in case of adverse event.
HHE	Spontaneous data	
Convulsion (with or without fever)	Spontaneous data	• Subjects will be followed up to 6 months after receipt of the vaccine to check for any safety signal. • Parent(s)/LAR(s) should report any untoward symptoms their child experiences after receiving the vaccine immediately to the investigator. • All SAEs should be reported by the investigator immediately to GSK.
	Comparator (*Prevenar 13*)	
See Package Insert		
	Study Procedures	
Allergic reaction to the vaccine.	Spontaneous data	Subjects will be observed for at least 30 minutes after vaccine administration, with medical attention available in case of anaphylaxis reactions.
Redness, swelling, pain at injection site	Clinical trial data	• Parent(s)/LAR(s) should report any untoward symptoms their child experiences after receiving the vaccine to the investigator. • All SAEs should be reported by the investigator immediately to GSK.

1.3.2. Benefit Assessment

By receiving the HRV vaccine the subject may become protected against 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 PCV-free HRV vaccine co-administration with routinely administered childhood vaccines.

Routine childhood vaccinations *Pediarix*, *Hiberix* and *Prevenar 13* will be administered to subjects according to their prescribed vaccination schedule, as part of the study.

In addition, the subjects will undergo a history directed 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 the 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. Co-Primary objectives

- To demonstrate the non-inferiority of the immune responses to three doses of *Pediarix*, *Hiberix* and *Prevenar 13* when co-administered with two doses of the PCV-free liquid HRV vaccine, as compared to when co-administered with the currently licensed lyophilized HRV vaccine, 1 month after Dose 3 of routine infant vaccines.

Criteria for non-inferiority:

  - *Lower limits of the two-sided standardized asymptotic 95% confidence intervals (CIs) on the differences between groups (HRV Liq group minus HRV Lyo group) in the percentages of subjects with seroprotective concentrations (≥0.1 IU/mL) for each of anti-diphtheria (anti-D) and anti-tetanus (anti-T) antibodies are ≥-10% (clinical limit for non-inferiority),*
  - *The lower limit of the two-sided standardized asymptotic 95% CI on the difference between groups (HRV Liq group minus HRV Lyo group) in the percentages of subjects with seroprotective concentration (≥10 mIU/mL) for antibodies against hepatitis B surface antigen (anti-HBs) is ≥-10% (clinical limit for non-inferiority),*
  - *Lower limits of the two-sided standardized asymptotic 95% CIs on the differences between groups (HRV Liq group minus HRV Lyo group) in the*

percentages of subjects with seroprotective titers (≥8 ED_{50}) for each of anti-poliovirus serotypes 1, 2 and 3 antibodies are ≥-5% (clinical limit for non-inferiority),

- *Lower limits of the two-sided 95% CIs on the geometric mean antibody concentrations (GMC) ratios (HRV Liq group over HRV Lyo group) for antibodies against each of the pertussis toxoid (PT), filamentous hemagglutinin (FHA) and pertactin (PRN) antigens [anti-PT, anti-FHA and anti-PRN] are ≥0.67 (clinical limit for non-inferiority),*
- *Lower limits of the two-sided 95% CIs on the GMC ratios (HRV Liq group over HRV Lyo group) for each of the 13 Streptococcus pneumoniae (S. pneumoniae) serotypes are ≥0.5 (clinical limit for non-inferiority),*
- *The lower limit of the two-sided standardized asymptotic 95% CI on the difference between groups (HRV Liq group minus HRV Lyo group) in the percentages of subjects with concentration of antibodies against polyribosyl-ribitol-phosphate antigen (anti-PRP) ≥0.15 μg/mL is ≥- 5%,*
- *The lower limit of the two-sided standardized asymptotic 95% CI on the difference between groups (HRV Liq group minus HRV Lyo group) in the percentages of subjects with concentration of antibodies against anti-PRP ≥1.0 μg/mL is ≥-10% (clinical limit for non-inferiority).*

- To rule out a 10% decrease in seroresponse to PT, FHA and PRN antigen when *Pediarix* is co-administered with PCV-free-liquid HRV vaccine compared to when *Pediarix* is co-administered with the currently licensed lyophilized HRV vaccine.
  - seroresponse is defined as the percentage of subjects showing an antibody concentration above a threshold that leads to 95% seroresponse in the control group (lyophilized HRV vaccine),
  - p-value on the difference in seroresponse between groups is <2.5% for each PT, FHA and PRN antigen (p-value is computed by integrating on the p-value for the null hypothesis that the seroresponse rate in the liquid group is <85% and the a-posteriori probability of the threshold in the lyophilized group).

Refer to Section 10.1 for the definition of the primary endpoints.

2.2. Secondary objectives

Immunogenicity:

- To assess the immunogenicity of the PCV-free liquid HRV vaccine and currently licensed lyophilized HRV vaccine in terms of serum anti-rotavirus immunoglobulin A (IgA) antibody seropositivity rate at Visit 4, 3 months after Dose 2 of the HRV vaccine.
- To assess the immunogenicity of routine infant vaccines *Pediarix*, *Hiberix* and *Prevenar 13* when co-administered with the PCV-free liquid HRV vaccine and

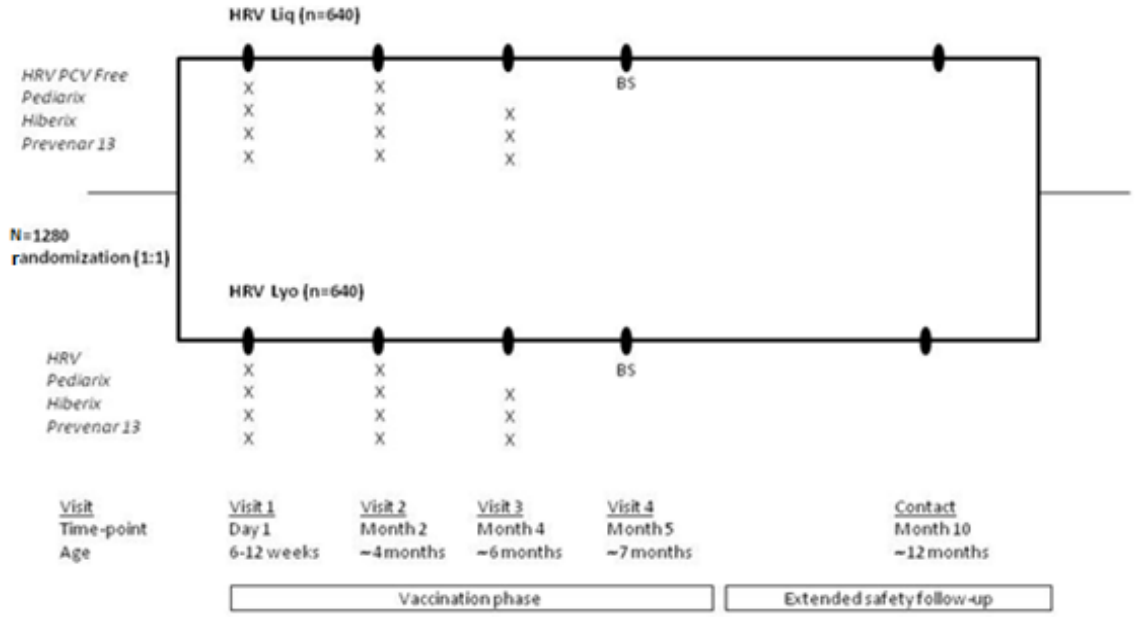
currently licensed lyophilized HRV vaccine at Visit 4, 1 month after Dose 3 of routine infant vaccines.

Reactogenicity and safety:

- To assess the reactogenicity of the PCV-free liquid HRV vaccine and currently licensed lyophilized HRV vaccine in terms of general solicited adverse events (AEs) during the 8 day (Day 1-Day 8) follow-up period after each dose of HRV vaccine.
- To assess the safety of the study vaccines in terms of unsolicited AEs during the 31 day (Day 1-Day 31) follow-up period after each dose of HRV vaccine 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, n = Number of subjects in each study group, BS = blood sample. Contact (by telephone call or any other convenient procedure) will take place 6 months after Visit 3 for safety follow-up. An IDMC will review the safety data by treatment group periodically. Details of the review will be described in an IDMC charter.

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 IIIA, single-blind, randomized, controlled, multi-centric study with two parallel groups.

- Duration of the study: The total duration of the study, per subject, will be approximately 10 months including the 6 months of Extended Safety Follow-Up (ESFU) period after the last dose of study vaccine administered.
  - Epoch 001: Primary starting at Visit 1 (Day 1) and ending at the ESFU contact (Month 10).
- Primary completion Date (PCD): Last subject attending Visit 4.

Refer to glossary of terms for the definition of PCD.

- End of Study (EoS): Last testing results released for samples collected at Visit 4. If the last testing results are available before the Last Subject Last Visit (LSLV), i.e., before the ESFU, the EoS will then be the LSLV.

Refer to glossary of terms for the definition of EoS.

The study groups and the 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	640	6 weeks-12 weeks	•
HRV Lyo	640	6 weeks-12 weeks	•

The study groups and the treatment planned for the study are provided in Table 2.

Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine name	Study Groups	
		HRV Liq	HRV Lyo
HRV Liquid	HRV PCV-free	X	
HRV Lyophilized	HRV*		x
Pediarix	DTPa-HBV-IPV	X	x
Hiberix	*Hib*	X	x
Prevenar 13	*Prevenar 13*	X	x

*Licensed lyophilized HRV vaccine.

- Control: active control-GSK Biologicals' currently licensed lyophilized HRV vaccine.
- Vaccination schedule: Two doses of HRV vaccine to be administered according to a 0, 2 month schedule as per the immunization schedule for HRV vaccine administration in the US.

  Co-administration of routine childhood vaccines *Pediarix*, *Hiberix* and *Prevenar 13* will be performed as follows:
  - All the subjects will receive a dose each of *Pediarix*, *Hiberix* and *Prevenar 13* at Visit 1 (Day 1), Visit 2 (Month 2) and Visit 3 (Month 4).
  - The routine booster dose for the co-administered vaccines will not be administered to subjects as a part of this study. Subject's parent(s)/LARs will be

reminded at Visit 4 to consult their primary health care provider regarding the booster dose of the vaccines for their child.

- Treatment allocation: Randomized 1:1 using GSK Biologicals' Randomization System on Internet (SBIR).
- Blinding: single-blind

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

Table 3 Blinding of study epoch

Study Epoch	Blinding
Epoch 001	single-blind

- Sampling schedule: Details of the samples to be collected are as follows:
  - Blood samples will be collected from all subjects at Visit 4 (Month 5) to measure serum anti-RV IgA antibody concentrations and antibody concentrations/titers against all the antigens in the co-administered vaccines.
- Type of study: self-contained.
- Data collection: Electronic Case Report Form (eCRF).
- Safety monitoring: An IDMC consisting of clinical experts and a biostatistician will review the safety data by treatment group periodically to monitor the safety aspects of the PCV-free liquid HRV vaccine.

4. STUDY COHORT

4.1. Number of subjects/centers

The target sample size is 832 subjects evaluable for immunogenicity analyses at Visit 4 (416 subjects in each group). Assuming that approximately 35% of enrolled subjects might withdraw or not be evaluable for analyses of immunogenicity, the target sample size to be enrolled is 1280 eligible subjects (640 subjects in each group).

Overview of the recruitment plan

- The enrollment will be terminated when 1280 eligible subjects have been enrolled.
- The recruitment and randomization will be monitored by SBIR.

4.2. Inclusion criteria for enrollment

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).
- A male or female between, and including, 6 and 12 weeks (42-90 days) of age at the time of the first study vaccination.
- Written or witnessed/thumb printed informed consent obtained from the parent(s)/LAR(s) of the subject prior to performing any study specific procedure.
- Healthy subjects as established by medical history and clinical examination before entering into the study.

4.3. Exclusion criteria for enrollment

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 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).
- Planned administration/administration of a vaccine not foreseen by the study protocol within the period starting 30 days before the first dose of vaccine administration and ending at Visit 4, with the exception of the inactivated influenza vaccine, which is allowed at any time during the study, if administered at a site which is different from the sites used to administer the co-administered vaccines.
- 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 vaccine/product (pharmaceutical product or device).

- 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).
- Family history of congenital or hereditary immunodeficiency.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination (no laboratory testing required).
- Major congenital defects or serious chronic illness.
- Previous vaccination against Haemophilus type b (Hib), diphtheria, tetanus, pertussis, pneumococcus, RV and/or poliovirus.
- Previous confirmed occurrence of RV GE, Hib, diphtheria, tetanus, pertussis, pneumococcus, hepatitis B and/or polio disease.
- Any medical condition that in the judgment of the investigator would make intramuscular injection unsafe.
- GE within 7 days preceding the study vaccine administration (warrants deferral of the vaccination).
- History of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccines.
- Hypersensitivity to latex.
- History of any neurological disorders or seizures.
- History of SCID.
- Acute disease and/or fever at the time of enrollment.
  - 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 diarrhea, mild upper respiratory infection) without fever may be enrolled at the discretion of the investigator.

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 Harmonisation (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 Harmonised Tripartite Guideline for clinical investigation of medicinal products in the pediatric 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 as appropriate.
- 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 HRV vaccine 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 centers/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, an over-randomization of 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 1280 eligible subjects who will be randomly assigned to two study groups in a (1:1) ratio (approximately 640 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 the 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, the randomization system will determine the study group and 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 each dose subsequent to the first 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 study is single-blind where the investigator and/or his staff will be aware of the treatment assignment and the subject's parents/LARs will not be aware of the treatment assignment.

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	6-12 weeks	~4 months	~6 months	~7 months	~12 months
Epoch	**Epoch 001**				
Type of contact	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**ESFU (Phone contact)**
Time-points	**Day 1**	**Month 2**	**Month 4**	**Month 5**	**Month 10**
Sampling time-points	**Pre-Vacc**			**Post-Vacc**	
Informed consent	●				
Check inclusion/exclusion criteria	●				
Collect demographic data	●				
Record gestational age	●				
Medical history	●				
Hepatitis B vaccination history	●				
History of previous vaccination from birth other than Hepatitis B vaccination	●				
Physical examination including length and weight measurement	●				
Check contraindications and warnings and precautions to vaccination	●	●	●		
Pre-vaccination body temperature	●	●	●		
Vaccines					
Randomization and treatment number allocation	●				
Treatment number allocation for subsequent doses		●	●		
Recording of administered treatment number	●	●	●		
HRV vaccine administration	●	●			
Record regurgitation	●	●			
HRV vaccine replacement dose administration in case of regurgitation*	●	●			
Co-administration of Pediarix, Hiberix and Prevenar 13	●	●	●		
Lab Assays					
Blood sampling for antibody determination (~5 mL)				●	
Safety Assessments					
Distribution of diary cards	O	O			
Record any concomitant medications/vaccinations	●	●	●	●	
Record any intercurrent medical conditions	●	●	●	●	
Recording of solicited adverse events within 8 days after each dose of HRV vaccination (Days 1-Day 8)	●	●			
Recording of non-serious adverse events within 31 days after each dose of HRV vaccination (Days 1-31)	●	●			
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		

Age	6-12 weeks	~4 months	~6 months	~7 months	~12 months
Epoch	Epoch 001				
Type of contact	Visit 1	Visit 2	Visit 3	Visit 4	ESFU (Phone contact)
Time-points	Day 1	Month 2	Month 4	Month 5	Month 10
Sampling time-points	Pre-Vacc			Post-Vacc	
Diary card transcription by investigator or designee		●	●		
Phone Contact					●
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.
Post-Vacc = blood sample collected from subjects, one month after the third dose of the co-administered vaccines (Visit 4); ESFU: Extended Safety Follow-up; AE = Adverse Event; SAE = Serious Adverse Events; LAR = Legally Acceptable Representative.
*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.

Advisory Committee on Immunization Practices (ACIP) recommends administering monovalent Hepatitis B vaccine to all newborns before hospital discharge. Infants who did not receive a birth dose should receive three doses of a Hepatitis-B-containing vaccine on a schedule of 0, 1 to 2 months, and 6 months starting as soon as feasible. Administer the second dose, 1 to 2 months after the first dose (minimum interval of four weeks), administer the third dose at least eight weeks after the second dose AND at least 16 weeks after the first dose. The final (third or fourth) dose in the Hepatitis B vaccine series should be administered no earlier than age 24 weeks.

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

Table 5 Intervals between study visits

Interval	Optimal length of interval
Visit 1→Visit 2	60 days
Visit 2→Visit 3	60 days
Visit 3→Visit 4	30 days
Visit 3→ESFU ‡	6 months

‡A safety follow-up contact (by telephone call or any other convenient procedure) to collect information on SAEs and medication taken for treatment of the same.

Refer to Table 19 for intervals between study visits that determine subjects' eligibility for inclusion in the per-protocol analysis.

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 enrollment.

5.6.3. Collect demographic data

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

5.6.4. Medical and vaccination history

Obtain the subject's medical and vaccination history (including Hepatitis B 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 length 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 within the allowed interval for this visit (see Table 19).

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 [fever is defined as temperature ≥38.0°C/100.4°F regardless the location of measurement] on the day of vaccination, the vaccination visit will be rescheduled within the allowed interval for this visit (see Table 19).

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 assessments

Blood samples will be taken during Visit 4 as specified in Section 5.5, outline of study procedures:

- A volume of approximately 5 mL of whole blood (to provide approximately 1.75 mL of serum) should be drawn from all subjects for antibody determination at Visit 4 (Post-Vacc). After centrifugation, serum samples should be kept at -20°C/-4°F or below until shipment. Refer to the SPM for more details on sample storage conditions.

5.6.10. Study Vaccines administration

- After completing all prerequisite procedures prior to vaccination, two oral doses of the HRV vaccine will be administered at an approximate 2-months interval and three doses of co-administered vaccines will be given intramuscularly (IM) at 2, 4 and 6 months (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 19).
- The subjects will be observed closely for at least 30 minutes following the administration of the vaccines, 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 8.3 for procedures for the investigator to record AEs and SAEs. Refer to Section 8.4 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 HRV 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 HRV vaccination and during the next 7 days) or any unsolicited AEs (i.e., on the day of HRV vaccination and during the next 30 days occurring 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. Phone Contact

A safety follow-up contact will be done by a telephone call to collect information on SAEs and medication taken for treatment of the same.

5.6.15. Study conclusion

The investigator will:

- review data collected to ensure accuracy and completeness
- complete the Study Conclusion screen 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 in countries where this is allowed 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 the US 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).

If additional testing is performed, the marker priority ranking given in Section 5.7.4 may be changed.

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 (including 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 sample to be collected from subjects is described in Table 6.

Table 6 Biological sample

Sample Type	Quantity	Unit	Sampling time-point	No of subjects
Blood	Approximately 5	ml	Visit 4 (Post-Vacc)	All

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.

All serological assays will be performed at GSK Biologicals' laboratory or in a validated laboratory designated by GSK Biologicals using standardized and validated procedures.

The laboratory assays to be performed are presented in 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	20	GSK Biologicals [1]
SER	Bordetella pertussis.Filamentous Hemaglutinin Ab.IgG	ELI	NA	IU/ml	2.046	GSK Biologicals [1]
SER	Bordetella pertussis.Pertussis Toxin Ab.IgG	ELI	NA	IU/ml	2.693	GSK Biologicals [1]
SER	Bordetella pertussis.Pertactin Ab.IgG	ELI	NA	IU/ml	2.187	GSK Biologicals [1]
SER	Hepatitis B Virus.Surface Ab	CLIA	ADVIA Centaur anti-HBs2 (Siemens Healthcare)	mIU/ml	6.2	GSK Biologicals [1]
SER	Haemophilus influenzae type b.Polyribosyl Ribitol Phosphate Ab	ELI	NA	µg/ml	.15[3]	GSK Biologicals [1]
SER	Corynebacterium diphtheriae.Diphtheria Toxoid Ab.IgG	ELI	NA	IU/ml	.1	GSK Biologicals [1]
SER	Clostridium tetani.Tetanus Toxoid Ab.IgG	ELI	NA	IU/ml	.1	GSK Biologicals [1]
SER	Poliovirus Sabin Type 1 Ab	NEU	NA	ED50	8	GSK Biologicals [1]
SER	Poliovirus Sabin Type 2 Ab	NEU	NA	ED50	8	GSK Biologicals [1]
SER	Poliovirus Sabin Type 3 Ab	NEU	NA	ED50	8	GSK Biologicals [1]
SER	S pneu.PS01 IgG	ECL	in-house	µg/ml	.08	GSK Biologicals [1]
SER	S pneu.PS03 IgG	ECL	in-house	µg/ml	.075	GSK Biologicals [1]
SER	S pneu.PS04 IgG	ECL	in-house	µg/ml	.061	GSK Biologicals [1]

System	Component	Method	Kit/Manufacturer	Unit	Cut-off	Laboratory
SER	S pneu.PS05 IgG	ECL	in-house	µg/mll	.198	GSK Biologicals [1]
SER	S pneu.PS06A IgG	ECL	in-house	µg/ml	.111	GSK Biologicals [1]
SER	S pneu.PS06B IgG	ECL	in-house	µg/mll	.102	GSK Biologicals [1]
SER	S pneu.PS07F IgG	ECL	in-house	µg/ml	.063	GSK Biologicals [1]
SER	S pneu.PS09V IgG	ECL	in-house	µg/ml	.066	GSK Biologicals [1]
SER	S pneu.PS14 IgG	ECL	in-house	µg/ml	.16	GSK Biologicals [1]
SER	S pneu.PS18C IgG	ECL	in-house	µg/ml	.111	GSK Biologicals [1]
SER	S pneu.PS19A IgG	ECL	in-house	µg/ml	.199	GSK Biologicals [1]
SER	S pneu.PS19F IgG	ECL	in-house	µg/ml	.163	GSK Biologicals [1]
SER	S pneu.PS23F IgG	ECL	in-house	µg/ml	.073	GSK Biologicals [1]

[1] GSK Biologicals laboratory refers to the Clinical Laboratories Sciences (CLS) in Rixensart, Belgium; Wavre, Belgium or a validated laboratory designated by GSK Biologicals.
SER = Serum
S pneu = Streptococcus pneumoniae
IgA = Immunoglobulin A
IgG = Immunoglobulin G
ELI = Enzyme-Linked Immunosorbent Assay
NEU = Neutralization Assay
CLIA = ChemiLuminescence ImmunoAssay
NA = Not applicable
mIU = milli International Units; U = Units; IU = International Units
ED 50 = Estimated dose 50%
ECL: ElectroChemiLuminescence
[3] The assay cut-off for Hib may be subject to change.

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 priority rank and immunological read-outs are given in Table 8.

Table 8 Immunological read-outs

Blood sampling time-point		No. subjects	Component	Components priority rank
Type of contact and time-point	Sampling time-point			
Visit 4 (Month 5)	Post-Vacc	All	PRP	1
			D	2
			T	3
			Poliovirus serotypes 1, 2 and 3	4
			HBs	5
			PT	6
			FHA	7
			PRN	8
			13 S pneumoniae serotypes	9
			HRV	10

PRP: polyribosyl-ribitol-phosphate, PT: pertussis toxoid, FHA: filamentous hemagglutinin, PRN: pertactin, HBs: hepatitis B surface antigen, D: diphtheria, T: tetanus, HRV: human rotavirus.

In case of insufficient blood sample volume to perform assays for all antibodies, the samples will be analyzed according to priority ranking provided in Table 8.

5.7.5. Immunological correlates of protection

The following cut-offs are accepted as immunological correlates of protection:

- Specific antibodies against diphtheria toxoid and tetanus toxoid will be measured by Enzyme Linked Immunosorbent Assay (ELISA). The assay cut-offs for anti-D and anti-T is currently set at 0.1 IU/mL (ELISA), which provides a conservative estimate of the percentage of subjects deemed to be protected [Camargo, 1984; Melville-Smith, 1983].
- Antibodies to the anti-HBs will be measured using CLIA. The cut-off of the test is set at 6.2 mIU/mL. An antibody concentration ≥10 mIU/mL defines seroprotection [CDC, 1991; WHO, 1988].
- Antibodies against poliovirus types 1, 2 and 3 will be determined by a virus micro-neutralization test adapted from the World Health Organization Guidelines for WHO/EPI Collaborative Studies on Poliomyelitis [WHO, 1993]. The lowest dilution at which serum samples will be tested is 1:8, from which a test will be considered positive. Titers will be expressed in terms of the reciprocal of the dilution resulting in 50% inhibition. Antibody titers greater than or equal to this value are considered as protective.
- Data from subjects given unconjugated Hib vaccine suggest that, in the absence of induction of immunological memory, a concentration of 0.15 µg/mL is indicative of short-term protection, with 1 µg/mL considered indicative of long-term protection [Käyhty, 1983; Anderson, 1984].
- No correlate of protection is defined for the immune response to pertussis antigens. Antibodies against the pertussis components PT, FHA and PRN will be measured by ELISA technique. The current cut-offs for all three pertussis antibodies are the

Lower Limit Of Quantification (LLOQ) of the assays which are respectively 2.693 IU/mL for PT, 2.046 IU/mL for FHA and 2.187 IU/mL for PRN. Subjects with antibody concentration below this cut-off will be considered seronegative.

- Pneumococcal serotype specific total IgG antibodies (antibodies against 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19 A, 19F and 23F) will each be measured by multiplex ElectroChemiLuminescence (ECL) assays. The LLOQ (in µg/mL) determined for each of these strains are shown in Table 7. No correlate of protection is defined for the immune response to pneumococcal antigens.
- No immunological correlate of protection has been demonstrated so far for the antigen used as part of the HRV vaccine.

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

For the subjects identified as non-responders, it remains the responsibility of the investigator in charge of the subject's clinical management to determine the medical need for re-vaccination and to re-vaccinate the subjects as per local/regional practices.

6. STUDY VACCINES AND ADMINISTRATION

6.1. Description of study vaccines

All candidate vaccines to be used have been developed and manufactured by GSK Biologicals.

The Quality Control Standards and Requirements for each candidate vaccine 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 vaccines

Treatment name	Vaccine/product name	Formulation	Presentation	Volume to be administered	Number of doses
HRV Liquid	HRV PCV-free	PCV-free HRV RIX4144 live attenuated >=$10^{6 \cdot 0}$$CCID_{50}$	Liquid vaccine in a pre-filled oral applicator.	1.5 ml	2
HRV Lyophilized	HRV	HRV RIX4144 live attenuated >=$10^{6 \cdot 0}$$CCID_{50}$	Lyophilized vaccine in a monodose glass vial.	1 ml	2
	HRV Diluent	$CaCO_3$=60mg	Diluent for lyophilized vaccine (calcium carbonate liquid antacid) supplied separately in a prefilled oral applicator.		
Pediarix	DTPa-HBV-IPV	DT>=30IU; TT>=40IU; PT=25µg; FHA=25µg; PRN=8µg; HBsAg=10µg; Inactivated Poliovirus type 1 (Mahoney strain)=40DU; Inactivated Poliovirus type 2 (MEF-1 strain)=8DU; Inactivated Poliovirus type 3 (Saukett strain); Aluminium=700µg Al3+	The DTPa-HBV-IPV component is presented as a turbid white suspension in a pre-filled syringe.	0.5 ml	3
Hiberix	Hib	PRP=10µg; TT~=25µg l.	The lyophilized Hib component is presented as a white pellet in a glass vial; it must be reconstituted before use. Sterile 0.9% saline solution	0.5 ml	3
	NaCl	NaCl=150mM			

Treatment name	Vaccine/product name	Formulation	Presentation	Volume to be administered	Number of doses
Prevenar 13	*Prevenar 13*	PS1=2.2µg CRM197; PS3=2.2µg CRM197; PS4=2.2µg CRM197; PS5=2.2µg CRM197; PS6A=2.2µg CRM197; PS6B=4.4µg CRM197; PS7F=2.2µg CRM197; PS9V=2.2µg CRM197; PS14=2.2µg CRM197; PS18C=2.2µg CRM197; PS19A=2.2µg CRM197; PS19F=2.2µg CRM197; PS23F=2.2µg CRM197; $AlPO_4$=125µg Al3+	Suspension for injection in a pre-filled syringe.	0.5 ml	3

DT: Diphtheria toxoid, TT: Tetanus toxoid, PT: Pertussis toxoid, FHA: Filamentous hemagglutinin, PRN: Pertactin
$CCID_{50}$ = median Cell Culture Infective Dose (quantity of virus causing infection in 50% of exposed cells)
ml = milliliter; mg = milligrams

6.2. Storage and handling of study vaccines

The study vaccines must be stored at the respective 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°C (for +2 to +8°C/+36 to +46°F 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°C down to 0.0°C impacting IMP(s) there is no need to report in (e)TDF, but adequate actions must be taken to restore the +2 to +8°C/+36 to +46°F 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 vaccines

Liquid formulation of HRV vaccine

The pre-filled oral applicator is 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 resuspend the vaccine. The entire volume of the resuspended product (approximately 1 mL) should be withdrawn into the same oral applicator and the resuspended product should then be administered promptly as a single oral dose.

Administration of the oral vaccines

In order to allow the swallowing of the entire volume of the single oral dose (of liquid or lyophilized formulation), 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. If regurgitation or vomiting occurs after vaccination, a single replacement dose may be given at the same vaccination visit. This information should be recorded in the eCRF. The subject may continue to participate in the study.

Administration of the intramuscular vaccines

In order to ensure proper IM injection of the co-administered vaccines, a needle of at least 1 inch (2.54 cm) length, 25 gauge will be used [Diggle, 2006; Zuckerman, 2000].

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	Route[1]	Site		
					Location	Directionality[2]	Laterality
Visit 1, Visit 2	HRV Liq	HRV Liquid	1.5 ml	O	Not applicable	Not applicable	Not applicable
Visit 1 , Visit 2	HRV Lyo	HRV Lyophilized	1 ml	O	Not applicable	Not applicable	Not applicable
Visit 1, Visit 2, Visit 3	HRV Liq, HRV Lyo	*Pediarix*	0.5 ml	IM	Thigh	Upper	Right
Visit 1, Visit 2, Visit 3	HRV Liq, HRV Lyo	*Hiberix*	0.5 ml	IM	Thigh	Anterolateral	Right
Visit 1, Visit 2, Visit 3	HRV Liq, HRV Lyo	*Prevenar 13*	0.5 ml	IM	Thigh	Lower	Left

[1]Oral (O), Intramuscular (IM)
[2] Directionality is a qualifier for further detailing the location of the vaccine administration.

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 the study vaccines. 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 8.5).

- Anaphylaxis following the administration of vaccine(s).
- Hypersensitivity reaction following the administration of the vaccine(s).
- Any uncorrected congenital malformation of the gastrointestinal tract (such as Meckel's diverticulum) that would predispose for IS.
- Any history of IS.
- SCID.
- Any condition that in the judgment of the investigator would make intramuscular injection unsafe.
- Contraindication for pertussis-containing vaccines:
  - Encephalopathy of unknown etiology, defined as an acute, severe central nervous system disorder, occurring within 7 days following previous vaccination with pertussis-containing vaccine and generally consisting of major alterations in consciousness, unresponsiveness, generalized or focal seizures that persist more than a few hours, with failure to recover within 24 hours.
  - Individuals with progressive neurologic disorder, including infantile spasms, uncontrolled epilepsy, or progressive encephalopathy should not receive a

pertussis-containing vaccine until a treatment regimen has been established and the condition has stabilized.

Refer to the approved product label/package inserts for warnings and precautions for the use of *Pediarix, Prevenar13, Rotarix* (currently licensed lyophilized HRV vaccine) and *Hiberix* vaccines.

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 8.5).

- 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 diarrhea, mild upper respiratory infection) without fever may be enrolled at the discretion of the investigator.
- GE within 7 days preceding the HRV vaccine administration.

6.6. Warnings and precautions

The HRV vaccine should under no circumstances be injected.

There are 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 7th day. In clinical trials, cases of transmission of excreted vaccine virus to seronegative contacts of vaccines 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 of the co-administered vaccines for information on the warnings and precautions concerning their administration.

6.7. Concomitant medications/products and concomitant vaccinations

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 following each dose of study vaccine.
- Any concomitant vaccination administered in the period from first study vaccination (Visit 1) and ending at Visit 4.
- 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 [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].
- 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 at any time during the study period for the treatment of a SAE. In addition, 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 per protocol set for analysis of immunogenicity.

- Any investigational or non-registered product (drug or vaccine) other than the study vaccines used during the study period between the first vaccination at Visit 1 to the blood sampling at Visit 4.
- Immunosuppressants or other immune-modifying drugs administered chronically (i.e., more than 14 days) during the study period between Visit 1 to Visit 4. For corticosteroids, this will mean prednisone ≥ 0.5 mg/kg/day, or equivalent. Inhaled and topical steroids are allowed.
- Immunoglobulins and/or any blood products administered during the study period between the first vaccination at Visit 1 to the blood sampling at Visit 4.
- Administration of long-acting immune-modifying drugs 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 vaccine administration and ending at Visit 4* blood sampling, with the exception of the inactivated influenza vaccine, which is allowed at any time during the study if administered at a site which is different from the sites used to administer the co-administered vaccines.

  * In case an emergency mass vaccination for an unforeseen public health threat (e.g.: a pandemic) is organised 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 SmPC or 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/the 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 if occurring up to Visit 4.

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 they/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.

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, hematology, 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.

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. Events or outcomes not qualifying as adverse events or serious adverse events

Not applicable.

8.3. Detecting and recording adverse events and serious adverse events

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

All AEs starting within 31 days following administration of Dose 1 and Dose 2 of the HRV vaccine (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 vaccines until the subject is discharged from the study. See Section 8.4 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 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*		Visit 1	7 d post V1	30 d post-V1		Visit 2	7 d post V2	30 d post-V2		Visit 3	7 d post V3	30 d post-V3	Visit 4		ESFU contact
			Day 1				Month 2				Month 4			Month 5		Month 10
Solicited general AEs																
Unsolicited AEs																
AEs/SAEs leading to withdrawal from the study																
SAEs																
SAEs related to study participation or concurrent GSK medication/vaccine																

* i.e., consent obtained. V: vaccination; Post-V: post-vaccination.

8.3.2. 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**.**

8.3.3. Evaluation of adverse events and serious adverse events

8.3.3.1. 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.

8.3.3.2. Assessment of adverse events

8.3.3.2.1. Assessment of intensity

Intensity of the following solicited AEs will be assessed as described in Table 13 and Table 14.

Table 13 Intensity scales to be used by the parent(s)/LAR(s) for solicited symptoms during the solicited follow-up period

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

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

Table 14 Intensity scales for diarrhea, vomiting and fever occurring during the solicited period

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

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

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.

8.3.3.2.2. 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)/product(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 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).

8.3.3.3. 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).

8.3.3.4. 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.

8.4. Reporting of serious adverse events

8.4.1. Prompt reporting of serious adverse events to GSK Biologicals

SAEs that occur in the time period defined in Section 8.3 will be reported promptly to GSK within the timeframes described in Table 15, once the investigator determines that the event meets the protocol definition of a SAE.

Table 15 Timeframes for submitting serious adverse 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.

8.4.2. 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 US sites only: Fax: PPD Canadian sites only: Fax: PPD

8.4.3. 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.

8.4.3.1. 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.

8.4.4. 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 15.

8.4.5. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to GSK in accordance with the procedures detailed in Section 8.4.1. 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.

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

8.5.1. 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 15).

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.

8.5.2. 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 a paper/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.

8.6. 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).

8.7. 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 is available for the concluding contact 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 not available for the concluding contact 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 he/she/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/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 8.5.2).

9.2.2. Subject withdrawal from study vaccines

A 'withdrawal' from the study vaccines 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 vaccines 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 page/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 endpoints

- Immunogenicity with respect to components of the routine infant vaccines, one month after Dose 3 of routine infant vaccines (Visit 4):
  - Anti-D antibody concentration ≥0.1 IU/mL,
  - Anti-T antibody concentration ≥0.1 IU/mL,
  - Anti-HBs antibody concentrations ≥10 mIU/mL,
  - Anti-poliovirus types 1, 2 and 3 antibody titers ≥8 ED_{50},
  - Anti-PT, anti-FHA and anti-PRN antibody concentrations expressed as GMCs,
  - Anti-pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) antibody concentrations expressed as GMCs,
  - Anti-PRP antibody concentrations ≥0.15 µg/mL,
  - Anti-PRP antibody concentrations ≥1.0 µg/mL.
- Difference in seroresponse with respect to PT, FHA and PRN antigen components one month after Dose 3 of routine infant vaccines (Visit 4):
  - Seroresponse to anti-PT, anti-FHA and anti-PRN.

10.2. Secondary endpoints

- Serum anti-rotavirus IgA antibody seropositivity 3 months after Dose 2 of HRV vaccine (Visit 4).
  - Serum anti-RV IgA antibody concentrations ≥20 U/mL and ≥90 U/mL 1-2 months after Dose 2.
- Immunogenicity with respect to components of the routine infant vaccines, one month after Dose 3 of routine infant vaccines (Visit 4):
  - PT, anti-FHA and anti-PRN antibody concentrations ≥2.693 IU/mL, ≥2.046 IU/mL and ≥2.187 IU/mL, respectively.
  - Anti-pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) antibody concentrations ≥0.35 µg/mL for the ELISA,
  - Anti-D, anti-T, anti-PRP and anti-HBs antibody concentrations expressed as GMCs and anti-poliovirus types 1, 2 and 3 antibody concentrations expressed as Geometric Mean Titers (GMTs).
- Occurrence of general solicited AEs during the 8 day (Day 1-Day 8) follow-up period after each dose of HRV vaccine.
- Occurrence of unsolicited AEs within 31 days (Day 1-Day 31) after any dose of HRV vaccine, according to the Medical Dictionary for Regulatory Activities (MedDRA) classification.
- Occurrence of SAEs from Dose 1 of study vaccines up to study end.

10.3. Determination of sample size

The target sample size is 832 subjects evaluable for immunogenicity analyses at Visit 4 (416 subjects in each group). Assuming that approximately 35% of enrolled subjects might withdraw or not be evaluable for analyses of immunogenicity, the target sample size to be enrolled is 1280 subjects (640 subjects in each group).

The primary objective of this study is to demonstrate the immune response elicited to all antigens contained in each of the routine infant vaccines *Pediarix*, *Hiberix* and *Prevenar 13* when co-administered with PCV-free liquid HRV vaccine is non-inferior to that elicited when co-administered with the lyophilized HRV vaccine.

10.3.1. Control on type I error

A 2.5% nominal type I error will be used for each co-primary evaluation. To control the type I error below 2.5%, a hierarchical procedure will be used for the multiple study objectives. That is, an objective will be reached if its associated criterion is met and the previous objectives were reached. The same order in which the study objectives are listed in Section 2.1 will be considered for hypothesis testing.

The sample size has been estimated in order to obtain at least 90% power to demonstrate the co-primary objectives. The power associated with the target sample size for the conclusion on each inferential objective of this study is detailed in Section10.3.3.

10.3.2. Reference for sample size:

References were chosen based on observed response and standard deviations in studies Rota-060 (107531) and Hib-097 (112957).

The percentage of subjects above the cut-off is detailed in Table 16.

Table 16 Percentage of subjects above the cut-off

Study	Timing	Cut-off	N	n	%	LL	UL
Rota-060 (Co-administered group)	Post dose 3	D ≥0.1 IU/mL	178	178	100	97.9	100
		T ≥0.1 IU/mL	178	178	100	97.9	100
		HBs≥10 mIU/mL#	169	169	100	97.8	100
		IPV1 ≥1:8	128	128	100	97.2	100
		IPV2 ≥1:8	139	139	100	97.4	100
		IPV3 ≥1:8	146	146	100	97.5	100
		PRP ≥0.15 µg/mL	180	177	98.3	95.2	99.7
		PRP ≥1 µg/mL	180	161	89.4	84.0	93.5
Hib-097 (ActHib group)	Post dose 3	D ≥0.1 IU/mL	273	273	100	98.7	100
		T ≥0.1 IU/mL	274	274	100	98.7	100
		HBs ≥10 mIU/mL#	258	257	99.6	97.9	100
		IPV1 ≥1:8	181	181	100	98.0	100
		IPV2 ≥1:8	192	188	97.9	94.8	99.5
		IPV3 ≥1:8	323	323	100	98.9	100
		PRP ≥0.15 µg/mL	1590	1536	96.6	95.6	97.4
		PRP≥1 µg/mL	274	246	89.8	85.6	93.1
Hib-097 (Pooled Hiberix group)	Post dose 3	D ≥0.1 IU/mL	393	393	100	99.1	100
		T ≥0.1 IU/mL	393	393	100	99.1	100
		HBs ≥10 mIU/mL#	363	362	99.7	98.5	100
		IPV1 ≥1:8	248	246	99.2	97.1	99.9
		IPV2 ≥1:8	280	275	98.2	95.9	99.4
		IPV3 ≥1:8	257	254	98.8	96.6	99.8
		PRP ≥0.15 µg/mL	1590	1536	96.6	95.6	97.4
		PRP≥1 µg/mL	1590	1291	81.2	79.2	83.1

N= Number of subjects with available results, n (%) = Number (percentage) of subjects with titer/concentration within the specified range, D = Diphtheria, T = Tetanus; IPV1 = Polio virus type 1; IPV2 = Polio virus type 2; IPV3 = Polio

virus type 3; PRP = Polyribosyl-Ribitol-Phosphate; HBs = Hepatitis B; LL/UL = 95% exact CI; # irrespective of whether it was given at birth or not.

The standard deviation for log_{10} transformed concentrations post-vaccination is presented in Table 17.

Table 17 Standard deviation for log_{10} transformed titer/concentration post-vaccination

Antigen	Rota-060 Post dose 3 (Co-administration group)	Hib-097 (ActHib group)	Hib-097 (Pooled Hiberix group)
PT	0.257	0.281	0.297
PRN	0.397	0.407	0.409
FHA	0.264	0.255	0.293
Anti-4	0.335	0.295	0.301
Anti-6B	0.549	0.524	0.496
Anti-9V	0.351	0.357	0.381
Anti-14	0.345	0.409	0.405
Anti-18C	0.376	0.336	0.375
Anti-19F	0.332	0.293	0.317
Anti-23F	0.444	0.426	0.447
Anti-1	-	0.337	0.354
Anti-19A	-	0.398	0.358
Anti-3	-	0.281	0.341
Anti-5	-	0.361	0.387
Anti-6A	-	0.350	0.347
Anti-7F	-	0.311	0.326

PT = Pertussis Toxoid, PRN = Pertactin, FHA = Filamentous Hemagglutinin

10.3.3. Power computation

The sample size has been estimated in order to obtain at least 90% power to demonstrate the primary objectives (Bonferroni adjustment of type II error). A hierarchical procedure will be used for the multiple study objectives. That is, an objective will be reached if its associated criterion is met and the previous objectives were reached. The same order in which the study objectives are listed in Section 2.1 will be considered for hypothesis testing.

The power computations were based on the following methods:

- Non-inferiority on percentage of subjects with titer/concentration above pre-specified cut-off: Type II error is obtained using PASS 2005, one-sided non-inferiority test for two proportions, under the alternative of equal proportions (Miettinen and Nurminen method).
- Non-inferiority on GMC: Type II error is obtained using PASS 2005, one-sided non-inferiority test for two from normal data with common variance, under the alternative of equal means;

To account for the multiplicity of comparison, the global type II errors were conservatively estimated as the sum of individual type II errors.

The power associated to the target sample size for the conclusion on each inferential objective of this study is detailed below.

Table 18 details the power for non-inferiority post-dose 3.

Table 18 Power for non-inferiority post-dose 3

	Margin	Reference	N HRV Liq Group	N HRV Lyo Group	Type II error
Anti-D ≥0.1 IU/mL	10.0%	99.5%	416	416	0.0%
Anti-T ≥0.1 IU/mL	10.0%	99.5%	416	416	0.0%
Anti-IPV1 ≥1:8	5.0%	99.5%	416	416	0.0%
Anti-IPV2 ≥1:8	5.0%	98.8%	416	416	0.1%
Anti-IPV3 ≥1:8	5.0%	99.5%	416	416	0.0%
Anti-HBs ≥10 mIU/mL	10.0%	98.0%	416	416	0.0%
Anti-PT GMC	0.67	0.297	416	416	0.0%
Anti-FHA GMC	0.67	0.293	416	416	0.0%
Anti-PRN GMC	0.67	0.409	416	416	0.0%
Anti-1 GMC	0.5	0.549	416	416	0.0%
Anti-3 GMC	0.5	0.549	416	416	0.0%
Anti-4 GMC	0.5	0.335	416	416	0.0%
Anti-5 GMC	0.5	0.549	416	416	0.0%
Anti-6A GMC	0.5	0.549	416	416	0.0%
Anti-6B GMC	0.5	0.549	416	416	0.0%
Anti-7F GMC	0.5	0.549	416	416	0.0%
Anti-9V GMC	0.5	0.381	416	416	0.0%
Anti-14 GMC	0.5	0.409	416	416	0.0%
Anti-18C GMC	0.5	0.376	416	416	0.0%
Anti-19A GMC	0.5	0.549	416	416	0.0%
Anti-19F GMC	0.5	0.332	416	416	0.0%
Anti-23F GMC	0.5	0.447	416	416	0.0%
Anti-PRP ≥0.15 µg/mL	5.0%	96.5%	416	416	4.8%
Anti-PRP ≥1 µg/mL	10.0%	81.2%	416	416	4.4%
Seroresponse to PT$	10.0%	0.297	416	416	0.1%
Seroresponse to FHA$	10.0%	0.293	416	416	0.1%
Seroresponse to PRN$	10.0%	0.409	416	416	0.1%
Global Power > 90.0%					

N = Number of evaluable subjects per group, Anti-D = anti-diphtheria antibody, Anti-T = anti-tetanus antibody, Anti-IPV1 = anti-poliovirus type 1, Anti-IPV2 = anti-poliovirus type 2, Anti-IPV3 = anti-poliovirus type 3, Anti-PT = anti-pertussis toxoid antibody, Anti-FHA = anti-filamentous hemagglutinin, Anti-PRN = anti-pertactin antibody, Anti-PRP =antibodies against polyribosyl-ribitol-phosphate; Anti-HBs =antibodies against hepatitis B.
For Anti-PRP, anti-D, anti-T, anti-IPV1, anti-IPV2 and anti-IPV3 antibodies, the reference used for power calculation is rate. For the rest of the antibodies, the standard deviation values were considered.
*For rates ~100%, a conservative reference of 99.5% is taken.
$Power results were based on the simulation assuming the distribution of immune response from *HRV Liq* group and *HRV Lyo group* are the same, and equal sero-response rate between groups under the alternative (seroresponse for

anti-PT, anti-FHA and anti-PRN in *HRV liq* group were defined as the percentage of subjects showing an antibody concentration above a threshold that led to 95% seroresponse in the *HRV lyo*), 1-sided, alpha=0.025.

10.4. Sets for Analyses

10.4.1. Exposed Set

- The Exposed Set (ES) will include all subjects with at least one study vaccine administration documented.A safety analysis based on the ES will include all vaccinated subjects.
- An immunogenicity analysis based on the ES will include all vaccinated subjects for whom immunogenicity data is available.

The ES analysis will be performed per treatment actually administered at Dose 1.

10.4.2. Per-protocol Set for analysis of immunogenicity

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

- who have received the study vaccines according to their random assignment,
- who comply with the vaccination schedule of routine infant vaccines and HRV vaccines as per Table 19,
- for whom the routine infant vaccines were administered according to the protocol as per Table 10,
- for whom the HRV vaccine liquid or lyophilized formulation was administered according to protocol,
- who have not received a vaccine not specified or forbidden in the protocol up to Visit 4 blood sampling,
- who had not received medication forbidden by the protocol up to Visit 4 blood sampling,
- whose underlying medical condition(s) was (were) not forbidden by the protocol up to Visit 4 blood sample,
- for whom data concerning immunogenicity endpoint measures are available. This will include subjects for whom assay results are available for antibodies against at least one routine infant vaccine antigen component,
- who comply with the blood sampling schedule after the 3rd dose of Pediarix, Hiberix and Prevenar 13 as per Table 19,
- who have no concomitant infection up to Visit 4 blood sample, which may influence the immune system.

Table 19 Maximum allowed interval between visits

Interval	Allowed length of interval
Visit 1→Visit 2	49 days-83 days after Dose 1 of study vaccines 1
Visit 2→Visit 3	56 days-83 days after Dose 2 of study vaccines 1
Visit 3 →Visit 4	21 days-48 days after Dose 3 of study vaccines 1
Visit 3 →ESFU	180 days-210 days after Dose 3 of study vaccines

[1] Subjects will not be eligible for inclusion in the Per-Protocol Set (PPS) for analysis of immunogenicity, if they make the study visit outside this interval. This is not applicable for the interval between Visit 3 and ESFU.

10.5. Derived and transformed data

- A seronegative subject is a subject whose antibody concentration is below the cut-off value.
- A seropositive subject is a subject whose antibody concentration is greater than or equal to the cut-off value. The applicable thresholds are presented in Table 7.
- A seroprotected subject is a subject whose antibody concentration is greater than or equal to the level defining clinical protection. The following seroprotection thresholds are applicable:
  - anti-D antibody concentrations ≥0.1 IU/mL.
  - anti-T antibody concentrations ≥0.1 IU/mL.
  - anti-HBs antibody concentrations ≥10 mIU/mL.
  - anti-poliovirus types 1, 2 and 3 antibody titers ≥8 ED50.
  - anti-PRP antibody concentrations ≥0.15 µg/mL.
- Other cut-offs to be considered:
  - anti RV IgA antibody concentration ≥90 U/mL.
  - anti-pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) antibody concentrations ≥0.35 µg/mL for the ELISA.
  - anti-PRP antibody concentrations ≥1.0 µg/mL.
- The GMC calculation will be performed by taking the anti-log of the mean of the log concentration transformations. Note that antibody concentrations below the cut-off of the assay will be given an arbitrary value of half the cut-off while antibody concentration above the assay cut-off but below the Limit of Quantification will be given the assay cut-off as value.

Handling of missing data:

Immunogenicity:

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

Reactogenicity and Safety:

- For analysis of solicited, unsolicited AEs (such as SAEs or AEs by primary MedDRA term), and for the analysis of concomitant medications, all vaccinated subjects will be considered. Subjects who did not report the event or the concomitant medication will be considered as subjects without the event or the concomitant medication respectively.

10.6. Analysis of demographics

The median, mean, range and standard deviation of age (in weeks) at each study vaccine dose and of the gestational age will be computed by group. The median, mean and standard deviation of length (in centimeters) and weight (in kilograms) at Visit 1 will computed by group. The racial and sex composition of the subjects will be presented.

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

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

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

10.7. Analysis of immunogenicity

The primary analysis will be based on the PPS for analysis of immunogenicity. An analysis on the ES will be performed only if, in any group, more than 5% of the vaccinated subjects with immunological data are excluded from the PPS for immunogenicity.

The following section describes the analyses that will be performed.

10.7.1. Within group analysis

For each treatment group, one month after Dose 3 of routine infant vaccines at Visit 4 (Month 5) time-point:

- Seroprotection rates against HBsAg, diphtheria toxoid, tetanus toxoid, PRP antigen and poliovirus types 1, 2 and 3 (with exact 95% CI) will be calculated.
- Seropositivity rates and their exact 95% CIs for antibodies against PT, FHA, PRN, anti-rotavirus, S. pneumoniae serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F and HBsAg will be tabulated.
- Percentage of subjects with anti-pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) antibody concentrations ≥0.35 µg/mL with 95% CI will be calculated.

- Percentage of subjects with anti-PRP antibody concentrations ≥1.0 μg/mL will be calculated with 95% CI.
- Percentage of subjects with anti RV IgA antibody concentration ≥90 U/mL will be calculated with 95% CI.
- GMC/GMT with 95% CI will be tabulated for antibodies against each antigen.

The above mentioned descriptive analyses will also be performed by race and sex.

The distribution of antibody concentrations/titers for each appropriate serotype/antigen will be displayed using tables and/or reverse cumulative distribution curves.

For anti-HBs antibodies, an analysis will be done according to vaccination history to Hepatitis B vaccine.

10.7.2. Between group assessment

For each treatment group, one month after Dose 3 of routine infant vaccines at Visit 4 (Month 5) time-point:

- Two-sided asymptotic standardized 95% CIs for the difference in the percentage of subjects with titer/concentration above or equal to pre-specified clinical thresholds will be computed (HRV Liq group minus HRV Lyo group).
- The two-sided 95% CIs for the group GMC/GMT ratio (HRV Liq group over HRV Lyo group) will be computed using an ANOVA model on the logarithm10 transformation of the concentrations. The ANOVA model will include the vaccine group as fixed effects. In addition, for anti-HBs, the model will include the Hepatitis B vaccination history as co-variable.
- For the group comparison in anti-PT, FHA, PRN seroresponse at one month post dose 3, P-value for testing H0: P≤ 85% vs. H1: P>85% (P= % of subjects in HRV liq group with seroresponse (above a threshold that leads to 95% seroresponse in the HRV lyo group) will be computed. P-value will be computed by integrating on the p-value for the null hypothesis that the seroresponse rate in the HRV lyo group is <85% and the a-posteriori probability of the threshold in the HRV liq group.

10.8. Analysis of safety

The ES will be used for the analysis of safety.

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 post-vaccination 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, those assessed as causally related to vaccination, those rated as grade 3 in intensity with causal relationship to vaccination and those that resulted in a 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 general solicited symptom rated as grade 3 in intensity, those assessed as causally related to vaccination, those rated as grade 3 in intensity with causal relationship to vaccination and those that resulted in a medically attended visit. For fever, additional analyses will be performed by 0.5°C increments. These calculations will also be performed by sex and race.

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 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 unsolicited AEs rated as grade 3 with causal relationship to vaccination and those that resulted in a medically attended visit.

The percentage of subjects who started taking at least one concomitant medication, antipyretic medication and prophylactic antipyretic medication during the 8 day (Days 1-8) and 31 day (Days 1-31) follow-up period post-vaccination will be tabulated by dose, overall per subject and over all the doses.

Subjects who experienced at least one SAE during the entire study period (from Dose 1 till ESFU Contact at Month 10) will be reported and the SAEs will be described in detail.

10.9. Interpretation of analyses

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

10.9.1. Statistical methods

- The exact 95% CIs for a proportion within a group will be based on the method by Clopper [Clopper, 1934].
- The standardized asymptotic CI for the group difference in proportion is the method 6 described in the Newcombe paper [Newcombe, 1998]
- The 95% CIs of the group GMC/GMT ratios will be computed using an ANOVA model on the logarithm10 transformation of the concentrations/titers. The ANOVA model will include the vaccine group as fixed effects. In addition, for anti-HBs, the model will include the Hepatitis B vaccination history as co-variable.
- The 95% CI for GMTs/GMCs will be obtained within each group separately. The 95% CI for the mean of log-transformed titer/concentration will be first obtained assuming that log-transformed values were normally distributed with unknown variance. The 95% CI for the GMTs/GMCs will then be obtained by exponential-transformation of the 95% CI for the mean of log-transformed titer/concentration.

- P-value for seroresponse endpoint will be computed by integrating on the p-value for the null hypothesis that the seroresponse rate in the HRV lyo group is <85% and the a-posteriori probability of the threshold in the HRV liq group.

10.10. Conduct of analyses

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.

10.10.1. Sequence of analyses

Safety data that is as clean as possible will be analyzed for IDMC review. Details of the review will be described in an IDMC charter. The final analyses of all data will be conducted after conclusion of the ESFU contact and will include the final analyses of immunogenicity, reactogenicity and safety.

An integrated clinical study report containing all data will be written and made available to the investigators and submitted to regulatory authorities as appropriate**.**

10.10.2. Statistical considerations for interim analyses

All analyses will be conducted on final data and therefore no statistical adjustment for interim analyses is required.

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 a 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

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APPENDIX A LABORATORY ASSAYS

Rotavirus Ab.IgA Determination

The anti-rotavirus antibody concentrations are determined by a validated anti-rotavirus IgA ELISA. Microtiter plates (96-well) are coated with an anti-rotavirus 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-rotavirus 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-rotavirus 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.

Poliovirus

Presence of antibodies against poliovirus types 1, 2 and 3 will be determined by a virus micro-neutralization test adapted from the World Health Organization Guidelines for WHO/EPI Collaborative Studies on Poliomyelitis. The starting dilution at which serum samples will be tested is 1/8, from which a test will be considered positive. Titers will be expressed in terms of the reciprocal of the dilution resulting in 50% inhibition.

Streptococcus

Pneumococcal serotype-specific immunoglobin G (IgG) antibodies (antibodies against serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) will be measured by ECL multiplex assays. The antibody concentration will be determined by logistic log comparison of the assay curves with a standard reference serum sp007 available from the US Food and Drug Administration (FDA) for which concentration of IgG to each of the 13 serotypes is known in µg/mL.

Anti-FHA ELISA (Bordetella pertussis, Filamentous Hemaglutinin Ab.IgG)

Antibody responses against FHA will be quantified by ELISA using purified FHA antigen extracted from *Bordetella Pertussis* culture in virulence Phase I as coating. The antigen is coated onto a 96-wells microplate. After a washing and a blocking step, dilutions of serum samples, controls and standard are incubated on the coated plate. The microplate is washed and mouse HorseRadishPeroxidase (HRP)-conjugated anti-human IgG monoclonal antibodies are added. After incubation, unbound antibodies are removed by washing and TetraMethylBenzidine (TMB) is incubated to reveal the enzyme activity. Color reaction is stopped by addition of sulfuric acid and the resulting yellow color is measured spectrophotometrically.

The intensity of the color is directly proportional to the concentration of the anti-FHA antibodies present in the sample.

Concentrations are calculated from a reference standard curve using a four parameters logistic fitting algorithm and expressed in International units (IU/mL).

Anti-PT ELISA (Bordetella pertussis, Pertussis Toxin Ab.IgG)

Antibody responses against PT will be quantified by ELISA using purified PT antigen extracted from *Bordetella Pertussis* culture in virulence Phase I as coating. The antigen is coated onto a 96-wells microplate. After a washing and a blocking step, dilutions of serum samples, controls and standard are incubated on the coated plate. The microplate is washed and mouse HorseRadishPeroxidase (HRP)-conjugated anti-human IgG monoclonal antibodies are added. After incubation, unbound antibodies are removed by washing and is incubated to reveal the enzyme activity. Color reaction is stopped by addition of sulfuric acid and the resulting yellow color is measured spectrophotometrically.

The intensity of the color is directly proportional to the concentration of the anti-PT antibodies present in the sample.

Concentrations are calculated from a reference standard curve using a 4 parameters logistic fitting algorithm and expressed in International units (IU/mL).

Anti-PRN ELISA (Bordetella pertussis, Pertactin Ab.IgG)

Antibody responses against PRN will be quantified by ELISA using purified PRN antigen extracted from *Bordetella Pertussis* culture in virulence Phase I as coating. The antigen is coated onto a 96-wells microplate. After a washing and a blocking step, dilutions of serum samples, controls and standard are incubated on the coated plate. The microplate is washed and mouse HorseRadishPeroxidase (HRP)-conjugated anti-human IgG monoclonal antibodies are added. After incubation, unbound antibodies are removed by washing and TetraMethylBenzidine (TMB) is incubated to reveal the enzyme activity. Color reaction is stopped by addition of sulfuric acid and the resulting yellow color is measured spectrophotometrically.

The intensity of the color is directly proportional to the concentration of the anti-PRN antibodies present in the sample.

Concentrations are calculated from a reference standard curve using a 4 parameters logistic fitting algorithm and expressed in International units (IU/mL).

Anti-D ELISA (Corynebacterium diphtheria, Diphtheria Toxoid Ab.IgG)

Antibody responses against D will be quantified by ELISA using purified D antigen as coating. The antigen is coated onto a 96-wells microplate. After a washing and a blocking step, dilutions of serum samples, controls and standard are incubated on the coated plate. The microplate is washed and mouse HorseRadishPeroxidase (HRP)-conjugated anti-human IgG monoclonal antibodies are added. After incubation, unbound antibodies are removed by washing and TetraMethylBenzidine (TMB) is incubated to reveal the enzyme activity. Color reaction is stopped by addition of sulfuric acid and the resulting yellow color is measured spectrophotometrically.

The intensity of the color is directly proportional to the concentration of the anti-D antibodies present in the sample.

Concentrations are calculated from a reference standard curve using a 4 parameters logistic fitting algorithm and expressed in International units (IU/mL).

Anti-T ELISA (Clostridium tetani, Tetanus Toxoid Ab.IgG)

Antibody responses against T will be quantified by ELISA using purified T antigen as coating. The antigen is coated onto a 96-wells microplate. After a washing and a blocking step, dilutions of serum samples, controls and standard are incubated on the coated plate. The microplate is washed and mouse HorseRadishPeroxidase (HRP)-conjugated anti-human IgG monoclonal antibodies are added. After incubation, unbound antibodies are removed by washing and TetraMethylBenzidine (TMB) is incubated to reveal the enzyme activity. Color reaction is stopped by addition of sulfuric acid and the resulting yellow color is measured spectrophotometrically.

The intensity of the color is directly proportional to the concentration of the anti-T antibodies present in the sample.

Concentrations are calculated from a reference standard curve using a 4 parameters logistic fitting algorithm and expressed in International units (IU/mL).

Anti-PRP ELISA (Haemophilus influenzae type b, Polyribosyl Ribitol Phosphate Ab)

Antibody responses against PRP will be quantified by ELISA using purified PRP antigen extracted from inactivated *Haemophilus influenzae type b* as coating. The antigen is coated onto a 96-wells microplate. After a washing and a blocking step, dilutions of serum samples, controls and standard are incubated on the coated plate. The microplate is washed and goat HorseRadishPeroxidase (HRP)-conjugated anti-human Ig to polyclonal antibodies are added. After incubation, unbound antibodies are removed by washing and TetraMethylBenzidine (TMB) is incubated to reveal the enzyme activity. Color reaction is stopped by addition of sulfuric acid and the resulting yellow color is measured spectrophotometrically.

The intensity of the color is directly proportional to the concentration of the anti-PRP antibodies present in the sample.

Concentrations are calculated from a reference standard curve using a 4 parameters logistic fitting algorithm and expressed in micrograms (µg/mL).

Hepatitis B virus

The ADVIA Centaur Anti-HBS2 assay is a sandwich immunoassay using direct, chemiluminometric technology. HBsAg (ad and ay)are covalently coupled to magnetic latex particules in the Solid Phase. In the Lite Reagent, the HBs Ag (ad and ay) is labelled with acridinum ester. Non-magnetic latex particules are added from the ancillary well. The sample is incubated simultaneously with Lite Reagent, Solis Phase and Acillary Reagent. Antibody-antigen complexes will form if anti-HBs is present in the sample. A

direct relationship exist between the amount of anti-HBs activity present in the patient sample and the amount of relative light units (RLUs) detected by the system.

APPENDIX B CLINICAL LABORATORIES

Table 20 GSK Biologicals' laboratories

Laboratory	Address
GSK Biological's Clinical Laboratories 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

Table 21 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