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

Primary Study vaccine and number	**GlaxoSmithKline (GSK) Biologicals':** • Hepatitis B vaccine, Engerix™-B Kinder (SKF103860) • Combined diphtheria-tetanus-acellular pertussis hepatitis B-inactivated poliovirus and *Haemophilus influenzae* type b (DTPa-HBVIPV/Hib) vaccine, Infanrix™ hexa (SB217744)
eTrack study number and Abbreviated Title	106794 (DTPA-HBV-IPV-115)
EudraCT number	2015-003391-74
Date of protocol	Final Version 01: 06 November 2015
Title	Persistence of hepatitis B antibodies, immunogenicity and safety of GSK Biologicals' hepatitis B vaccine, Engerix™-B Kinder (SKF103860) challenge dose, in adolescents vaccinated with four doses of Infanrix™ hexa (SB217744) during infancy.
Detailed Title	A phase IV, open-label, multicentre study to assess the long-term persistence of antibodies against hepatitis B and the immunogenicity and safety of a challenge dose of hepatitis B vaccine (Engerix-B™ Kinder SKF103860) in children aged 14-15 years, previously primed and boosted in the first two years of life with four doses of GSK Biologicals' DTPa-HBV-IPV/Hib (Infanrix™ hexa SB217744) vaccine.
Co-ordinating author	PPD Scientific Writer

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Contributing authors	• PPD Project-level Clinical Research and Development Lead • PPD Clinical Research and Development Lead • PPD Project Statistician • PPD Lead Statistician • PPD Study Delivery Lead • PPD Study Delivery Manager • PPD Vaccine Supply Coordinator • PPD Clinical Safety representative • PPD Project Data Manager • PPD Study Data Manager • PPD Global Clinical Regulatory Affairs Representative • PPD Clinical Laboratory Sciences Read-out Team Leader • PPD Clinical Laboratory Sciences Study Manager • PPD Global Patent Representative • PPD Local Delivery Lead

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Protocol Sponsor Signatory Approval

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Sponsor signatory	Narcisa Elena Mesaros, MD Project level Clinical Research and Development Lead, DTP/Polio Vaccines, Late Clinical Development, Vaccine Discovery and Development, 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' investigational vaccine 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.

eTrack study number and Abbreviated Title	106794 (DTPA-HBV-IPV-115)
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Investigator name	_____
Signature	_____
Date	_____
PPD **function and title**	_____
Signature	_____
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 IV, open-label, multicentre study to assess the long-term persistence of antibodies against hepatitis B and the immunogenicity and safety of a challenge dose of hepatitis B vaccine (Engerix-B™ Kinder SKF103860) in children aged 14-15 years, previously primed and boosted in the first two years of life with four doses of GSK Biologicals' DTPa-HBV-IPV/Hib (Infanrix™ hexa SB217744) vaccine.

Indication

Immunisation against infection caused by all known subtypes of hepatitis B virus.

Rationale for the study and study design

- **Rationale for the study**

In the global context of vaccination against hepatitis B, the European Medicines Agency (EMA) has requested GSK Biologicals to set up a long-term surveillance programme of vaccines containing a recombinant hepatitis B component. This study is the last study in a series of four studies, constituting a common follow-up programme of vaccination with *Infanrix hexa*. The previous studies conducted in this series are mentioned below:

- 106789 (DTPA-HBV-IPV-112): The study was conducted in healthy subjects 4-5 years of age at the time of enrolment, who received four consecutive doses of *Infanrix hexa* [Steiner, 2010].
- 112688 (DTPA-HBV-IPV-113): The study was conducted in healthy subjects 7-8 years of age at the time of enrolment who received four consecutive doses of *Infanrix hexa* [Meeren, 2014].
- 106793 (DTPA-HBV-IPV-114): The study was conducted in healthy subjects 12-13 years of age at the time of enrolment who received four consecutive doses of *Infanrix hexa.*

This study aims to determine the persistence, from childhood to adolescence of immunity to hepatitis B that is conferred by 4 doses vaccination with *Infanrix hexa* in the first 2 years of age. Persistent immunity to hepatitis B was assessed previously, in four to five year old, seven to eight year old and 12-13 year old children who were vaccinated with four doses of *Infanrix hexa*, as part of routine vaccination in infancy, in Germany. More than 60% of subjects showed seroprotective persistent antibody concentrations and at least 96% of subjects in all age groups showed an anamnestic response to the single hepatitis B challenge dose [Steiner, 2010; Meeren, 2014; GSK

Biologicals' Clinical Study Report (106793)]. *Infanrix hexa* was licensed for use in Germany in 2000. The results observed in these studies are in line with the results observed when children and adolescents were primed with three doses of a monovalent hepatitis B vaccine [Behre,2012]. *Infanrix hexa* was licensed for use in Germany in 2000. Thus, children who received routine vaccination in the year 2001 with *Infanrix hexa* will reach an age of 14 to 15 years by 2016. These children will be invited to participate in this study in order to collect persistence data for hepatitis B antibodies and to assess the anamnestic response, immunogenicity, safety and reactogenicity of a single challenge dose of the hepatitis B vaccine (*Engerix-B Kinder*).

- **Rationale for the study design**

This study will be conducted with a single group of subjects as all children recruited will receive a single challenge dose of HBV vaccine. A blood sample will be taken before and one month after the single challenge dose to evaluate:

- The persistence of anti-HBs antibody concentrations in subjects 14-15 years of age previously vaccinated with four doses of *Infanrix hexa* in the first two years of life.
- The ability to mount an anamnestic response to the single HBV challenge dose.
- The safety and reactogenicity of the single challenge dose of HBV vaccine.

Since this study involves a single study group and all subjects will be administered the same vaccine, it is planned to be conducted in an open and non-randomised manner.

Objectives

Primary

- To assess the immunological response to hepatitis B antigen, in terms of antibody concentrations ≥100 mIU/ml, one month after the single challenge dose of the HBV vaccine in subjects 14-15 years of age, previously vaccinated with four doses of *Infanrix hexa* in the first two years of life.

Secondary

- To assess the persistence of anti-HBs antibodies, in terms of seroprotection status and antibody concentrations, in subjects 14-15 years of age, previously vaccinated with four doses of *Infanrix hexa* in the first two years of life.

- To assess the immunological response to hepatitis B antigen, in terms of anamnestic response, one month after the single challenge dose of the HBV vaccine in subjects 14-15 years of age, previously vaccinated with four doses of *Infanrix hexa* in the first two years of life.
- To assess the immunological response to the hepatitis B antigen, in terms of seroprotection status and antibody concentrations, one month after the single challenge dose of the HBV vaccine in subjects 14-15 years of age, previously vaccinated with four doses of *Infanrix hexa* in the first two years of life.
- To evaluate the safety and reactogenicity of a single challenge dose of HBV vaccine (*Engerix-B Kinder*) in terms of solicited symptoms (local and general), unsolicited symptoms and serious adverse events (SAEs).

Study design

- Experimental design: Phase IV, open-label, non-randomised, multi-centric, single-country study with a single group.
- Duration of the study: the intended duration of the study, per subject, will be approximately one month. The study will have a single epoch as follows:
  - Epoch 001: Primary starting at Visit 1 (Day 0) and ending at Visit 2 (Month 1).
- Study groups: The study groups are presented in Synopsis Table 1 and Synopsis Table 2.

Synopsis Table 1 Study groups and epochs foreseen in the study

Study groups	Number of subjects	Age (Min/Max)	Epochs
			Epoch 001
HBV Group	300	14 years – 15 years	x

Synopsis Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine name	HBV Group
Engerix-B Kinder	HBV	•

- Control: uncontrolled
- Vaccination schedule: A single dose of HBV vaccine will be administered to all subjects, who were previously primed and boosted with four doses of *Infanrix hexa* in the first two years of life.
- Treatment allocation: non-randomised

- Blinding: open-label

Synopsis Table 3 Blinding of study epochs

Study Epochs	Blinding
Epoch 001	open

- Sampling schedule: Blood samples will be taken from each subject at the following time-points:
  - Pre-vaccination: at Visit 1, before the administration of the single challenge dose of HBV vaccine.
  - Post-vaccination: at Visit 2, approximately one month after the single challenge dose of HBV vaccine.
- Type of study: self-contained
- Data collection: Electronic Case Report Form (eCRF).

Number of subjects

Approximately 300 children aged 14-15 years will be enrolled in this study. They should have received four doses of *Infanrix hexa* with the three primary vaccination doses received by 9 months of age and the booster dose received between 11 and 18 months of age as part of routine vaccination practice in Germany.

Endpoints

Primary

- Immunogenicity to components of the study vaccine.
  - Anti-HBs antibody concentrations ≥100 mIU/ml, one month after the single challenge dose of HBV vaccine.

Secondary

- Anti-HBs antibody persistence after previous vaccination with *Infanrix hexa*.
  - Anti-HBs antibody concentrations ≥ 10 mIU/ml, ≥ 100 mIU/ml and anti-HBs antibody concentrations before the single challenge dose of HBV vaccine.
- Immunogenicity to the components of the study vaccine.
  - Anti-HBs antibody concentrations ≥ 10 mIU/ml and anti-HBs antibody concentrations one month after the single challenge dose of HBV vaccine.
  - Anamnestic response to the single challenge dose of HBV vaccines.

- Solicited local and general symptoms.
  - Occurrence of each solicited local and general symptom during the 4-day (Day 0–3) follow-up period after the single challenge dose of HBV vaccine.
- Unsolicited adverse events
  - Occurrence of unsolicited AEs during the 31-day (Day 0–30) follow-up period after the single challenge dose of HBV vaccine.
- Serious adverse events
  - Occurrence of serious adverse events after the single challenge dose of HBV vaccine up to study end.

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LIST OF ABBREVIATIONS

AE:	Adverse Event
Anti-HBc:	Antibodies against hepatitis B core antigen
Anti-HBs:	Antibodies against hepatitis B surface antigen
ATP:	According-To-Protocol
CDC:	Centers for Disease Control and Prevention, USA
CEVAC:	Center for Vaccinology, Ghent University Hospital
CI:	Confidence Interval
CLIA:	ChemiLuminescence ImmunoAssay
DTPa-HBV-IPV/Hib:	Combined diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated poliovirus vaccine and *Haemophilus influenzae* type b conjugate (*Infanrix hexa*)
eCRF:	electronic Case Report Form
EDD:	Estimated Date of Delivery
EGA:	Estimated Gestational Age
EMA:	European Medicines Agency
GSK:	GlaxoSmithKline
ICF:	Informed Consent Form
IAF:	Informed Assent Form
ICH:	International Conference on Harmonisation
IEC:	Independent Ethics Committee
IM:	Intramuscular
IMP:	Investigational Medicinal Product
IRB:	Institutional Review Board
IU/ml:	International Units per millilitre
LAR:	Legally Acceptable Representative

LL:	Lower Limit
LMP:	Last Menstrual Period
mg:	Milligram
µg:	Microgram
MACDP:	Metropolitan Atlanta Congenital Defects Program
MedDRA:	Medical Dictionary for Regulatory Activities
RCC:	Reverse Cumulative Curves
RDE:	Remote Data Entry
SAE:	Serious Adverse Event
SBIR:	Randomisation System on Internet
SDV:	Source Document Verification
SmPC:	Summary of Product Characteristics
SPM:	Study Procedures Manual
TVC:	Total vaccinated cohort
WHO:	World Health Organization

GLOSSARY OF TERMS

Adequate contraception: Adequate contraception is defined as a contraceptive method with failure rate of less than 1% per year when used consistently and correctly and when applicable, in accordance with the product label for example:

- abstinence from penile-vaginal intercourse, when this is their preferred and usual lifestyle,
- oral contraceptives, either combined or progestogen alone,
- injectable progestogen,
- implants of etenogestrel or levonorgestrel,
- estrogenic vaginal ring,
- percutaneous contraceptive patches,
- intrauterine device or intrauterine system,
- male partner sterilisation prior to the female subject's entry into the study, and this male is the sole partner for that subject,

  The information on the male sterility can come from the site personnel's review of the subject's medical records; or interview with the subject on her medical history.
- male condom combined with a vaginal spermicide (foam, gel, film, cream or suppository),
- male condom combined with a female diaphragm, either with or without a vaginal spermicide (foam, gel, film, cream, or suppository).

Adequate contraception does not apply to subjects of child bearing potential with same sex partners, when this is their preferred and usual lifestyle.

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 an open-label study, no blind is used. Both the investigator and the subject know the identity of the treatment assigned.
Child in care:	A child who has been placed under the control or protection of an agency, 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.
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
Epoch:	An epoch is a self-contained set of consecutive timepoints or a single timepoint from a single protocol. Self-contained means that data collected for all subjects at all timepoints within that epoch allows to draw a complete conclusion to define or precise the targeted label of the product. Typical examples of epochs are 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 according-to-protocol (ATP) analyses.
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:	A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial,

(Synonym of Investigational Medicinal Product)	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: **(The terms legal representative or legally authorized representative are used in some settings.)**	An individual or juridical or other body authorized under applicable law to consent, on behalf of a prospective subject, to the subject's participation in the clinical trial.
Menarche:	Menarche is the onset of menses for the first time in a young female and is preceded by several changes associated with puberty including breast development and pubic hair growth. Menarche usually occurs within 1-2 years of breast development, thelarche. However, a young female can become pregnant before her first menses. Thus, a conservative definition of non-childbearing potential in a pre-menarcheal female is a young female who has not yet entered puberty as evidenced by lack of breast development (palpable glandular breast tissue).
Randomisation:	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.
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.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the clinical study, either as a recipient of the vaccine or as a control.
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, identified by a unique number, according to the study randomisation or treatment allocation.
Unsolicited adverse	Any AE reported in addition to those solicited during the clinical study. Also any ‘solicited’ symptom with onset

event:	outside the specified period of follow-up for solicited symptoms will be reported as an unsolicited adverse event.

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 ™ and in *italics*.

Trademarks of the GlaxoSmithKline group of companies	Generic description
Engerix™-B Kinder	Recombinant hepatitis B vaccine
Infanrix™ hexa	Combined diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated poliovirus and *Haemophilus influenzae* type b vaccine

1. INTRODUCTION

1.1. Background

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus. It is a major global health problem and the most serious type of viral hepatitis. It can cause chronic liver disease and puts people at high risk of death from cirrhosis of the liver and liver cancer. An estimated 240 million people are chronically infected with hepatitis B. More than 780,000 people die every year due to the complications of hepatitis B, including cirrhosis and liver cancer [WHO, 2015].

A vaccine against hepatitis B has been available since 1982. The vaccine is 95% effective in preventing infection and the development of chronic disease and liver cancer due to hepatitis B. Protection against hepatitis B disease after immunisation relies on persistent protective serum antibodies and on the ability of the immune system to mount an anamnestic response when confronted with the hepatitis B virus (HBV). Persisting immune memory to the hepatitis B virus surface antigen (HBsAg) is explored by evaluating the effect of a HBV challenge vaccination of previously immunised subjects. Studies conducted after primary immunisation with HBV vaccine in different populations have shown long-term persistence and immune memory induced by *Engerix-B Kinder* against hepatitis B, extending up to 20 years after primary vaccination [Avdicova, 2012; Behre, 2012; Poovorawan, 2013].

Please refer to the Prescribing Information for information regarding the regarding the potential risks and benefits of *Engerix-B Kinder*.

1.2. Rationale for the study and study design

1.2.1. Rationale for the study

In the global context of vaccination against hepatitis B, the European Medicines Agency (EMA) has requested GSK Biologicals to set up a long-term surveillance programme of vaccines containing a recombinant hepatitis B component. This study is the last study in a series of four studies, constituting a common follow-up programme of vaccination with *Infanrix hexa*. The previous studies conducted in this series are mentioned below:

- 106789 (DTPA-HBV-IPV-112): The study was conducted in healthy subjects 4-5 years of age at the time of enrolment, who received four consecutive doses of *Infanrix hexa* [Steiner, 2010].
- 112688 (DTPA-HBV-IPV-113): The study was conducted in healthy subjects 7-8 years of age at the time of enrolment who received four consecutive doses of *Infanrix hexa* [Van Der Meeren, 2014].
- 106793 (DTPA-HBV-IPV-114): The study was conducted in healthy subjects 12-13 years of age at the time of enrolment who received four consecutive doses of *Infanrix hexa*.

This study aims to determine the persistence, from childhood to adolescence of immunity to hepatitis B that is conferred by 4 doses of vaccination with *Infanrix hexa* in the first 2 years of age. Persistent immunity to hepatitis B was assessed previously, in four to five year old, seven to eight year old and 12-13 year old children who were vaccinated with four doses of *Infanrix hexa*, as part of routine vaccination in infancy, in Germany. More than 60% of subjects showed seroprotective persistent antibody concentrations and at least 96% of subjects in all age groups showed an anamnestic response to the single hepatitis B challenge dose [Zinke M, 2010, Steiner, 2010; Van Der Meeren, 2014; GSK Biologicals' Clinical Study Report (106793)]. The results observed in these studies are in line with the results observed when children and adolescents were primed with three doses of a monovalent hepatitis B vaccine [Behre,2012]. *Infanrix hexa* was licensed for use in Germany in 2000. Thus, children who received routine vaccination in the year 2001 with *Infanrix hexa* will reach an age of 14 to 15 years by 2016. These children will be invited to participate in this study in order to collect persistence data for hepatitis B antibodies and to assess the anamnestic response, immunogenicity, safety and reactogenicity of a single challenge dose of the hepatitis B vaccine (*Engerix-B Kinder*).

1.2.2. Rationale for the study design

This study will be conducted with a single group of subjects as all children recruited will receive a single challenge dose of HBV vaccine. A blood sample will be taken before and one month after the single challenge dose to evaluate:

- The persistence of anti-HBs antibody concentrations in subjects 14-15 years of age previously vaccinated with four doses of *Infanrix hexa* in the first two years of life.
- The ability to mount an anamnestic response to the single HBV challenge dose.

The safety and reactogenicity of the single challenge dose of HBV vaccine will also be evaluated in this study.

Since this study involves a single study group and all subjects will be administered the same vaccine, it is planned to be conducted in an open and non-randomised manner.

1.3. Benefit : Risk Assessment

Engerix-B Kinder has demonstrated a good safety profile in the clinical studies performed to date.

Please refer to the Prescribing Information for information regarding the summary of potential risks and benefits of *Engerix-B Kinder*.

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

1.3.1. Risk Assessment

Based on substantial data available from clinical and non-clinical experience, the safety profile of *Engerix-B Kinder* has been established. The vaccine is well tolerated without key safety findings.

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Investigational study vaccine (*Engerix-B Kinder*)		
Hypersensitivity	Spontaneous data/ hypersensitivity after administration of hepatitis B containing vaccines or to any component of the vaccine	Subjects will be observed for at least 30 minutes after vaccine administration, with medical attention available in case of anaphylaxis reactions.
Adequate human data on use during pregnancy/lactation and adequate animal reproduction studies are not available	The effect of the vaccine on foetal development has not been assessed.	Pregnant subjects will not be included in this study (see Section 4.2). Female subjects of childbearing potential are to have a urine pregnancy test prior to the study vaccine administration and will not be vaccinated if the test is positive (see Section 4.2 and 4.3).
Study Procedures		
Syncope	Spontaneous Syncope (fainting) can occur following, or even before, any vaccination as a psychogenic response to the needle injection.	Section 6.6 highlights that procedures should be in place to avoid injuries from falls following syncope.

1.3.2. Benefit Assessment

When primed with *Infanrix hexa*, a vast majority of subjects get protected (>95%) against hepatitis B disease. The persistence studies suggest that immune memory persists for at least 20 years after vaccination. However, the immune response in some individuals might wane more rapidly than expected. By taking part in this study, the subject will know if he/she is still seroprotected against hepatitis 14-15 years after hepatitis B vaccination.

This study will provide information about the duration of protection offered by three primary doses and one booster dose in the first 2 years of life of *Infanrix hexa* against hepatitis B infection. This would also present an opportunity to facilitate better protection against the disease through hepatitis B vaccination, in the future.

The subject will also benefit from an additional vaccine dose, which is currently not routinely offered, and which may help to keep him/her protected against hepatitis B even longer.

1.3.3. Overall Benefit: Risk Conclusion

Taking into account the measures taken to minimise risk to subjects participating in this study, the potential or identified risks identified in association with *Engerix-B Kinder* are justified by the potential benefits that may be afforded to subjects receiving the vaccine for immunisation against hepatitis B infection.

2. OBJECTIVES

2.1. Primary objective

- To assess the immunological response to hepatitis B antigen, in terms of antibody concentrations ≥100 mIU/ml, one month after the single challenge dose of the HBV vaccine in subjects 14-15 years of age, previously vaccinated with four doses of *Infanrix hexa* in the first two years of life.

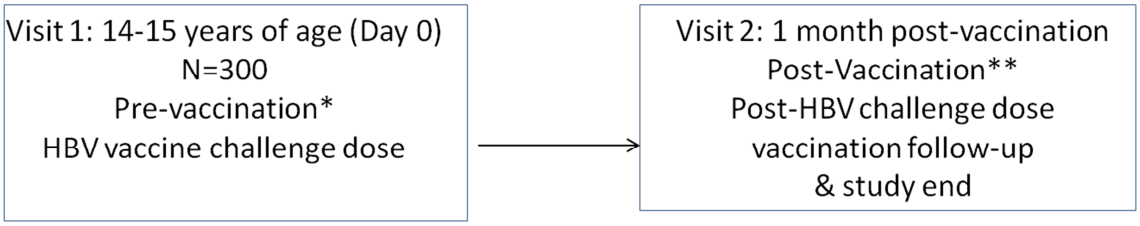
Refer to Section 10.1 for the definition of the primary endpoint.

2.2. Secondary objectives

- To assess the persistence of anti-HBs antibodies, in terms of seroprotection status and antibody concentrations, in subjects 14-15 years of age, previously vaccinated with four doses of Infanrix hexa in the first two years of life.
- To assess the immunological response to hepatitis B antigen, in terms of anamnestic response, one month after the single challenge dose of the HBV vaccine in subjects 14-15 years of age, previously vaccinated with four doses of Infanrix hexa in the first two years of life.
- To assess the immunological response to the hepatitis B antigen, in terms of seroprotection status and antibody concentrations, one month after the single challenge dose of the HBV vaccine in subjects 14-15 years of age, previously vaccinated with four doses of Infanrix hexa in the first two years of life.
- To evaluate the safety and reactogenicity of a single challenge dose of HBV vaccine (*Engerix-B Kinder*) in terms of solicited symptoms (local and general), unsolicited symptoms and serious adverse events (SAEs).

Refer to Section 10.2 for the definition of the secondary endpoints.

3. STUDY DESIGN OVERVIEW



N: Number of subjects planned to be enrolled
* Pre-vaccination: Blood sampling before the administration of the single HBV vaccine challenge dose
** Post-vaccination: Blood sampling one month after the administration of the single HBV vaccine challenge dose

Protocol waivers or exemptions are not allowed with the exception 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 IV, open-label, non-randomised, multi-centric, single-country study with a single group.
- Duration of the study: the intended duration of the study, per subject, will be approximately one month. The study will have a single epoch as follows:
  - Epoch 001: Primary starting at Visit 1 (Day 0) and ending at Visit 2 (Month 1).
- Study groups: the details are presented below in Table 1.

Table 1 Study groups and epochs foreseen in the study

Study groups	Number of subjects	Age (Min/Max)	Epochs
			Epoch 001
HBV Group	300	14 years – 15 years	x

The treatment group details are provided in Table 2.

Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine name	HBV Group
Engerix-B Kinder	HBV	•

- Control: uncontrolled
- Vaccination schedule: A single dose of HBV vaccine will be administered to all subjects, who were previously primed and boosted with four doses of *Infanrix hexa* in the first two years of life.
- Treatment allocation: non-randomised
- Blinding: Refer to Table 3.

Table 3 Blinding of study epochs

Study Epochs	Blinding
Epoch 001	open

- Sampling schedule: Blood samples will be taken from each subject at the following time-points:
  - Pre-vaccination: at Visit 1, before the administration of the single challenge dose of HBV vaccine.
  - Post-vaccination: at Visit 2, approximately one month after the single challenge dose of HBV vaccine.
- Type of study: self-contained
- Data collection: Electronic Case Report Form (eCRF).

4. STUDY COHORT

4.1. Number of subjects/centres

Approximately 300 children aged 14-15 years will be enrolled in this study. They should have received four doses of *Infanrix hexa* with the three primary vaccination doses received by 9 months of age and the booster dose received between 11 and 18 months of age as part of routine vaccination practice in Germany.

Parents/Legally Acceptable Representative(s) [LAR(s)] of these children will be contacted and their children will be invited to participate in this challenge dose study.

Refer to Section 10.3 for a detailed description of criteria used to estimate the sample size.

Overview of the recruitment plan

- The study will be conducted at multiple centres in Germany.
- Enrolment is expected to be completed within a period of approximately six months.
- The study duration per subject will be approximately one month.
- The follow-up of recruitment of subjects into the study will be performed using GSK Biologicals' central randomisation system on Internet (SBIR).
- The recruitment will be monitored by the site monitor.

4.2. Inclusion criteria for enrolment

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability 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)/Legally Acceptable Representative(s) [LAR(s)] who, in the opinion of the investigator, can and will comply with the requirements of the protocol (e.g. completion of the diary cards, return for follow-up visits).
- Written informed consent obtained from the parent(s)/LAR(s) of the subject prior to performance of any study specific procedure.
- In addition to the informed consent that will be signed by the parents/LAR(s), written informed assent of the subject will be sought.
- A male or female between the ages of 14 to 15 (from and including the 14th birthday, up to but excluding the 16th birthday) at the time of vaccination.
- Healthy subjects as established by medical history and clinical examination before entering into the study.
- Subjects with documented evidence of previous vaccination with four consecutive doses of *Infanrix hexa* as part of routine vaccination in Germany: three doses of primary vaccination received by 9 months of age and one booster dose received between 11 and 18 months of age.
- Female subjects of non-childbearing potential may be enrolled in the study.
  - Non-childbearing potential is defined as pre-menarche, current tubal ligation, hysterectomy or ovariectomy.

  Please refer to the glossary of terms for the definition of menarche.
- Female subjects of childbearing potential may be enrolled in the study, if the subject:
  - has practiced adequate contraception for 30 days prior to vaccination, and
  - has a negative pregnancy test on the day of vaccination, and
  - has agreed to continue adequate contraception during the entire treatment period and for 2 months after completion of the vaccination series.

Please refer to the glossary of terms for the definition of adequate contraception.

4.3. Exclusion criteria for enrolment

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability 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 vaccine during the period starting 30 days before the dose of study vaccine (Day -29 to Day 0), or planned use during the study period.
- Any medical condition that in the judgment of the investigator would make intramuscular injection unsafe.
- Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs during the period starting six months prior to the vaccine dose. For corticosteroids, this will mean prednisone $\geq$ 0.5 mg/kg/day (for paediatric subjects), or equivalent. Inhaled and topical steroids are allowed.
- 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 in the period starting 30 days before the dose and ending 30 days after the dose of HBV vaccine administration with the exception of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (dTpa) vaccine, which can be given as part of routine vaccination practice. Seasonal or pandemic influenza vaccine can be given at any time during the study, and according to the Summary of Product Characteristics and national recommendations.
- 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).
- Evidence of previous hepatitis B booster vaccination since administration of the fourth dose of *Infanrix hexa* booster in the second year of life.
- History of or intercurrent hepatitis B disease.
- Hepatitis B vaccination at birth.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination (no laboratory testing required).
- Family history of congenital or hereditary immunodeficiency.
- History of any reaction or hypersensitivity (hypotone-hyperresponsiveness reaction) likely to be exacerbated by any component of the vaccine.
- Major congenital defects or serious chronic illness including thrombocytopenia and bleeding disorders.
- History of any neurological disorders or seizures.
- Acute disease and/or fever at the time of enrolment.
  - Fever is defined as temperature $\geq$ 37.5°C for oral, axillary or tympanic route, or $\geq$ 38.0°C for rectal route.
  - Subjects with a minor illness (such as mild diarrhoea, mild upper respiratory infection) without fever may be enrolled at the discretion of the investigator.

- Acute or chronic, clinically significant pulmonary, cardiovascular, hepatic or renal functional abnormality, as determined by physical examination or laboratory screening tests.
- Administration of immunoglobulins and/or any blood products during the period starting 3 months before the dose of study vaccine or planned administration during the study period.
- Pregnant or lactating female.
- Female planning to become pregnant or planning to discontinue contraceptive precautions.

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 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 paediatric population (ICH E11) and all other applicable ethical guidelines.

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

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

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favourable opinion/approval of study protocol and any subsequent amendments.
- Subject's parent(s)/LAR(s) informed consent and subject informed assent, 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) and subject informed assent, 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.

In accordance with the ICH Harmonised Tripartite Guidelines for Good Clinical Practice, those subjects who can only be enrolled in the study with the consent of the subject's parent(s)/legally acceptable representative (e.g. minors), should be informed about the study to the extent compatible with the subject's understanding and, if capable, the subject should sign and personally date a written Informed Assent Form (IAF). It is required that the assent be signed by each subject, if capable, in addition to the informed consent that is to be signed by his/her legal representative.

The investigator has the final responsibility for the final presentation of the ICF and IAF, respecting the mandatory requirements of local regulations. The ICF and IAF generated by the investigator with the assistance of the sponsor's representative must be acceptable to GSK Biologicals and be approved (along with the protocol, and any other necessary documentation) by the IRB/IEC.

5.2. Subject identification and randomisation of treatment

5.2.1. Subject identification

Subject identification numbers will be assigned sequentially to the subjects whose parent(s)/LAR(s) have consented to allow their child/ward to participate in the study, according to the range of subject identification numbers allocated to the study centre.

5.2.2. Randomisation of treatment

5.2.2.1. Randomisation of supplies

This is a single group non- randomised study. All eligible subjects will receive the challenge dose of HBV. A sequential list of treatment numbers will be generated by MATerial EXcellence (MATEX). The Randomisation System on Internet (SBIR) will be used to allocate treatment numbers to the subjects and also to track enrolment in the study.

To allow GSK Biologicals to take advantage of greater rates of recruitment than anticipated at individual centres in this multi-centre study and to thus reduce the overall study recruitment period, an over-randomisation 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

Allocation of a treatment number to the subject at the investigator site will be performed using a randomisation system on internet (SBIR). The randomisation algorithm will use a minimisation procedure accounting for centre.

After obtaining the signed and dated ICF/IAF from the subject/subject's parent(s)/LAR(s) 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 randomisation system will provide the treatment number to be used for the 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.

5.3. Method of blinding

This study is an open label study i.e. all subjects will receive a dose of the same vaccine, known to the investigator and to the parents/ LAR(s) of the subject.

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 burden of the study for the subject will be minimised as much as possible. For taking blood samples, three attempts at most should be performed. If the physician is not successful after the third attempt, he/she will make no further attempts. A local numbing cream or patch will also be offered at the discretion of the investigator prior to blood sampling, in order to minimise pain when blood samples are drawn.

The list of study procedures is presented in Table 4.

Table 4 List of study procedures

Epoch	Epoch 001	
Age	**14-15 years**	
Visit	**Visit 1**	**Visit 2**
Timing	**Day 0**	**Month 1**
Sampling time-points	**Pre-HBV challenge**	**Post-HBV challenge**
Written informed consent from parent(s)/LAR(s)	●	
Written informed assent from subject	O*	
Check inclusion/exclusion criteria	●	
Collect demographic data	●	
Medical and Vaccination history	●	
Physical examination	●	
Urine pregnancy test	●**	
Check contraindications, warnings and precautions	O	
Measure/record height and weight	●	
Pre-vaccination body temperature	●	
Vaccine		
Treatment number allocation	O	
Hepatitis B vaccine challenge dose administration	●	
Recording of administered treatment number	●	
Laboratory Assays		
Blood sampling for antibody determination (at least 2.5 mL)	●	●
Safety assessments		
Recording of any concomitant medication/vaccination	●	●
Record any intercurrent medical conditions		●
Distribution of diary cards to the parents/LAR(s)	O	
Recording of solicited symptoms (Days 0–3)	●	
Recording of any non-serious adverse events within 30 days post-vaccination	●	●
Recording of serious adverse events and pregnancies	●	●
Recording of SAEs related to study participation or to a concurrent GSK medication/vaccine	●	●
Return of diary cards		O
Diary card transcription by investigator		●
Study Conclusion		●

● is used to indicate a study procedure that requires documentation in the individual eCRF.
○ is used to indicate a study procedure that does not require documentation in the individual eCRF.
*This study procedure does not require documentation in the individual eCRF. The study purpose and procedures will be explained to the subject by appropriately trained personnel and the written informed assent will be obtained from the subject.
**This study procedure is to be performed for only female subjects of child bearing potential.

The intervals between study visits are presented in Table 5.

Table 5 Intervals between study visits

Interval	Optimal length of interval*	Maximum interval allowed**
Birth → Visit 1	14-15 days of age	From the 14th up to, but excluding the 16th birthday
Visit 1 → Visit 2	30 days	21 – 48 days

* Whenever possible the investigator should arrange study visits within this interval

** Subjects will not be eligible for inclusion in one or more cohorts for analyses if they make the study visit outside this interval. An interval of 21–48 days will be considered for the According-to-Protocol (ATP) cohort of immunogenicity. Refer to Section 10.4 for the definition of the cohorts for analyses.

If a subject returns for the Visit 2 blood draw prior to completion of the 31-day safety follow-up period, the subject should continue to record this information on the diary card until 31 days post-vaccination and mail the diary card to the site. The investigator will make an attempt to obtain this information as soon as possible after the 31-day follow-up period if it is not mailed.

5.6. Detailed description of study procedures

The following procedure will be conducted prior to study entry.

5.6.1. Informed consent and informed assent

The signed/witnessed/thumb printed informed consent of the subject's parent(s)/LAR(s) must be obtained before study participation. The signed informed assent of the subject below the age of consent (i.e. minor) should be obtained in addition to the signed informed consent by his/her parent(s)/LAR(s) according to local rules and regulations. Refer to Section 5.1 for the requirements on how to obtain informed consent and assent, as appropriate.

The following procedures will be conducted at the time of study entry.

5.6.2. Check inclusion and exclusion criteria

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

5.6.3. Collect demographic data

Record demographic data such as age [in years], gender, geographic ancestry, height [in cm] and weight [kg] in the subject's eCRF.

5.6.4. Medical and Vaccination history

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

5.6.5. Physical examination

Perform a physical examination of the subject, including assessment of body temperature. Collected information needs to be recorded in the eCRF.

Physical examination at the study visit subsequent to the vaccination visit, will be performed only if the subject indicates during questioning that there might be some underlying pathology(ies) or if deemed necessary by the Investigator or delegate. Treatment of any abnormality observed during this examination has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

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. Urine pregnancy test

Female subjects of childbearing potential are to have a urine pregnancy test prior to any study vaccine administration. The study vaccine may only be administered if the pregnancy test is negative. Note: The urine pregnancy test must be performed even if the subject is menstruating at the time of the study visit.

5.6.7. Check contraindications, warnings and precautions to vaccination

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

5.6.8. Assess pre-vaccination body temperature

The axillary, rectal, oral or tympanic body temperature of all subjects needs to be measured prior to any study vaccine administration. The preferred route for recording temperature in this study will be axillary. If the subject has fever [fever is defined as temperature ≥ 37.5°C for oral, axillary or tympanic route, or ≥ 38.0°C for rectal route] on the day of vaccination, the vaccination visit will be rescheduled within the allowed interval for this visit (see Table 5).

5.6.9. Sampling

As specified in the List of Study Procedures (see Table 4), blood samples will be taken during both study visits. Refer to the Module on Biospecimen Management in the SPM for detailed instructions for the collection, handling and processing of the blood samples.

5.6.9.1. Blood sampling for safety or immune response assessments

Blood samples will be taken during Visit 1 and Visit 2 as specified in Section 5.5.

A volume of at least 2.5 mL of whole blood (to provide at least 0.8 mL of serum) should be drawn from all subjects included in the study for analyses of humoral immune response. After centrifugation, serum samples should be kept at –20°C or below until shipment. Refer to the SPM for more details on sample storage conditions.

5.6.10. Study Vaccine administration

- After completing all prerequisite procedures prior to vaccination, one dose of study vaccine will be administered intramuscularly (IM) in the deltoid region of the non-dominant arm (refer to Section 6.3 for detailed description of the vaccine administration procedure). If the investigator or delegate determines that the subject's health on the day of administration temporarily precludes vaccine administration, the visit will be rescheduled within the allowed interval for this visit (see Table 5)
- The subjects will be observed closely for at least 30 minutes following the administration of the vaccine, with appropriate medical treatment readily available in case of anaphylaxis.

5.6.11. 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.

5.6.12. Recording of AEs, SAEs and pregnancies

- Refer to Section 8.3 for procedures for the investigator to record AEs, SAEs and pregnancies. Refer to Section 8.4 for guidelines and how to report SAE and pregnancy 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 the vaccination visit (Visit 1), diary cards will be provided to the subject's parent(s)/LAR(s). The subject's parent(s)/LAR(s) will record body (axillary) temperature and any solicited local/general AEs (i.e. on the day of vaccination and during the next 3 days) or any unsolicited AEs (i.e. on the day of vaccination and during the next 30 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.

  Note: Diary cards can be filled in by a minor subject under the supervision of the subject's parent(s)/LAR(s) provided that the minor has the competency to assess and report the information to be provided in the diary card. The ultimate accountability for the completion of the diary cards remains with the subject's parent(s)/LAR(s).

The investigator should discuss this accountability with the subject's parent(s)/LAR(s).

- Collect and verify completed diary cards during discussion with the subject's parent(s)/LAR(s) on Visit 2.

  Note: If the diary card has been filled in by a minor subject, the investigator or delegate should verify the reported information during a discussion with the minor subject preferably in the presence of his/her 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.

5.6.13. Study conclusion

At Visit 2 the investigator will:

- review data collected to ensure accuracy and completeness.
- complete the Study Conclusion screen in the eCRF.

5.7. Biological sample handling and analyses

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 respective countries and will only be performed once an independent Ethics Committee 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 ATP analyses (See Section 10.4 for the definition of cohorts to be analysed). 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 samples

The biological samples to be collected in the study are presented in Table 6.

Table 6 Biological samples

Sample type	Quantity	Unit	Timepoint
Blood	At least 2.5	mL	Visit 1 (pre-HBV challenge) Visit 2 (post-HBV challenge)

5.7.3. Laboratory assays

Please refer to APPENDIX A for the address of the clinical laboratories used for sample analyses.

Serological assays for the determination of anti-HBc and anti-HBs antibodies will be performed by ChemiLuminescence ImmunoAssay (CLIA) at a GSK Biologicals' laboratory or in a laboratory designated by GSK Biologicals using standardised and validated procedures (see Table 7).

Table 7 Humoral Immunity (Antibody determination)

System	Component	Scale	Method	Test kit/ Manufacturer	Unit	Cut-off	Laboratory*
SER	Hepatitis B Virus.Core Ab	Ordinal	CLIA	Immulite (Siemens Healthcare)	No unit	N/A	CEVAC
SER	Hepatitis B Virus.Surface Ab	Quantitative	CLIA	Centaur (Siemens Healthcare)	mIU/ml	6.2	GSK Biologicals**

*Refer to APPENDIX A for the laboratory addresses
CEVAC: Center for Vaccinology
**GSK Biologicals laboratory refers to the Clinical Laboratory Sciences (CLS) in Rixensart, Belgium Wavre, Belgium
SER: Serum
CLIA: ChemiLuminescence ImmunoAssay (CLIA)
mIU/ml: Milli-International units per millilitre
N/A: Not applicable

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

5.7.4. Biological samples evaluation

5.7.4.1. Immunological read-outs

The immunological read-outs are presented in Table 8.

Table 8 Immunological read-outs

Blood sampling timepoint		No. subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint			
Visit 1 (Day 0)	Pre-HBV vaccination	Approximately 300	Anti-HBs	(1)
Visit 2 (Month 1)	Post-HBV vaccination	Approximately 300	Anti-HBs Anti-HBc	(1) (2)

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

5.7.5. Immunological correlates of protection

The assay cut-off used for the detection of anti-HBs antibodies has been set at 6.2 mIU/ml. The cut-off of 10 mIU/ml provides a conservative estimate of the percentage of subjects deemed to be protected for antibodies against hepatitis B antibodies [CDC, 1991; WHO, 1988; Frisch-Niggemeyer, 1986].

Assessment of the protection level will be done at least 4 weeks after the administration of the challenge dose.

The immunological assay results will be communicated to the investigator as soon as they become available.

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.

Non-responders are defined as subjects with anti-HBs antibody concentration < 10 mIU/mL, one month after the HBV challenge.

6. STUDY VACCINE AND ADMINISTRATION

6.1. Description of study vaccine

The HBV vaccine (*Engerix-B Kinder*) to be used has been developed and manufactured by GSK Biologicals.

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

The vaccine is labelled and packed according to applicable regulatory requirements.

The commercial vaccine is assumed to comply with the specifications given in the manufacturer's Summary of Product Characteristics.

The description of study vaccine is presented in Table 9.

Table 9 Study vaccine

Treatment name	Vaccine/product name	Formulation	Presentation	Volume	Number of doses
Engerix-B Kinder	HBV	HBsAg=10µg; $Al(OH)_3$=250µg Al3+	Suspension for injection in pre-filled syringes	0.5 ml	1

6.2. Storage and handling of study vaccine

The study vaccine 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 vaccine.

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 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 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 vaccine.

6.3. Dosage and administration of study vaccine

All subjects will receive one dose of the HBV vaccine. The vaccine will be administered as an IM injection at a 90-degree angle into the deltoid region of the non-dominant arm (preferably). In order to ensure proper IM injection of the study vaccine, a needle of at least 1 inch (2.54 cm) length, 25 gauge will be used [CDC, 2002].

The dosage and administration of study vaccines is presented in Table 10.

Table 10 Dosage and administration

Type of contact and timepoint	Volume to be administered	Treatment name	Route [1]	Site	Side
Visit 1 (Day 0)	0.5 ml	HBV	IM	Deltoid region of arm	Preferably Non-dominant

[1]IM: Intramuscular

6.4. Replacement of unusable vaccine doses

In addition to the vaccine doses provided for the planned number of subjects (including over-randomisation when applicable), at least 5% additional vaccine doses will be supplied to replace those that are unusable.

6.5. Contraindications to vaccination

Since this is a single-dose study, contraindications have been included as part of the exclusion criteria (see Section 4.3)

The following events constitute contraindications to administration of HBV vaccine 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 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 ≥ 37.5°C for oral, axillary or tympanic route, or ≥ 38.0°C for rectal route. The preferred route for recording temperature in this study will be axillary.
  - Subjects with a minor illness (such as mild diarrhoea, mild upper respiratory infection) without fever can be administered all vaccines.

6.6. Warnings and precautions

- *Engerix-B Kinder* should not be administered in the buttock or intradermally since this may result in lower immune response.
- *Engerix-B Kinder* should under no circumstances be administered intravenously.
- Syncope (fainting) can occur following, or even before any vaccination especially in adolescents as a psychogenic response to the needle injection. It is important that procedures are in place to avoid injury from syncope.
- Due to the long incubation period of hepatitis B, it is possible for unrecognised infection to be present at the time of vaccination. The vaccine may not prevent hepatitis B in such cases.

Refer to the approved product label/package insert.

6.7. Concomitant medications/products and concomitant vaccinations

At each study visit, the investigator should question the subject's parent(s)/LAR(s) about any medication/product taken and vaccination 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 from the administration of the study vaccine (Day 0) up to 30 days post-vaccination (Day 30).
- Any concomitant vaccination administered in the period starting 30 days before the dose of study vaccine and ending at the last study visit [Day 30 (Visit 2)].
- 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 ≥ 37.5°C for oral, axillary or tympanic route, or ≥ 38.0°C for rectal route].

- 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.

6.7.2. Concomitant medications/products/vaccines that may lead to the elimination of a subject from ATP 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 ATP analyses. See Section 10.4 for cohorts to be analysed.

- Any investigational or non-registered product (drug or vaccine) other than the study vaccine used during the study period.
- Immunosuppressants or other immune-modifying drugs administered chronically (i.e. more than 14 days in total) during the study period. For corticosteroids, this will mean prednisone ≥ 0.5 mg/kg/day (for paediatric subjects) or equivalent. Inhaled and topical steroids are allowed
- Long-acting immune-modifying drugs administered at any time during the study period (e.g. infliximab).
- A vaccine not foreseen by the study protocol administered during the period starting from the dose of the study vaccine and ending 30 days after the dose of vaccine administration (Visit 2)*, with the exception of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (dTpa) vaccine, which can be given as part of routine vaccination practice. Seasonal or pandemic influenza vaccine can be given at any time during the study, and according to the Summary of Product Characteristics and national recommendations.

  *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 immunisation 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.
- Immunoglobulins and/or any blood products administered during the study period.

6.8. Intercurrent medical conditions that may lead to elimination of a subject from ATP analyses

At each study visit subsequent to 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.

- Subjects may be eliminated from the ATP cohort for immunogenicity if, during the study, they incur a condition that has the capability of altering their immune response (i.e. HBV infection) or are confirmed to have an alteration of their initial immune status.
- Previous hepatitis B infection as evidenced by testing seropositive for antibodies against hepatitis B core antigen (anti-HBc) at post-HBV vaccine challenge blood sampling time point.

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 adverse event (AE) or serious adverse event (SAE) as provided in this protocol.

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

8.1. Safety definitions

8.1.1. Definition of an adverse event

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

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

Examples of an AE include:

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

- New conditions detected or diagnosed after investigational vaccine 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 investigational vaccine or a concurrent medication (overdose per se should not be reported as an AE/SAE).
- Signs, symptoms temporally associated with vaccine 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 procedure (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 hospitalisation or prolongation of existing hospitalisation,

   Note: In general, hospitalisation 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 hospitalisation are also considered AEs. If a complication prolongs hospitalisation or fulfils any other serious criteria, the event will also be considered

serious. When in doubt as to whether 'hospitalisation' occurred or was necessary, the AE should be considered serious.

Hospitalisation 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,

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, diarrhoea, 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.

e. Is a congenital anomaly/birth defect in the offspring of a study subject.

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 hospitalisation 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 hospitalisation.

8.1.3. Solicited adverse events

8.1.3.1. Solicited local (injection-site) adverse events

The following local (injection-site) AEs will be solicited:

Table 11 Solicited local adverse events

Pain at injection site
Redness at injection site
Swelling at injection site

8.1.3.2. Solicited general adverse events

The following general AEs will be solicited:

Table 12 Solicited general adverse events

Fatigue
Fever
Gastrointestinal symptoms †
Headache

†Gastrointestinal symptoms include nausea, vomiting, diarrhoea and/or abdominal pain.

Note: Temperature will be recorded in the evening. Should additional temperature measurements be performed at other times of day, the highest temperature will be recorded in the eCRF.

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

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

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

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

8.2.1. Pregnancy

Female subjects who become pregnant after the vaccination may continue the study at the discretion of the investigator.

While pregnancy itself is not considered an AE or SAE, any adverse pregnancy outcome or complication or elective termination of a pregnancy for medical reasons will be recorded and reported as an AE or a SAE.

Note: The pregnancy itself should always be recorded on an electronic pregnancy report.

The following should always be considered as SAE and will be reported as described in Sections 8.4.1 and 8.4.3:

- Spontaneous pregnancy loss, including:
  - spontaneous abortion, (spontaneous pregnancy loss before/at 22 weeks of gestation)
  - ectopic and molar pregnancy
  - stillbirth (intrauterine death of foetus after 22 weeks of gestation).

Note: the 22 weeks cut-off in gestational age is based on WHO-ICD 10 noted in the EMA Guideline on pregnancy exposure [EMA, 2006]. It is recognized that national regulations might be different.

- Any early neonatal death (i.e. death of a live born infant occurring within the first 7 days of life).
- Any congenital anomaly or birth defect (as per [CDC MACDP] guidelines) identified in the offspring of a study subject (either during pregnancy, at birth or later) regardless of whether the foetus is delivered dead or alive. This includes anomalies identified by prenatal ultrasound, amniocentesis or examination of the products of conception after elective or spontaneous abortion.

Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered by the investigator to be reasonably related to the investigational vaccine will be reported to GSK Biologicals as described in Section 8.4.3. While the investigator is not obligated to actively seek this information from former study participants, he/she may learn of a pregnancy through spontaneous reporting.

8.3. Detecting and recording adverse events, serious adverse events and pregnancies

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

All AEs starting 30 days following administration of the dose of study vaccine (Day 0 to Day 30) must be recorded into the appropriate section of the eCRF, irrespective of intensity or whether or not they are considered vaccination-related.

The time period for collecting and recording SAEs will begin at the receipt of study vaccine and will end 30 days following administration of the dose of study vaccine for each subject. 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 vaccine.

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.

The time period for collecting and recording pregnancies will begin at the receipt of study vaccine and will end 30 days following administration of the dose of study vaccine. See section 8.4 for instructions on reporting of pregnancies.

An overview of the protocol-required reporting periods for AEs, SAEs, and pregnancies is given in Table 13.

Table 13 Reporting periods for collecting safety information

Event	Pre HBV challenge(Consent obtained)	HBV challenge (Visit 1)	4-day post HBV challenge follow-up	31-day post HBV challenge follow-up (Visit 2) Study conclusion
Time in study		Day 0	Day 3	Day 30
Solicited local and general AEs				
Unsolicited AEs				
AEs/SAEs leading to withdrawal from the study				
SAEs				
SAEs related to study participation or concurrent GSK medication/vaccine				
Pregnancies				

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 13. 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 investigational vaccine, 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 vaccine 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

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

Table 14 Intensity scales for solicited symptoms in children of 14-15 years of age

Child (14-15 years)		
Adverse Event	**Intensity grade**	**Parameter**
Pain at injection site	0	None
	1	Mild: Any pain neither interfering with nor preventing normal every day activities.
	2	Moderate: Painful when limb is moved and interferes with every day activities.
	3	Severe: Significant pain at rest. Prevents normal every day activities.
Redness at injection site		Record greatest surface diameter in mm
Swelling at injection site		Record greatest surface diameter in mm
Fever*		Record temperature in °C
Headache	0	Normal
	1	Mild: Headache that is easily tolerated
	2	Moderate: Headache that interferes with normal activity
	3	Severe: Headache that prevents normal activity
Fatigue	0	Normal
	1	Mild: Fatigue that is easily tolerated
	2	Moderate: Fatigue that interferes with normal activity
	3	Severe: Fatigue that prevents normal activity
Gastrointestinal symptoms (nausea, vomiting, diarrhoea and/or abdominal pain)	0	Normal
	1	Mild: Gastrointestinal symptoms that are easily tolerated
	2	Moderate: Gastrointestinal symptoms that interfere with normal activity
	3	Severe: Gastrointestinal symptoms that prevent normal activity

*Fever is defined as temperature ≥ 37.5°C for oral, axillary or tympanic route, or ≥ 38.0°C for rectal route. The preferred route for recording temperature in this study will be axillary.

The maximum intensity of local injection site redness/swelling will be scored at GSK Biologicals as follows:

0	:	Absent
1	:	≤ 20 mm
2	:	>20 and ≤ 50 mm
3	:	> 50 mm

The maximum intensity of fever will be scored at GSK Biologicals as follows:

		Axillary
0	:	< 37.5°C
1	:	≥37.5°C and ≤ 38.0°C
2	:	> 38.0°C and ≤ 39.0°C
3	:	> 39.0°C

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 adolescents, such an AE would, for example, prevent attendance at school and would necessitate the administration of corrective therapy.

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 investigational vaccine and the occurrence of each AE/SAE. The investigator will use clinical judgement to determine the relationship. 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 investigational vaccine will be considered and investigated. The

investigator will also consult the 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.

In case of concomitant administration of multiple vaccines, it may not be possible to determine the causal relationship of general AEs to the individual vaccine administered. The investigator should, therefore, assess whether the AE could be causally related to vaccination rather than to the individual vaccines.

All solicited local (injection site) reactions will be considered causally related to vaccination. Causality of all other 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 investigational vaccine?

YES	:	There is a reasonable possibility that the vaccine contributed to the AE.
NO	:	There is no reasonable possibility that the AE is causally related to the administration of the study vaccine. There are other, more likely causes and administration of the study vaccine 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 vaccine, 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 hospitalisation, 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, pregnancies, and other events

8.4.1. Prompt reporting of serious adverse events, pregnancies, and other 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.

Pregnancies 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 becomes aware of the pregnancy.

Table 15 Timeframes for submitting serious adverse event, pregnancy and other events reports to GSK Biologicals

Type of Event	Initial Reports		Follow-up of Relevant Information on a Previous Report	
	Timeframe	Documents	Timeframe	Documents
SAEs	24 hours*‡	electronic Expedited Adverse Events Report	24 hours*	electronic Expedited Adverse Events Report
Pregnancies	2 weeks*	electronic pregnancy report	2 weeks*	electronic pregnancy 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, pregnancies

Study Contact for Reporting SAEs and pregnancies
Refer to the local study contact information document.
Back-up Study Contact for Reporting SAEs and pregnancies
24/24 hour and 7/7 day availability: **GSK Biologicals Clinical Safety & Pharmacovigilance** Fax: PPD or PPD Email address: 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. Completion and transmission of pregnancy reports to GSK Biologicals

Once the investigator becomes aware that a subject is pregnant, the investigator (or designate) must complete the required information onto the electronic pregnancy report WITHIN 2 WEEKS.

Note: Conventionally, the estimated gestational age (EGA) of a pregnancy is dated from the first day of the last menstrual period (LMP) of the cycle in which a woman conceives. If the LMP is uncertain or unknown, dating of EGA and the estimated date of delivery (EDD) should be estimated by ultrasound examination and recorded in the pregnancy report.

8.4.5. Updating of SAE and pregnancy information after removal of write access to the subject's eCRF

When additional SAE or pregnancy 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.

8.4.6. 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 investigational vaccine 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, serious adverse events, and pregnancies

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

8.5.1.1. 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 end of the study.

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.

8.5.1.2. 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.
- with other non-serious AEs, until they are lost to follow-up.

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

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

8.5.2. Follow-up of pregnancies

Pregnant subjects will be followed to determine the outcome of the pregnancy. At the end of the pregnancy, whether full-term or premature, information on the status of the mother and child will be forwarded to GSK Biologicals electronic pregnancy report and the Expedited Adverse Events Report if applicable. Generally, the follow-up period doesn't need to be longer than six to eight weeks after the estimated date of delivery.

Regardless of the reporting period for SAEs for this study, if the pregnancy outcome is a SAE, it should always be reported as SAE.

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 an AE 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.

9. SUBJECT COMPLETION AND WITHDRAWAL

9.1. Subject completion

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

9.2. Subject withdrawal

Withdrawals will not be replaced.

9.2.1. Subject withdrawal from the study

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

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

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 subject's parent(s)/LAR(s) 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:

- Serious adverse event.
- Non-serious adverse event.
- Protocol violation (specify).
- Consent withdrawal, not due to an adverse event*.
- Moved from the study area.
- Lost to follow-up.

- Other (specify).

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

Subjects who are withdrawn from the study because of SAEs/AEs must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn from the study as result of a SAE/AE until resolution of the event (see Section 8.5.1.2).

10. STATISTICAL METHODS

10.1. Primary endpoint

- Immunogenicity to components of the study vaccine.
  - Anti-HBs antibody concentrations ≥100 mIU/ml, one month after the single challenge dose of HBV vaccine.

10.2. Secondary endpoints

- Anti-HBs antibody persistence after previous vaccination with *Infanrix hexa*.
  - Anti-HBs antibody concentrations ≥ 10 mIU/ml, ≥ 100 mIU/ml and anti-HBs antibody concentrations before the single challenge dose of HBV vaccine.
- Immunogenicity to the components of the study vaccine.
  - Anti-HBs antibody concentrations ≥ 10 mIU/ml and anti-HBs antibody concentrations one month after the single challenge dose of HBV vaccine.
  - Anamnestic response to the single challenge dose of HBV vaccines.

  For the definition of anamnestic response, refer to Section 10.5.
- Solicited local and general symptoms.
  - Occurrence of each solicited local and general symptom during the 4-day (Day 0–3) follow-up period after the single challenge dose of HBV vaccine.
- Unsolicited adverse events
  - Occurrence of unsolicited AEs during the 31-day (Day 0–30) follow-up period after the single challenge dose of HBV vaccine.
- Serious adverse events
  - Occurrence of serious adverse events after the single challenge dose of HBV vaccine up to study end.

10.3. Determination of sample size

The primary objective of the study is to assess the anti-HBs antibody response to a single challenge dose of HBV vaccine (*Engerix-B Kinder*) in subjects at 14-15 years of age, previously vaccinated with four doses of *Infanrix hexa* in the first two years of life. The objectives of the study are descriptive as given in Section 2. However for the computation of sample size, the anti-HBs antibody response to the challenge dose of *Engerix-B Kinder* is expected to be above 90%.

Approximately 300 children aged 14-15 years who were previously primed and boosted with four doses of *Infanrix hexa* in the first two years of life will be enrolled in this study.

It is assumed that 10% of subjects enrolled in this study may be non-evaluable for the analyses of the primary endpoint (e.g., drop-outs, non-compliance with protocol, etc.). Thus, a sample size of 270 evaluable subjects provides a power of at least 86% for the lower limit (LL) of the 95% confidence interval (CI) for the percentage of subjects who are expected to respond to the HBV vaccine challenge (i.e. anti-HBs antibody concentrations ≥ 100 mIU/ml one month after HBV vaccine challenge) to be more than 90%, if the true percentage of subjects who have responded to the HBV vaccine challenge is 95%, as shown in Table 16.

In addition, should the true percentage of response to the HBV vaccine challenge be greater than 95% (for example 96% to 98%), Table 16 shows that the study is adequately powered to lead to a LL of the 95% CI not more than 5% below that true percentage (i.e. a LL above 91% to 93%, respectively). For instance, if the true percentage of response to the HBV vaccine challenge is 97%, a sample size of 270 subjects provides a power of at least 93% for the LL of the 95% CI, for the percentage of subjects who have responded to the HBV vaccine challenge (i.e. anti-HBs antibody concentrations ≥ 100 mIU/ml one month after HBV vaccine challenge) to be more than 92%.

Table 16 Power to obtain a lower limit for the 95 percent CI for the response to HBV vaccine challenge (anti-HBs antibody concentration greater than or equal to 100 mIU per ml) greater than 90 percent or greater than a margin that would be 5 percent below the expected percentage for a sample size of 270 evaluable subjects

Expected percentage	Power (%) to obtain a lower limit greater than 5% below the expected percentage	Power (%) to obtain a lower limit greater than 90%
**94%	80%	64%
^95%	**86%**	**86%**
96%	92%	97%
97%	93%	99%
*97.5%	95%	99%
98%	97%	> 99%

Note: All computations were performed in Pass 2005 based on a one-sided binomial test with a target one sided significance level of 0.025.
References data from studies:
^DTPa-HBV-IPV-112 (106789): Subjects who received a single dose of HBV vaccine in the age group of 4-5 years and were previously primed and boosted with four doses of *Infanrix hexa* in the first two years of life. (Percentage of subjects ≥ 100 mIU/ml post challenge dose 95.8%, 95%CI [92.8%, 97.8%])
*DTPA-HBV-IPV-113 (112688): Subjects who received a single dose of HBV vaccine in the age group of 7-8 years and were previously primed and boosted with four doses of *Infanrix hexa* in the first two years of life. (Percentage of subjects ≥ 100 mIU/ml post challenge dose 95.8%, 95%CI [92.6%, 97.9%])
**DTPA-HBV-IPV-114 (106793): Subjects who received a single dose of HBV vaccine in the age group of 12-13 years and were previously primed and boosted with four doses of Infanrix hexa in the first two years of life. (Percentage of subjects ≥ 100 mIU/ml post challenge dose 94.1%, 95%CI [90.7%, 96.5%])

10.4. Cohorts for Analyses

Three cohorts are defined for the purpose of analyses:

- Total vaccinated cohort (TVC)
- ATP cohort for analyses of immunogenicity
- ATP cohort for analyses of antibody persistence

10.4.1. Total vaccinated cohort

The TVC will include all subjects who received the single challenge dose of HBV vaccine.

- The TVC for the analyses of safety will include all subjects with study vaccine administration documented.
- The TVC for the analyses of immunogenicity will include all subjects who have received a single challenge dose of HBV vaccine and for whom data concerning immunogenicity endpoint measures are available at the post-HBV challenge blood sampling time point.

10.4.2. According-to-protocol cohort for analyses of immunogenicity

The ATP cohort for analyses of immunogenicity will include all subjects who satisfy the below criteria:

- who meet all eligibility criteria
- who comply with the procedures and intervals defined in the protocol.
- who do not meet any of the elimination criteria during the study
- for whom post-vaccination immunogenicity (Visit 2) results are available.

The interval between vaccination at Visit 1 and blood sampling at Visit 2, considered for inclusion of a subject in the ATP cohort for analyses of immunogenicity will be 21-48 days.

10.4.3. According-to-protocol cohort for analyses of antibody persistence

The ATP cohort for analyses of antibody persistence will include all enrolled subjects:

- aged 14–15 years (from and including the 14th birthday up to but excluding the 16th birthday) at the time of enrolment.
- who have not received any additional dose of hepatitis B vaccine (or any other vaccine with this antigen component) other than the four doses of *Infanrix hexa* during the first two years of life.
- with no evidence of hepatitis B infection or disease (including anti-HBc at post-HBV challenge dose time point).
- for whom serological results are available at the pre-HBV challenge blood sampling time point.

10.5. Derived and transformed data

- A seronegative subject is a subject with anti-HBs antibody concentration below the assay cut-off (< 6.2 mIU/ml).
- A seropositive subject is a subject with anti-HBs antibody concentrations above the assay cut-off (≥ 6.2 mIU/ml).
- A seroprotected subject is a subject with anti-HBs antibody concentrations above the protection level (≥ 10 mIU/ml).
- Anamnestic response to the single challenge dose is defined as:
  - At least (i.e. greater than or equal to) 4-fold rise in post-vaccination anti-HBs antibody concentrations in subjects seropositive at the pre-vaccination time point.
  - Post-vaccination, anti-HBs antibody concentrations ≥ 10 mIU/ml in subjects seronegative at the pre-vaccination time point.

- The Geometric mean antibody concentrations (GMCs) calculations will be performed by taking the anti-log of the mean of the $\log_{10}$ concentration transformations. All subjects will be considered. Subjects whose antibody concentrations are below the cut-off of the assay will be given an arbitrary value of half the cut-off for the purpose of GMC calculation. Note that as per assay specification, results between the assay cut-off of 6.2 mIU/ml and 7.65 mIU/ml (= Lower limit of Quantification) will be quantified as 6.2 mIU/ml.
- For a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, an analysis will exclude subjects with missing or non-evaluable measurements.

Safety/Reactogenicity

- For the analyses of solicited symptoms, missing or non-evaluable measurements will not be replaced. Therefore the analyses of the solicited symptoms based on the TVC will include only subjects with documented safety data (i.e. symptom screen completed).
- For the analyses of unsolicited adverse events/concomitant medication, all vaccinated subjects will be considered and subjects who did not report an event will be considered as subjects without an event.

10.6. Analyses of demographics

Demographic characteristics (age at study entry [in years], gender, geographic ancestry, height [in cm] and weight [kg]), cohort description and drop outs/withdrawals will be summarised using descriptive statistics:

- Frequency tables will be generated for categorical variable such as centre.
- Mean, median, standard deviation will be provided for continuous variable such as age.

10.7. Analyses of immunogenicity

Analyses for antibody persistence

The analyses for antibody persistence will be performed on the ATP cohort for analyses of antibody persistence.

- Prior to the single dose of HBV vaccine:
  - Percentage of subjects with anti-HBs antibody concentrations ≥ 6.2 mIU/ml, ≥ 10 mIU/ml, ≥ 10 mIU/ml to < 100 mIU/ml, and ≥ 100 mIU/ml, with exact 95% CI will be calculated [Clopper, 1934].
  - GMCs with 95% CI will be calculated for anti-HBs antibodies.
  - The distribution of anti-HBs antibody concentrations will be displayed using reverse cumulative curves (RCCs).

Response to challenge dose

The primary analyses will be based on the ATP cohort for analyses of immunogenicity. If, the percentage of vaccinated subjects with serological results excluded from the ATP cohort for analyses of immunogenicity is 5% or more, a second analyses based on the TVC will be performed to complement the ATP analyses.

- One month after the single challenge dose of HBV vaccine:
  - Percentage of subjects with anti-HBs antibody concentrations ≥ 6.2 mIU/ml, ≥ 10 mIU/ml and ≥ 100 mIU/ml, with exact 95% CI will be calculated [Clopper, 1934].
  - GMCs with 95% CI will be calculated for anti-HBs antibodies.
  - The distribution of anti-HBs antibody concentrations will be displayed using RCCs.
- The percentage of subjects with anti-HBs concentrations ≥ 6.2 mIU/ml, ≥ 10 mIU/ml, and ≥ 100 mIU/ml (with exact 95% CI) and GMCs at the post-HBV challenge dose time-point, in relation to their pre-challenge dose status (< 6.2 mIU/ml, < 10 mIU/ml, ≥ 10 mIU/ml) will be tabulated [Clopper, 1934].
- Percentage of subjects (with 95% CI) who mount an anamnestic response to the single challenge dose of HBV vaccine will be calculated overall and in relation to their pre-vaccination status (< 6.2 mIU/ml, < 10 mIU/ml, ≥ 10 mIU/ml) [Clopper, 1934].
- Post-challenge anti-HBs antibody concentrations as a function of pre-challenge concentrations, with regression line will be computed using linear regression method for ATP cohort for immunogenicity only. The model will include pre-challenge log transformed concentrations as regressor (independent or explanatory variable) and post-challenge log-transformed concentrations as an outcome (dependent or response) variable.antibody concentrations observed at the pre-challenge dose time point will be presented graphically.

10.8. Analyses of safety

The primary analyses of safety and reactogenicity will be based on the TVC.

- The percentage of subjects with at least one local AE (solicited and unsolicited), with at least one general AE (solicited and unsolicited) and with any AE during the 4-day (Day 0 to Day 3) follow-up period after the vaccination will be tabulated with exact 95% CI [Clopper, 1934]. The same calculations will be performed for any Grade 3 (solicited or unsolicited) symptoms, relationship to the vaccination and any symptoms requiring medical attention.
- The percentage of subjects reporting each individual solicited symptom during the 4-day follow-up period with exact 95% CI [Clopper, 1934], by type of adverse event; by severity (any grade, Grade 3 only); by relationship to vaccination (any relationship, related only) will be tabulated.

- The occurrence of fever will be tabulated per 0.5°C cumulative increments as well as the occurrence of Grade 3 fever (> 39.0 °C axillary temperature) with causal relationship to vaccination.
- The percentage of subjects with at least one report of unsolicited adverse event classified by the Medical Dictionary for Regulatory Activities and reported within the 31-day (Day 0 to Day 30) follow-up period after vaccination will be tabulated with exact 95% CI [Clopper, 1934]. The same tabulation will be performed for Grade 3 unsolicited adverse events and for unsolicited adverse events with a causal relationship to vaccination.
- The percentage of subjects who started receiving at least one concomitant medication (i.e. any medication, antipyretic medication, prophylactic antipyretics) during the 4-day and 31-day follow-up period after vaccination will be tabulated (with exact 95% CI) [Clopper, 1934].
- SAEs and withdrawals due to AEs and SAEs reported during the study will be described in detail

10.9. 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.

10.9.1. Sequence of analyses

The analyses will be performed when all data up to one month after the vaccine dose will be available. All analyses and associated individual data will be presented in a Clinical Study Report, which will be shared with the investigator(s) involved in the conduct of this study.

10.9.2. 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 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 analyses 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 of omissions or inconsistencies with documentation and approval 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 others, 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 monitor's and 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.

For eCRF, the monitor freezes completed and approved screens at each visit.

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. However, the investigator/institution should seek the written approval of the sponsor before proceeding with the disposal of these records. 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.

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

Study information from this protocol will be posted on publicly available clinical trial registers before enrolment of subjects begins.

Summaries of the results of GSK interventional studies (phase I-IV) are posted on publicly available results registers within 6 months of the primary completion date for studies of authorised vaccines and 18 months for studies of non-authorised vaccines.

GSK also aims to publish the results of these studies in the searchable, peer reviewed scientific literature. Manuscripts are submitted for publication within 24 months of the last subject's last visit. At the time of publication, this protocol will be fully disclosed.

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.

12. COUNTRY SPECIFIC REQUIREMENTS

Refer to the Explanatory Statement concerning Gender Distribution.

13. REFERENCES

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Centers for Disease Control (CDC). Hepatitis B virus: A comprehensive strategy for eliminating transmission in the United States through universal childhood vaccination: Recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR*. 1991; 40(RR-13): 1–19.

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EMA Guideline on the exposure to medicinal products during pregnancy: need for post-authorization data (Doc. Ref. EMEA/CHMP/313666/2005) 'adopted at Community level in May 2006); *http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/11/WC500011303.pdf.*

Frisch-Niggemeyer W, Ambrosch F and Hofmann H. The assessment of immunity against hepatitis B after vaccination. *J Biol Stand*. 1986; 14: 255–258.

GSK Biologicals' Clinical Study Report (106793) DTPA-HBV-IPV-114. An open, phase IV, multicentre study to assess the long-term persistence of antibodies against hepatitis B and the immune response to a hepatitis B (Engerix-B Kinder) vaccine challenge in children aged 12-13 years, previously primed and boosted in the first two years of life with GlaxoSmithKline (GSK) Biologicals' DTPa-HBV-IPV/Hib (Infanrix hexa) vaccine. Data on file.

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Poovorawan Y, Chongsrisawat, Theamboonlers A, et al. Long-term anti-HBs antibody persistence following infant vaccination against hepatitis B and evaluation of anamnestic response: a 20-year follow-up study in Thailand. *Hum Vaccin Immunother*. 2013; 9(8):1679-84.

Steiner M, Ramakrishnan G, Gartner B et al. Lasting immune memory against hepatitis B in children after primary immunization with 4 doses of DTPa-HBV-IPV/Hib in the first and 2nd year of life. *BMC Infect Dis*. 2010; 10:9.

World Health Organization (WHO). Progress in the control of viral hepatitis: Memorandum from a WHO Meeting. *Bull WHO* 1988; 66(4): 443–445.

WHO. Hepatitis B Fact sheet N°204. July 2015. http://www.who.int/mediacentre/factsheets/fs204/en/. Accessed on 06 November 2015.

Zinke M, Disselhoff J, Gartner B, et al. Immunological persistence in 4–6 and 7–9 year olds previously vaccinated in infancy with hexavalent DTPa-HBV-IPV/Hib. *Hum Vacci* 2010;6(2):189-93.

APPENDIX A CLINICAL LABORATORIES

Table 17 GSK Biologicals' laboratories

Laboratory	Address
GSK Biologicals Clinical Laboratory Sciences, Rixensart	Biospecimen Reception - B7/44 Rue de l'Institut, 89 - B-1330 Rixensart - Belgium
GSK Biologicals Clinical Laboratory, Sciences, Wavre-Nord Noir Epine	Avenue Fleming, 20 - B-1300 Wavre - Belgium

Table 18 Outsourced laboratories

Laboratory	Address
CEVAC - University of Gent	De Pintelaan, 185 Gent Belgium
Néomed Lab Inc	Biospecimen Reception - Clinical Serology 525 Cartier blvd West - Laval - Quebec - Canada - H7V 3S8

Protocol Sponsor Signatory Approval

eTrack study number and Abbreviated Title	106794 (DTPA-HBV-IPV-115)
EudraCT number	2015-003391-74
Date of protocol	Final Version 01: 06 November 2015
Detailed Title	A phase IV, open-label, multicentre study to assess the long-term persistence of antibodies against hepatitis B and the immunogenicity and safety of a challenge dose of hepatitis B vaccine (Engerix-B™ Kinder SKF103860) in children aged 14-15 years, previously primed and boosted in the first two years of life with four doses of GSK Biologicals' DTPa-HBV-IPV/Hib (Infanrix™ hexa SB217744) vaccine.
Sponsor signatory	Narcisa Elena Mesaros, MD Project level Clinical Research and Development Lead, DTP/Polio Vaccines, Late Clinical Development, Vaccine Discovery and Development, GlaxoSmithKline Biologicals, SA
Signature	PPD
Date	12. 11. 2015

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