

**Clinical Study Protocol**

Sponsor:

GlaxoSmithKline Biologicals

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Primary Study vaccine/product and number	GlaxoSmithKline (GSK) Biologicals' hepatitis B vaccine, Engerix™-B (103860)
eTrack study number and Abbreviated Title	116811 (HBV-322)
EudraCT number	2015-004099-31
Date of protocol	Final Version 1: 02 February 2016
Title	Long-term persistence of immunity to hepatitis B in adults vaccinated 20 to 30 years ago with Engerix™-B (103860).
Detailed Title	A phase IV, open-label, non-randomised, multicentre study to assess the long-term persistence of immunity to hepatitis B in adults vaccinated 20 to 30 years ago with 3 or 4 doses of GSK Biologicals' hepatitis B vaccine, Engerix™-B.
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GSK Biologicals' Protocol DS v 14.1.1

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Protocol Sponsor Signatory Approval

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Sponsor signatory Dominique Descamps,
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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 vaccines and other study-related duties and functions as described in the protocol.
- To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.
- To ensure that no clinical samples (including serum samples) are retained onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject and/or the subject's legally acceptable representative.
- To perform no other biological assays on the clinical samples except those described in the protocol or its amendment(s).
- To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.
- That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the sponsor or the investigational vaccine, and more generally about his/her financial ties with the sponsor. GSK Biologicals will use and disclose the information solely for the purpose of complying with regulatory requirements.

Hence I:

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

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116811 (HBV-322)
Protocol Final Version 1

**eTrack study number and
Abbreviated Title** 116811 (HBV-322)

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Investigator name

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 [7.4.2](#).

SYNOPSIS

Detailed Title	A phase IV, open-label, non-randomised, multicentre study to assess the long-term persistence of immunity to hepatitis B in adults vaccinated 20 to 30 years ago with 3 or 4 doses of GSK Biologicals' hepatitis B vaccine, Engerix™-B.
Indication	Immunisation against infection caused by all known subtypes of hepatitis B virus.
Rationale for the study and study design	<ul style="list-style-type: none">Rationale for the study <p>Studies conducted in different populations have shown long-term persistence and immune memory induced by <i>Engerix-B</i> against hepatitis B, extending up to 20 years after primary vaccination in infants [Poovorawan, 2010; Van Damme, 2010; Poovorawan, 2012; Poovorawan, 2013], and up to 15 years after vaccination of 8-10 year old children [Gilca, 2013].</p> <p>This study will assess the persistence of immunity 20 to 30 years after primary vaccination of healthy adults, aged between 18-40 years, with <i>Engerix-B</i>.</p> <ul style="list-style-type: none">Rationale for the study design <p>This study will assess the long-term protection against HBV infection in adult subjects vaccinated with three or four doses (2+1 or 3+1 schedule) of <i>Engerix-B</i> 20 to 30 years ago by measuring the circulating antibodies against hepatitis B surface antigen (anti-HBs), by checking for occult infection (presence of antibodies against hepatitis B core antigen [anti-HBc]), by evaluating the anamnestic response to a challenge dose of the vaccine, and by testing for T and memory B cell activation.</p> <p>Long-term protection against HBV infection after immunisation relies primarily on the ability of the immune system to mount an anamnestic response when exposed to HBV [European Consensus Group on Hepatitis B Immunity, 2000; WHO, 2010]. The strongest assessment for persistence of immunity to hepatitis B in this study will be from the evaluation of anamnestic response in vaccinated individuals.</p> <p>Subjects who received three or four doses of <i>Engerix-B</i> vaccine as adults 20 to 30 years ago will be enrolled. Stratification of age will be carried out in order to ensure that the age of enrolled subjects is balanced across the participating countries. Hence, equal number of subjects will be enrolled into the two strata: 40-50 years and 51-60 years of</p>

age in all of the participating countries.

Since all of the subjects will have previously received vaccination with *Engerix-B*, they will be assessed for antibody persistence and long-term immunity, and will receive a single challenge dose of *Engerix-B*. The study will be non-randomised and open-label.

Objectives

Primary

- To assess the persistence of immunity to hepatitis B in terms of anti-HBs anamnestic response to an *Engerix-B* challenge dose, in adult subjects vaccinated with three or four doses of *Engerix-B* 20 to 30 years ago.

Secondary

- To assess the persistence of immunity to hepatitis B in terms of Geometric Mean Concentrations (GMCs), seropositivity rates, seroprotection rates, and percentage of subjects with anti-HBs antibody concentrations ≥ 100 mIU/ml, before or after the *Engerix-B* challenge dose, in subjects vaccinated with three or four doses of *Engerix-B* 20 to 30 years ago.
- To assess the persistence of immunity to hepatitis B in terms of T cell and memory B cell mediated immune responses specific to hepatitis B surface antigen, before and after the *Engerix-B* challenge dose, in adult subjects vaccinated with three or four doses of *Engerix-B* 20 to 30 years ago.
- To evaluate the safety and reactogenicity of *Engerix-B* challenge dose in terms of solicited symptoms, unsolicited symptoms and serious adverse events (SAEs).

Study design

- Experimental design: Phase IV, open-label, non-randomised, multi-centric, multi-country study with a single group.
- Duration of the study: Approximately one month per subject, starting from the administration of the challenge dose.

- Epoch 001: Primary starting at Visit 1 (Day 0) and ending at Visit 3 (Day 30).

Study group:

- LTFU: Subjects who received 3 or 4 doses of *Engerix-B* 20 to 30 years ago.

Synopsis Table 1 Study group and epoch foreseen in the study

Study Group	Number of subjects	Age (Min/Max)*	Epoch
			Epoch 001
LTFU	Approximately 100	40 years – 60 years	x

*Age at visit 1

Synopsis Table 2 Study group and treatment foreseen in the study

Treatment Name	Vaccine Name	Study Group
		LTFU
Engerix-B	HBV	x

- Control: uncontrolled.
- Vaccination schedule: A single dose of *Engerix-B* will be administered to all subjects at Visit 1 (Day 0).
- Treatment allocation: Non-randomised.
- Blinding: Open label.

Synopsis Table 3 Blinding of study epoch

Study Epoch	Blinding
Epoch 001	open

- Sampling schedule: Blood samples will be taken from all subjects in order to evaluate the immunogenicity endpoints. The following blood samples will be taken at each study visit (Visit 1, Visit 2 and Visit 3):
 - Three heparinised tubes of whole blood, of approximately 9 ml each, will be sampled for the assessment of memory B cells and T cells.
 - One tube of approximately 5 ml of blood will be taken, from which approximately 1.7 ml of serum will be extracted for the measurement of antibodies.
- Type of study: self-contained.
- Data collection: Electronic Case Report Form (eCRF).

Number of subjects Approximately 100 adults between 40-60 years of age will be enrolled in the study.

Endpoints**Primary**

- Persistence of immunity to hepatitis B in adult subjects vaccinated with three or four doses of *Engerix-B* 20 to 30 years ago.
 - Percentage of adult subjects with an anamnestic response 7 days and 30 days after the challenge dose.

Secondary

- Persistence of immunity to hepatitis B in adult subjects vaccinated with three or four doses of *Engerix-B* 20 to 30 years ago.
 - Percentage of adult subjects with anti-HBs antibody concentrations \geq 6.2 mIU/ml, \geq 10 mIU/ml and \geq 100 mIU/ml, at the pre-challenge dose time-point and Day 7 and Day 30 post-challenge dose time-points.
 - Anti-HBs antibody concentrations, at the pre-challenge dose time-point and Day 7 and Day 30 post-challenge dose time-points.
 - Hepatitis B specific memory B cell-mediated immune responses (frequency of HBs-specific memory B cells) at the pre-challenge dose time-point and Day 7 and Day 30 post-challenge dose time-points.
 - Hepatitis B specific T cell-mediated immune responses (frequency of HBs-specific CD4 T-lymphocytes) at the pre-challenge dose time-point and Day 7 and Day 30 post-challenge dose time-points.
- Solicited local and general symptoms.
 - Occurrence of solicited local symptoms during the 4-day (Days 0-3) follow-up period after the challenge dose.
 - Occurrence of solicited general symptoms during the 4-day (Days 0-3) follow-up period after the challenge dose.
- Unsolicited adverse events.
 - Occurrence of unsolicited symptoms during the 31-day (Days 0-30) follow-up period after the challenge dose.
- Serious adverse events.
 - Occurrence of SAEs after the challenge dose, up to the study end.

TABLE OF CONTENTS

	PAGE
SPONSOR INFORMATION	6
SYNOPSIS	7
LIST OF ABBREVIATIONS	17
GLOSSARY OF TERMS	19
TRADEMARKS	22
1. INTRODUCTION	23
1.1. Background	23
1.2. Rationale for the study and study design	23
1.2.1. Rationale for the study	23
1.2.2. Rationale for the study design	23
1.3. Benefit: Risk Assessment	24
1.3.1. Risk Assessment	24
1.3.2. Benefit Assessment	25
1.3.3. Overall Benefit: Risk Conclusion	25
2. OBJECTIVES	25
2.1. Primary objective	25
2.2. Secondary objectives	25
3. STUDY DESIGN OVERVIEW	26
4. STUDY COHORT	27
4.1. Number of subjects/centres	27
4.2. Inclusion criteria for enrolment	28
4.3. Exclusion criteria for enrolment	28
5. CONDUCT OF THE STUDY	30
5.1. Regulatory and ethical considerations, including the informed consent process	30
5.2. Subject identification and randomisation of treatment	31
5.2.1. Subject identification	31
5.2.2. Randomisation of treatment	31
5.2.2.1. Randomisation of supplies	31
5.2.2.2. Treatment allocation to the subject	31
5.2.2.2.1. Study group and treatment number allocation	31
5.3. Method of blinding	32
5.4. General study aspects	32
5.5. Outline of study procedures	32
5.6. Detailed description of study procedures	34
5.6.1. Informed consent	34
5.6.2. Collect demographic data	34
5.6.3. Medical history	34

5.6.4.	Hepatitis B vaccination history	34
5.6.5.	Physical examination	34
5.6.6.	Check inclusion and exclusion criteria	35
5.6.7.	Record height and weight	35
5.6.8.	Check contraindications to vaccination	35
5.6.9.	Pre-vaccination urine pregnancy test	35
5.6.10.	Assess pre-vaccination body temperature	35
5.6.11.	Treatment number allocation	35
5.6.12.	Sampling	35
5.6.12.1.	5.6.12.1. Blood sampling for antibody determination and CMI response	36
5.6.13.	5.6.13. Study Vaccine administration and observation of subjects following administration	36
5.6.14.	5.6.14. Check and record concomitant medication/vaccination and intercurrent medical conditions	36
5.6.15.	5.6.15. Recording of solicited AEs and non-serious AEs	36
5.6.16.	5.6.16. Medically attended visit	37
5.6.17.	5.6.17. Return of diary cards and diary card transcription by investigator	37
5.6.18.	5.6.18. Recording of AEs, SAEs, and pregnancies	37
5.6.19.	5.6.19. Study conclusion	37
5.7.	5.7. Biological sample handling and analysis	37
5.7.1.	5.7.1. Use of specified study materials	38
5.7.2.	5.7.2. Biological samples	38
5.7.3.	5.7.3. Laboratory assays	39
5.7.4.	5.7.4. Biological samples evaluation	40
5.7.4.1.	5.7.4.1. Immunological read-outs	40
5.7.5.	5.7.5. Immunological correlates of protection	40
6.	STUDY VACCINE AND ADMINISTRATION	41
6.1.	6.1. Description of study vaccine	41
6.2.	6.2. Storage and handling of study vaccine	41
6.3.	6.3. Dosage and administration of study vaccine	42
6.4.	6.4. Replacement of unusable vaccine doses	42
6.5.	6.5. Contraindications to vaccination	42
6.5.1.	6.5.1. Absolute contraindications	42
6.5.2.	6.5.2. Temporary contraindications	43
6.6.	6.6. Warnings and precautions	43
6.7.	6.7. Concomitant medications/products and concomitant vaccinations	43
6.7.1.	6.7.1. Recording of concomitant medications/products and concomitant vaccinations	43
6.7.2.	6.7.2. Concomitant medications/products/vaccines that may lead to the elimination of a subject from ATP analyses	44
6.8.	6.8. Intercurrent medical conditions that may lead to elimination of a subject from ATP analyses	44
7.	SAFETY	45
7.1.	7.1. Safety definitions	45
7.1.1.	7.1.1. Definition of an adverse event	45
7.1.2.	7.1.2. Definition of a serious adverse event	46
7.1.3.	7.1.3. Solicited adverse events	47
7.1.3.1.	7.1.3.1. Solicited local (injection-site) adverse events	47

7.1.3.2. Solicited general adverse events	47
7.1.4. Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events	47
7.2. Events or outcomes not qualifying as adverse events or serious adverse events	48
7.2.1. Pregnancy	48
7.3. Detecting and recording adverse events, serious adverse events and pregnancies	49
7.3.1. Time period for detecting and recording adverse events, serious adverse events and pregnancies	49
7.3.2. Post-Study adverse events and serious adverse events	50
7.3.3. Evaluation of adverse events and serious adverse events	51
7.3.3.1. Active questioning to detect adverse events and serious adverse events	51
7.3.3.2. Assessment of adverse events	51
7.3.3.2.1. Assessment of intensity	51
7.3.3.2.2. Assessment of causality	53
7.3.3.3. Assessment of outcomes	54
7.3.3.4. Medically attended visits	55
7.4. Reporting of serious adverse events, pregnancies, and other events	55
7.4.1. Prompt reporting of serious adverse events, pregnancies, and other events to GSK Biologicals	55
7.4.2. Contact information for reporting serious adverse events, pregnancies	56
7.4.3. Completion and transmission of SAE reports to GSK Biologicals	56
7.4.3.1. Back-up system in case the electronic reporting system does not work	56
7.4.4. Completion and transmission of pregnancy reports to GSK Biologicals	56
7.4.5. Updating of SAE and pregnancy information after removal of write access to the subject's eCRF	57
7.4.6. Regulatory reporting requirements for serious adverse events	57
7.5. Follow-up of adverse events, serious adverse events, and pregnancies	57
7.5.1. Follow-up of adverse events and serious adverse events	57
7.5.1.1. Follow-up during the study	57
7.5.1.2. Follow-up after the subject is discharged from the study	58
7.5.2. Follow-up of pregnancies	58
7.6. Treatment of adverse events	58
7.7. Subject card	58
8. SUBJECT COMPLETION AND WITHDRAWAL	59
8.1. Subject completion	59
8.2. Subject withdrawal	59
8.2.1. Subject withdrawal from the study	59
9. STATISTICAL METHODS	60
9.1. Primary endpoint	60

9.2. Secondary endpoints	60
9.3. Determination of sample size	61
9.4. Cohorts for Analyses	62
9.4.1. Total vaccinated cohort	62
9.4.2. According-to-protocol cohort for analysis of immunogenicity	62
9.5. Derived and transformed data	62
9.6. Analysis of demographics	63
9.7. Analysis of immunogenicity	64
9.8. Exploratory Analysis:	65
9.9. Analysis of safety	65
9.10. Interpretation of analyses	65
9.11. Conduct of analyses	65
9.11.1. Sequence of analyses	66
9.11.2. Statistical considerations for interim analyses	66
10. ADMINISTRATIVE MATTERS	66
10.1. Electronic Case Report Form instructions	66
10.2. Study Monitoring by GSK Biologicals	66
10.3. Record retention	67
10.4. Quality assurance	68
10.5. Posting of information on publicly available clinical trial registers and publication policy	68
10.6. Provision of study results to investigators	68
11. COUNTRY SPECIFIC REQUIREMENTS	68
12. REFERENCES	69

LIST OF TABLES

	PAGE
Table 1	26
Table 2	27
Table 3	27
Table 4	31
Table 5	33
Table 6	34
Table 7	39
Table 8	39
Table 9	39
Table 10	40
Table 11	41
Table 12	42
Table 13	47
Table 14	47
Table 15	50
Table 16	52
Table 17	55
Table 18	61
Table 19	62
Table 20	71
Table 21	71

LIST OF APPENDICES

	PAGE
APPENDIX A CLINICAL LABORATORIES	71

LIST OF ABBREVIATIONS

AE:	Adverse Event
Ag:	Antigen
Anti-HBc:	Antibodies against Hepatitis B core antigen
Anti-HBs:	Antibodies against Hepatitis B surface antigen
ATP:	According-To-Protocol
BMI	Body Mass Index
CFC:	Cytokine Flow Cytometry
CI:	Confidence Interval
CLIA:	ChemiLuminescence ImmunoAssay
CLS	Clinical Laboratory Sciences
CMI:	Cell Mediated Immunity
eCRF:	Electronic Case Report Form
ELISPOT:	Enzyme-Linked Immunosorbent Spot
eTDF:	Electronic Temperature excursion Decision Form
GMC:	Geometric Mean Concentration
GSK:	GlaxoSmithKline
HBsAg:	Hepatitis B surface antigen
HBV:	Hepatitis B virus
IU/ml:	International Units per millilitre
ICF:	Informed Consent Form
ICH:	International Conference on Harmonisation
IEC:	Independent Ethics Committee
IRB:	Institutional Review Board
MedDRA:	Medical Dictionary for Regulatory Activities

PBMC:	Peripheral Blood Mononuclear Cell
SAE:	Serious Adverse Event
SBIR:	Randomisation System on Internet
SDV:	Source Document Verification
SPM:	Study Procedures Manual

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,
 - current tubal ligation,
 - 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.
- a contraceptive method with failure rate of more than 1% per year but still considered as acceptable birth control method.
 - 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.

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 time-points or a single time-point from a single protocol. Self-contained means that data collected for all subjects at all time-points 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) analysis (see Sections 6.7.2 and 9.4 for details on criteria for evaluability).
Immunological correlate of protection:	The defined immune response above which there is a high likelihood of protection in the absence of any host factors that might increase susceptibility to the infectious agent.
Investigational vaccine: (Synonym of Investigational Medicinal Product)	A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorisation when used in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.
Menopause:	Menopause is the age associated with complete cessation of menstrual cycles, menses, and implies the loss of reproductive potential by ovarian failure. A practical definition accepts menopause after 1 year without menses with an appropriate clinical profile at the appropriate age e.g. > 45 years.
Primary completion date:	The date that the final subject was examined or received an intervention for the purpose of final collection of data for the primary outcome, whether the clinical trial concluded according to the pre-specified protocol or was terminated.
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.
Site Monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of clinical studies at one or more investigational sites.
Solicited adverse event:	AEs to be recorded as endpoints in the clinical study. The presence/occurrence/intensity of these events is actively solicited from the subject or an observer during a specified post-vaccination follow-up period.
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.
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.
Treatment:	Term used throughout the clinical study to denote a set of investigational product(s) or marketed product(s) or placebo intended to be administered to a subject, identified by a unique number, according to the study randomisation or treatment allocation.
Treatment number:	A number identifying a treatment to a subject, according to the study randomisation or treatment allocation.
Unsolicited adverse event:	Any AE reported in addition to those solicited during the clinical study. Also any 'solicited' symptom with onset outside the specified period of follow-up for solicited symptoms will be reported as an unsolicited adverse event.

TRADEMARKS

The following trademark is used in the present protocol.

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

Trademarks of the GlaxoSmithKline group of companies	Generic description
Engerix TM -B	Hepatitis B vaccine

1. INTRODUCTION

1.1. Background

Hepatitis B is a life threatening liver infection caused by the Hepatitis B virus (HBV). An estimated 240 million people are chronically infected with HBV and around 780,000 people die yearly due to HBV [Lozano, 2012].

In 2012, the European Centre for Disease Prevention and Control (ECDC) estimated a hepatitis B incidence of 3.5 per 100,000 population [ECDC, 2012] in 29 countries.

Vaccination against hepatitis B is effective in preventing HBV infection. Universal infant vaccination was recommended by WHO in 1992 and more than 183 countries currently vaccinate infants against hepatitis B as part of their vaccination schedules [WHO, 2015].

GSK Biologicals' hepatitis B vaccine (*Engerix-B*) has a strongly documented safety and immunogenicity profile both in monitored clinical trials and under field conditions [Van Damme, 2010].

1.2. Rationale for the study and study design

1.2.1. Rationale for the study

Studies conducted in different populations have shown long-term persistence and immune memory induced by *Engerix-B* against hepatitis B, extending up to 20 years after primary vaccination in infants [Poovorawan, 2010; Van Damme, 2010; Poovorawan, 2012; Poovorawan, 2013], and up to 15 years after vaccination of 8-10 year old children [Gilca, 2013].

This study will assess the persistence of immunity 20 to 30 years after primary vaccination of healthy adults, aged between 18-40 years, with *Engerix-B*.

1.2.2. Rationale for the study design

This study will assess the long-term protection against HBV infection in adult subjects vaccinated with three or four doses (2+1 or 3+1 schedule) of *Engerix-B* 20 to 30 years ago by measuring the circulating antibodies against hepatitis B surface antigen (anti-HBs), by checking for occult infection (presence of antibodies against hepatitis B core antigen [anti-HBc]), by evaluating the anamnestic response to a challenge dose of the vaccine, and by testing for T and memory B cell activation.

Long-term protection against HBV infection after immunisation relies primarily on the ability of the immune system to mount an anamnestic response when exposed to HBV [European Consensus Group on Hepatitis B Immunity, 2000; WHO, 2010]. The strongest assessment for persistence of immunity to hepatitis B in this study will be from the evaluation of anamnestic response in vaccinated individuals.

Subjects who received three or four doses of *Engerix-B* vaccine as adults 20 to 30 years ago will be enrolled. Stratification of age will be carried out in order to ensure that the age of enrolled subjects is balanced across the participating countries. Hence, equal number of subjects will be enrolled into the two strata: 40-50 years and 51-60 years of age in all of the participating countries.

Since all subjects will have previously received vaccination with *Engerix-B*, they will be assessed for antibody persistence and long-term immunity and will receive a single challenge dose of *Engerix-B*. The study will be non-randomised and open-label.

1.3. Benefit: Risk Assessment

Engerix-B 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*.

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* has been established. The vaccine is well tolerated in all age groups and has been safely administered in schedules of 2+1 and 3+1 doses, including subjects with chronic medical conditions such as end-stage renal disease.

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Investigational study vaccine/product: <i>Engerix-B</i>		
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. In addition, subjects should be observed for 30 minutes after administration of the vaccine.

1.3.2. Benefit Assessment

By taking part in this study, the subject will know if he/she still has circulating antibodies 20 to 30 years after vaccination which is information that is not routinely available.

This study will provide detailed immunogenicity information about the antibody persistence and immune memory after primary vaccination with *Engerix-B* 20 to 30 years ago. This would also present an opportunity to facilitate better protection against the disease, 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* 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 persistence of immunity to hepatitis B in terms of anti-HBs anamnestic response to an *Engerix-B* challenge dose, in adult subjects vaccinated with three or four doses of *Engerix-B* 20 to 30 years ago.

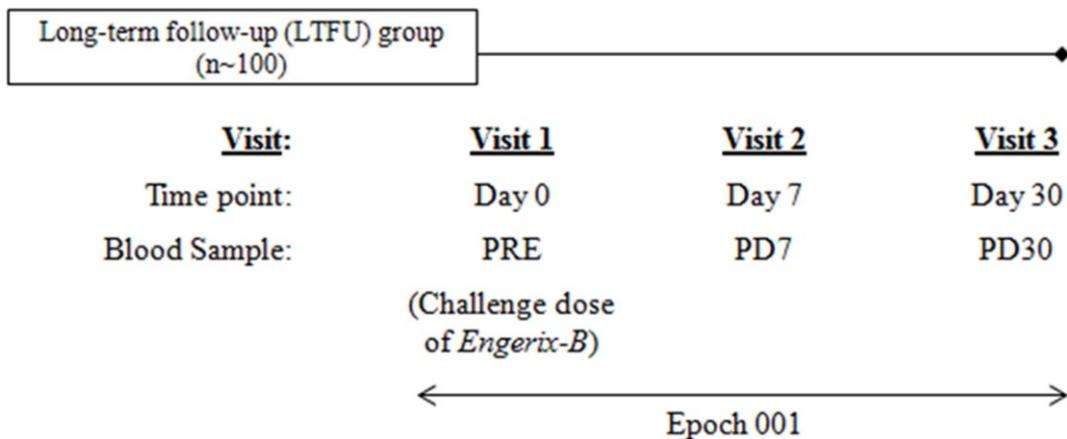
Refer to Section 9.1 for the definition of the primary endpoint.

2.2. Secondary objectives

- To assess the persistence of immunity to hepatitis B in terms of Geometric Mean Concentrations (GMCs), seropositivity rates, seroprotection rates, and percentage of subjects with anti-HBs antibody concentrations ≥ 100 mIU/ml, before or after the *Engerix-B* challenge dose, in subjects vaccinated with three or four doses of *Engerix-B* 20 to 30 years ago.
- To assess the persistence of immunity to hepatitis B in terms of T cell and memory B cell mediated immune responses specific to hepatitis B surface antigen, before and after the *Engerix-B* challenge dose, in adult subjects vaccinated with three or four doses of *Engerix-B* 20 to 30 years ago.
- To evaluate the safety and reactogenicity of *Engerix-B* challenge dose in terms of solicited symptoms, unsolicited symptoms and serious adverse events (SAEs).

Refer to Section 9.2 for the definition of the secondary endpoints.

3. STUDY DESIGN OVERVIEW



n = Total number of subjects, PRE = Blood sample to be collected before vaccination, PD7 = Blood sample to be collected 7 days after vaccination, PD30 = Blood sample to be collected 30 days after vaccination.

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, multi-country study with a single group.
- Duration of the study: Approximately one month per subject, starting from the administration of the challenge dose.
 - Epoch 001: Primary starting at Visit 1 (Day 0) and ending at Visit 3 (Day 30).
- Study group:
 - LTFU: Subjects who received 3 or 4 doses of *Engerix-B* 20 to 30 years ago.

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

Table 1 Study group and epoch foreseen in the study

Study group	Number of subjects	Age (Min/Max)‡	Epoch
			Epoch 001
LTFU	Approximately 100	40 years - 60 years	x

‡ Age at vaccination.

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

Table 2 Study group and treatment foreseen in the study

Treatment name	Vaccine name	Study Group
		LTFU
Engerix-B	HBV	x

- Control: uncontrolled.
- Vaccination schedule: A single dose of *Engerix-B* will be administered to all subjects at Visit 1 (Day 0).
- Treatment allocation: Non-randomised.
- Blinding: Open-label.

Table 3 Blinding of study epoch

Study Epoch	Blinding
Epoch 001	open-label

- Sampling schedule: Blood samples will be taken from all subjects in order to evaluate the immunogenicity endpoints. The following blood samples will be taken at each study visit (Visit 1 [PRE], Visit 2 [PD7] and Visit 3 [PD30]):
 - Three heparinised tubes of whole blood, of approximately 9 ml each, will be sampled for the assessment of memory B cells and T cells.
 - One tube of approximately 5 ml of blood will be taken, from which approximately 1.7 ml of serum will be extracted for the measurement of antibodies.
- Type of study: self-contained
- Data collection: electronic Case Report Form (eCRF).

4. STUDY COHORT

4.1. Number of subjects/centres

Approximately 100 adults between 40-60 years of age will be enrolled in study.

Overview of the recruitment plan

- The study will be conducted at multiple centres in Canada and Europe.
- Enrolment will be stopped when approximately 100 eligible subjects have been enrolled in all participating countries.
- Recruitment of subjects into the study will be tracked using the randomisation system on Internet (SBIR).
- 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 jeopardise 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 who, in the opinion of the investigator, can and will comply with the requirements of the protocol (e.g. completion of the diary cards, return for follow-up visits).
- A male or female between, and including, 40 and 60 years of age (from and including the 40th birthday up to, but excluding, the 61st birthday) at the time of the vaccination.
- Written informed consent obtained from the subject.
- Documented evidence of previous vaccination with three or four consecutive doses of *Engerix-B* administered in adulthood (i.e. at least 18 years of age) with
 - the last dose received 4 to 12 months after the previous one,
 - no subsequent booster dose ever received later, and
 - the last dose received 20 to 30 years before enrolment.
- Female subjects of non-childbearing potential may be enrolled in the study.
 - Non-childbearing potential is defined as pre-menarche, hysterectomy, ovariectomy or post-menopause.

Please refer to the [glossary of terms](#) for the definition of menopause.

- 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 one month after vaccination.

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 jeopardise 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:

- 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, or planned use during the study period.
- Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs within six months prior to the vaccine dose. For corticosteroids, this will mean prednisone ≥ 20 mg/day, 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).
- Previous hepatitis B booster vaccination since completion of the primary vaccination series with three or four doses of *Engerix-B*.
- Planned administration of a vaccine not foreseen by the study protocol within 30 days preceding the dose of study vaccine, or planned administration during the study period, with the exception of seasonal influenza vaccine.
- Any medical condition that in the judgment of the investigator places the subject at undue risk by participating in the study.
- 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).
- History of hepatitis B disease or episode of jaundice with unknown etiology.
- History of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccine.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination (no laboratory testing required).
- Major congenital defects or serious chronic illness (including insulin-dependent diabetes).
- Acute disease and/or fever at the time of enrolment.
 - Fever is defined as temperature $\geq 37.5^{\circ}\text{C}$ for oral, axillary or tympanic route, or $\geq 38.0^{\circ}\text{C}$ on 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.
- 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.
- Drug and/ or alcohol abuse within the last 5 years.

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.

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 informed consent.
- Investigator reporting requirements as stated in the protocol.

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

Freely given and written or witnessed/ thumb printed informed consent must be obtained from each subject 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.

The investigator has the final responsibility for the final presentation of the ICF, respecting the mandatory requirements of local regulations. The ICF 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 who have consented to participate in the study, according to the range of subject identification numbers allocated to each study centre.

5.2.2. Randomisation of treatment

5.2.2.1. Randomisation of supplies

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

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

The enrolment will be performed to ensure equal distribution of approximately 100 subjects across the two age strata (40 to 50 years and 51-60 years). Therefore, the expected distribution of subjects is as shown in [Table 4](#).

Table 4 Number of subjects required for enrolment

Age Stratum	Vaccine	N
40-50 years	Engerix B	50
51-60 years	Engerix B	50

N = number of subjects to be enrolled

Allocation of a treatment number to the subject at the investigator site will be performed using a randomisation system on internet (SBIR).

After obtaining the signed and dated ICF from the subject and having checked the eligibility of the subject, the study staff in charge of the vaccine administration will access SBIR. Upon providing the age (40 to 50 years and 51 to 60 years) and the subject identification number, the randomisation system will determine the age group and 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.

Note that as soon as the target number of approximately 50 subjects in a specific age group has been reached, the enrolment will be frozen for this age group.

5.3. Method of blinding

This is an open-label study as all the subjects will receive a single challenge dose of *Engerix-B*.

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

5.4. General study aspects

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

5.5. Outline of study procedures

The list of study procedures are provided in [Table 5](#).

Table 5 List of study procedures

Epoch	Epoch 001		
	Visit 1	Visit 2	Visit 3
Type of contact	Day 0	Day 7	Day 30
Sampling time-points	PRE	PD7	PD30
Informed consent	●		
Check inclusion/exclusion criteria	●		
Collect demographic data	●		
Medical history	●		
Hepatitis B vaccination history [†]	●		
Physical examination*	●	0	0
Measure/record height and weight	●		
Check contraindications to vaccination	0		
Pre-vaccination urine pregnancy test for female subjects of child bearing potential	●		
Pre-vaccination body temperature measurement	●		
Treatment number allocation	0		
Blood sampling for antibody determination (~5 ml)	●	●	●
Blood sampling for CMI response (~27 ml)	●	●	●
Vaccine administration	●		
Observation of the subjects for at least 30 minutes after vaccine administration	0		
Record any concomitant medication/vaccination	●	●	●
Record any intercurrent medical conditions		●	●
Distribution of diary cards	0		
Recording of solicited adverse events (Days 0–3) by subjects	●		
Recording of non-serious adverse events (Days 0–30) post-vaccination, by investigator or subjects	●	●	●
Medically attended visits		●	●
Return of diary cards		0	0
Diary card transcription by investigator [‡]		●	●
Recording of serious adverse events (SAEs)	●	●	●
Recording of SAEs related to study participation or to a concurrent GSK medication/vaccine	●	●	●
Recording of pregnancies and pregnancy outcomes	●	●	●
Study Conclusion			●

PRE = Blood sample to be collected before vaccination, PD7 = Blood sample to be collected 7 days after vaccination, PD30 = Blood sample to be collected 30 days after vaccination

● is used to indicate a study procedure that requires documentation in the individual eCRF.

0 is used to indicate a study procedure that does not require documentation in the individual eCRF.

* Physical examination after 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.

† Documentation regarding previous hepatitis B vaccination should be checked for subjects.

‡ Diary card will be returned to the subject to continue recording adverse events until the last Visit.

The intervals to be considered within the study are provided in [Table 6](#).

Table 6 Intervals between study visits

Interval	Optimal length of interval	Allowed interval ¹
Visit 1→ Visit 2	7 days	5 days - 12 days
Visit 1→ Visit 3	30 days	21 days - 48 days

¹ Subjects will not be eligible for inclusion in the ATP cohort for analysis of immunogenicity if they make the study visit outside this interval.

5.6. Detailed description of study procedures

5.6.1. Informed consent

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

5.6.2. Collect demographic data

Demographic data such as age in years at challenge dose, gender, geographic ancestry, height, weight and body mass index (BMI) will be recorded in the subject's eCRF at Visit 1.

5.6.3. Medical history

Obtain the subject's medical 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.

5.6.4. Hepatitis B vaccination history

Review the subject's hepatitis B vaccination history and record the actual date of vaccination and brand of vaccine. This information should be recorded in the eCRF.

5.6.5. Physical examination

Perform a physical examination of the subject, including assessment of body temperature and resting vital signs: systolic/diastolic blood pressure, heart rate and respiratory rate after at least 10 minutes of rest. Collected information needs to be recorded in the eCRF.

Physical examination at each 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 physical examination has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

5.6.6. Check inclusion and exclusion criteria

Check all inclusion and exclusion criteria as described in Sections [4.2](#) and [4.3](#) before enrolment.

5.6.7. Record height and weight

Height and weight of the subjects will be recorded at Visit 1.

5.6.8. Check contraindications to vaccination

Contraindications, warnings and precautions to vaccination must be checked at the vaccination visit. Refer to Sections [6.5](#) for more details.

5.6.9. Pre-vaccination urine pregnancy test

Female subjects of childbearing potential are to have a urine pregnancy test prior to 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.

5.6.10. Assess pre-vaccination body temperature

The axillary or oral body temperature of all subjects needs to be measured prior to study vaccine administration. The preferred routes for recording temperature in this study will be axillary or oral. If the subject has fever [fever is defined as temperature $\geq 37.5^{\circ}\text{C}$ for oral, axillary or tympanic route or $\geq 38.0^{\circ}\text{C}$ on rectal route] on the day of vaccination, the vaccination visit will be rescheduled within the allowed interval for this visit (refer [Table 6](#)).

5.6.11. Treatment number allocation

Treatment number allocation will be performed as described in Section [5.2.2](#). The number of each administered treatment must be recorded in the eCRF.

5.6.12. Sampling

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

5.6.12.1. Blood sampling for antibody determination and CMI response.

Blood samples will be taken during all study visits as specified in Section 5.5; List of Study Procedures.

- A volume of approximately 5 ml of whole blood (to provide at least 1.7 ml of serum) should be drawn from all subjects for analysis of humoral immune response at each visit (PRE, PD7 and PD30). 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.
- A volume of approximately 27 ml of whole blood should be drawn from all subjects for analysis of cell-mediated immune (CMI) response at each visit (PRE, PD7 and PD 30). The blood should be stored at the investigator's site at room temperature and it must not be centrifuged. Samples will be shipped/transferred at room temperature (15 to 25°C) to the designated laboratory for cell separation to be performed within 24 hours of blood collection. Refer to the SPM for more details on sample storage conditions.

5.6.13. Study Vaccine administration and observation of subjects following administration

- After completing all prerequisite procedures prior to vaccination, the study vaccine will be administered intramuscularly (IM) in the deltoid, preferably 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 (refer to Table 6).
- 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.

5.6.14. 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 also be checked and recorded in the eCRF as described in Section 6.8.

5.6.15. Recording of solicited AEs and non-serious AEs

- At Visit 1, the diary card will be provided to the subject. The subject will record body (preferred routes are axillary or oral) 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 days)

occurring after vaccination. The subject will be instructed to return the completed diary card to the investigator at the next study visit.

5.6.16. Medically attended visit

For each solicited and unsolicited symptom the subject experiences, the subject will be asked if he/she 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.

5.6.17. Return of diary cards and diary card transcription by investigator

- Collect and verify completed diary cards during discussion with the subject at Visit 2 and Visit 3.
- Any unreturned diary cards will be sought from the subject through telephone call(s) or any other convenient procedure. The investigator or delegate will transcribe the collected information into the eCRF in English.

5.6.18. Recording of AEs, SAEs, and pregnancies

- Refer to Section 7.3 for procedures for the investigator to record AEs, SAEs, and pregnancies. Refer to Section 7.4 for guidelines and how to report SAE and pregnancy reports to GSK Biologicals. Refer to section 7.5.2 for guidelines on how to report pregnancy outcomes to GSK Biologicals.
- The subjects will be instructed to contact the investigator immediately should the subjects manifest any signs or symptoms they perceive as serious.

5.6.19. Study conclusion

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 analysis

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

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

- Collected samples will be used for protocol mandated research and purposes related to the improvement, development and quality assurance of the laboratory tests described in this protocol. This may include the management of the quality of these tests, the maintenance or improvement of these tests, the development of new test

methods, as well as making sure that new tests are comparable to previous methods and work reliably.

- It is also possible that future findings may make it desirable to use the samples acquired in this study for future research, not described in this protocol. Therefore, all subjects in countries where this is allowed, will be asked to give a specific consent to allow GSK or a contracted partner to use the samples for future research. Future research will be subject to the laws and regulations in the 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.

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 analysis (See Section [9.4](#) 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 type and quantity of biological samples that will be collected for analysis has been provided in [Table 7](#).

Table 7 Biological samples

Sample type	Quantity	Unit	Time-point
Blood sample for antibody determination	Approximately 5	ml	PRE (Visit 1), PD7 (Visit 2), PD30 (Visit 3)
Blood sample for CMI	Approximately 27	ml	PRE (Visit 1), PD7 (Visit 2), PD30 (Visit 3)

PRE = Blood sample to be collected before vaccination

PD7 = Blood sample to be collected 7 days after vaccination

PD30 = Blood sample to be collected 30 days after vaccination.

5.7.3. Laboratory assays

Please refer to [APPENDIX A](#) for the address of the clinical laboratories used for sample analysis.

Serological assays for the determination of antibodies against HBV will be performed by ELISA at a GSK Biologicals' laboratory or in a laboratory designated by GSK Biologicals using standardised and validated procedures (refer to [Table 8](#) and [Table 9](#)).

Table 8 Humoral Immunity (Antibody determination)

System	Component	Method	Kit / Manufacturer	Unit	Cut-off	Laboratory*
SER	Hepatitis B Virus.Core Ab	CLIA	Immulite (Siemens Healthcare)	No unit	N/A	CEVAC
SER	Hepatitis B Virus.Surface Ab	CLIA	ADVIA Centaur anti-HBs2 (Siemens Healthcare)	mIU/ml	6.2	GSK Biologicals**

*Refer to [APPENDIX A](#) for the laboratory addresses.

SER = Serum

CLIA = ChemiLuminescence ImmunoAssay

N/A = Not Applicable

CEVAC = Centre for Vaccinology, University of Gent

**GSK Biologicals laboratory refers to the Clinical Laboratory Sciences (CLS) in Rixensart, Belgium; Wavre, Belgium.

Table 9 Cell-Mediated Immunity (CMI)

System	Component	Challenge	Method	Unit	Laboratory*
PBMC	memory B-cells	HBV03	ELIB	SFC/10E6 SFC IgG	GSK Biologicals**
PBMC	Cells CD4.CD40L(+) + Interleukin-2(-) + Tumor Necrosis Factor alpha(-) + Interferon gamma(-)	HBV02	CFC	Events/10E6 cells	GSK Biologicals**

*Refer to [APPENDIX A](#) for the laboratory addresses.

PBMC = Peripheral Blood Mononuclear Cell

ELIB = B cell Enzyme-linked Immunosorbent Spot

SFC/10E6 SFC IgG = Frequency of specific memory B cells per million of memory B cells

Events/10E6 cells = frequency of specific CD4+ T Cells per million of CD4+ T cells

CFC = Cytokine Flow Cytometry

**GSK Biologicals laboratory refers to the Clinical Laboratory Sciences (CLS) in Rixensart, Belgium. Alternatively, the testing may be outsourced to a laboratory designated by GSK Biologicals using standardised and validated procedures.

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 provided in [Table 10](#).

Table 10 Immunological read-outs

Blood sampling time-point		No. subjects	Component	Components priority rank
Type of contact and time-point	Sampling time-point			
Visit 1 (Day 0)	PRE	ALL	Anti-HBs*	1
		ALL	Anti-HBc*	2
		ALL	Memory B cells	1
		ALL	T cells	2
Visit 2 (Day 7)	PD7	ALL	Anti-HBs*	1
		ALL	Memory B cells	1
		ALL	T cells	2
Visit 3 (Day 30)	PD30	ALL	Anti-HBs*	1
		ALL	Memory B cells	1
		ALL	T cells	2

* The results of these testings might potentially impact the subject medical care.

PRE = Blood sample to be collected before vaccination, PD7 = Blood sample to be collected 7 days after vaccination, PD30 = Blood sample to be collected 30 days after vaccination.

Anti-HBs = Antibodies against hepatitis B surface antigen

Anti-HBc = Antibodies against hepatitis B core antigen

In case of insufficient blood sample volume to perform assays for all components, the samples will be analysed according to priority ranking provided in [Table 10](#).

5.7.5. Immunological correlates of protection

An anti-HBs antibody concentration ≥ 10 mIU/ml defines seroprotection [[Centers for Disease Control and Prevention, 1991](#); [WHO, 1988](#)].

The immunological assay results will be communicated to the investigator when available, for the data collected up to Visit 3.

The investigator is encouraged to share the immunological assay results for non-responders with the study subjects.

For subjects identified as non-responders, it remains the responsibility of the study investigator in charge of the subject's clinical management to determine the medical need

for re-vaccination, and to re-vaccinate the subjects according to local recommendations and practices.

For subjects for whom an occult infection is identified, it is the responsibility of the study investigator in charge of the subject's clinical management to determine the medical care of the subjects according to local recommendation.

6. STUDY VACCINE AND ADMINISTRATION

6.1. Description of study vaccine

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

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

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

The details of the study vaccine are presented in [Table 11](#).

Table 11 Study vaccine

Treatment name	Vaccine name	Formulation	Presentation	Volume to be administered	Number of doses
<i>Engerix-B</i>	HBV	HBsAg=20 µg; Al(OH) ₃ =500µg Al3+	The vaccine is presented as a suspension for injection in vials or prefilled syringes.	1 ml	1

HBsAg: Hepatitis B surface antigen.

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 authorised 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/+36 to +46°F label storage condition) impacting investigational medicinal products (IMPs) must be reported in the appropriate (electronic) temperature excursion decision form ([e]TDF).

The impacted IMPs must not be used and must be stored in quarantine at label temperature conditions until usage approval has been obtained from the sponsor.

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

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

6.3. Dosage and administration of study vaccine

All subjects will receive a single dose of *Engerix-B*.

In order to ensure proper intramuscular (IM) injection of the study vaccine, a needle of at least 1 inch (2.54 cm) length, 25 gauge will be used [[Centers for Disease Control and Prevention, 2002](#)]. IM injections should be administered at a 90-degree angle into the deltoid region, preferably in the non-dominant arm.

The details of dosage and administration of study vaccine are presented in [Table 12](#).

Table 12 Dosage and administration

Type of contact and time-point	Volume to be administered	Study group	Treatment name	Route ¹	Site	Side
Visit 1 (Day 0)	1 ml	LTFU	<i>Engerix-B</i>	IM	Deltoid	Non-dominant

¹Intramuscular (IM)

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 50% additional vaccine doses will be supplied to replace those that are unusable.

6.5. Contraindications to vaccination

6.5.1. Absolute contraindications

Engerix-B should not be administered to subjects with known hypersensitivity to any component of the vaccine, or to subjects having shown signs of hypersensitivity after previous *Engerix-B* administration. Since this is a single-dose study, the contraindications are included in the exclusion criteria (See Section 4.3).

6.5.2. Temporary contraindications

The following events constitute temporary contraindications to administration of *Engerix-B* at that point in time. If any of these events occur at the time scheduled for vaccination, the subject may be vaccinated at a later date, within the time window specified in the protocol (see Section 5.5), or the subject may be withdrawn at the discretion of the investigator (see Section 7.5).

- Acute disease and/or fever at the time of vaccination.
 - Fever is defined as temperature $\geq 37.5^{\circ}\text{C}$ for oral, axillary or tympanic route, or $\geq 38.0^{\circ}\text{C}$ for rectal route. The preferred route for recording temperature in this study will be axillary or oral.
 - Subjects with a minor illness (such as mild diarrhoea, mild upper respiratory infection) without fever can be administered the vaccine.

6.6. Warnings and precautions

As with all injectable vaccines, appropriate medical treatment and supervision should always be readily available in case of a rare anaphylactic reaction following the administration of the study vaccine.

Syncope (fainting) can occur following, or even before, any vaccination as a psychogenic response to the needle injection. It is important that procedures are in place to avoid injury from faints.

Refer to the approved product label/package insert for more information.

6.7. Concomitant medications/products and concomitant vaccinations

At each study visit, the investigator should question the subject about any medications/products taken and vaccinations received by the subject.

6.7.1. Recording of concomitant medications/products and concomitant vaccinations

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

- All concomitant medications/products, except vitamins and dietary supplements, administered during the period starting 30 days before and following the dose of study vaccine (Day 0 to 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 0 to Day 30).
- Prophylactic medication (i.e. medication administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination).

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 $\geq 37.5^{\circ}\text{C}$ for oral, axillary or tympanic route, or $\geq 38.0^{\circ}\text{C}$ 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 evaluable in the ATP analysis. See Section 9.4 for cohorts to be analysed.

- Any investigational or non-registered product (drug or vaccine) other than the study vaccine used during the study period.
- All concomitant medications/products, except vitamins and dietary supplements, administered during the period starting 30 days before and following the dose of study vaccine.
- Any concomitant vaccination administered in the period starting 30 days before the dose of study vaccine and ending at the last study visit.
- Immunosuppressants or other immune-modifying drugs administered chronically (i.e. more than 14 days) during the study period. For corticosteroids, this will mean prednisone ≥ 20 mg/day or equivalent. Inhaled and topical steroids are allowed.
- Immunoglobulins and/or any blood products administered from 3 months before the dose of study vaccine and during the study period.
- Long-acting immune-modifying drugs administered at any time during the study period (e.g. infliximab).

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 who are anti-HBc positive.
- Occurrence of a condition that has the capability of altering the subject's immune response.
- Any confirmed or suspected immunosuppressive or immunodeficiency condition based on medical history and physical examination (no laboratory tests required).

7. 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 will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

7.1. Safety definitions

7.1.1. 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 7.1.3. All other AEs will be recorded as UNSOLICITED AEs.

Examples of an AE DO NOT include:

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

7.1.2. 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, OR

Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, 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.

7.1.3. **Solicited adverse events**

7.1.3.1. **Solicited local (injection-site) adverse events**

The following local (injection-site) AEs will be solicited ([Table 13](#)):

Table 13 Solicited local adverse events

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

7.1.3.2. **Solicited general adverse events**

The following general AEs will be solicited ([Table 14](#)):

Table 14 Solicited general adverse events

Fatigue
Fever
Gastrointestinal symptoms [†]
Headache

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

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

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

In absence of diagnosis, abnormal laboratory findings (e.g. clinical chemistry, haematology, urinalysis) or other abnormal assessments (e.g. vital signs, etc.) that are judged by the investigator to be clinically significant will be recorded as AE or SAE if they meet the definition of an AE or SAE (refer to Sections [7.1.1](#) and [7.1.2](#)). Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs.

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

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

7.2.1. 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 7.4.1 and 7.4.3:

- 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 recognised 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 7.4.3. 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.

7.3. Detecting and recording adverse events, serious adverse events and pregnancies**7.3.1. Time period for detecting and recording adverse events, serious adverse events and pregnancies**

All AEs following the 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 [7.4](#) 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.

SAEs that are related to the investigational vaccine will be collected and recorded from the time of the receipt of study vaccine until the subject is discharged from the study.

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 [7.4](#) for instructions on reporting of pregnancies.

An overview of the protocol-required reporting periods for AEs, SAEs, and pregnancies is given in [Table 15](#).

Table 15 Reporting periods for collecting safety information

Event	ICF sign off*	Visit 1 Vaccination	4 d (Days 0-3) post-vaccination	Visit 2	Visit 3 31 d (Days 0-30) post-vaccination Study Conclusion
		D0		D7	D30
Solicited local and general AEs					
Unsolicited AEs					
AEs/SAEs leading to withdrawal from the study					
SAEs related to the investigational vaccine					
SAEs related to study participation or concurrent GSK medication/vaccine					
Pregnancies					

* i.e. consent obtained. D: Day.

7.3.2. 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 15](#). 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.

7.3.3. Evaluation of adverse events and serious adverse events**7.3.3.1. Active questioning to detect adverse events and serious adverse events**

As a consistent method of collecting AEs, the subject should be asked a non-leading question such as:

‘Have you felt different in any way since receiving the vaccine or since the previous 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.

7.3.3.2. Assessment of adverse events**7.3.3.2.1. Assessment of intensity**

The intensity of the following solicited AEs will be assessed as described (refer [Table 16](#)):

Table 16 Intensity scales for solicited symptoms in adults (40-60 years)

Adults (40-60 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 $\geq 37.5^{\circ}\text{C}$ for oral, axillary or tympanic route, or $\geq 38.0^{\circ}\text{C}$ for rectal route. The preferred route for recording temperature in this study will be axillary or oral.

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

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

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 (for example, prevent attendance at work 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 7.1.2.

7.3.3.2.2. *Assessment of causality*

The definitions for ‘NO’ and ‘YES’ have been written in such a way that all events that have been attributed a ‘NO’ can be pooled with events which in the primary vaccination study were determined to be ‘not related’ or ‘unlikely to be related’ to vaccination. Those events that are attributed a ‘YES’ can be pooled with those events that in the past were determined to have a ‘suspected’ or ‘probable’ relationship to vaccination in the primary vaccination study.

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 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/products, 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 7.1.2), 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).

7.3.3.3. 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).

7.3.3.4. Medically attended visits

For each solicited and unsolicited symptom the subject experiences, the subject will be asked if he/she 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.

7.4. Reporting of serious adverse events, pregnancies, and other events

7.4.1. Prompt reporting of serious adverse events, pregnancies, and other events to GSK Biologicals

SAEs that occur in the time period defined in Section 7.3 will be reported promptly to GSK within the timeframes described in [Table 17](#), once the investigator determines that the event meets the protocol definition of a SAE.

Pregnancies that occur in the time period defined in Section 7.3 will be reported promptly to GSK within the timeframes described in [Table 17](#), once the investigator becomes aware of the pregnancy.

The timeframes for submitting serious adverse event, pregnancy and other event reports to GSK Biologicals are presented in [Table 17](#).

Table 17 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.

7.4.2. 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 Outside US & Canada sites: Fax: PPD [REDACTED] or PPD [REDACTED] Email address: PPD [REDACTED]	

7.4.3. 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.

7.4.3.1. 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.

7.4.4. 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.

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

When additional SAE and 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 17](#).

7.4.6. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to GSK in accordance with the procedures detailed in Section [7.4.1](#). 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.

7.5. Follow-up of adverse events, serious adverse events, and pregnancies

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

7.5.1.1. 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 17](#)).

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 vaccination.

7.5.1.2. 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.

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

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

7.5.2. 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 using 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.

7.6. 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).

7.7. Subject card

Study subjects 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. 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 must be instructed to keep subject cards in their possession for the duration of the study.

8. SUBJECT COMPLETION AND WITHDRAWAL

8.1. Subject completion

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

8.2. Subject withdrawal

Withdrawals will not be replaced.

8.2.1. Subject withdrawal from the study

From an analysis 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 analysis.

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

Investigators will attempt to contact those subjects who do not return for scheduled visits or follow-up. To re-establish contact, the investigator will make 3 documented attempts (3 phone calls) followed by a certified letter to the last known address.

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 himself/herself, 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 he/she has withdrawn consent, the investigator will document the reason for withdrawal of consent, if specified by the subject, 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 7.5.1.2).

9. STATISTICAL METHODS

9.1. Primary endpoint

- Persistence of immunity to hepatitis B in adult subjects vaccinated with three or four doses of *Engerix-B* 20 to 30 years ago.
 - Percentage of adult subjects with an anamnestic response 7 days and 30 days after the challenge dose.

Refer to Section 9.5 for the definition of anamnestic response.

9.2. Secondary endpoints

- Persistence of immunity to hepatitis B in adult subjects vaccinated with three or four doses of *Engerix-B* 20 to 30 years ago.
 - Percentage of adult subjects with anti-HBs antibody concentrations ≥ 6.2 mIU/ml, ≥ 10 mIU/ml and ≥ 100 mIU/ml, at the pre-challenge dose time-point and Day 7 and Day 30 post-challenge dose time-points.
 - Anti-HBs antibody concentrations, at the pre-challenge dose time-point and Day 7 and Day 30 post-challenge dose time-points.
 - Hepatitis B specific memory B cell-mediated immune responses (frequency of HBs-specific memory B cells) at the pre-challenge dose time-point and Day 7 and Day 30 post-challenge dose time-points.
 - Hepatitis B specific T cell-mediated immune responses (frequency of HBs-specific CD4 T-lymphocytes) at the pre-challenge dose time-point and Day 7 and Day 30 post-challenge dose time-points.
- Solicited local and general symptoms.
 - Occurrence of solicited local symptoms during the 4-day (Days 0-3) follow-up period after the challenge dose.
 - Occurrence of solicited general symptoms during the 4-day (Days 0-3) follow-up period after the challenge dose.

- Unsolicited adverse events.
 - Occurrence of unsolicited symptoms during the 31-day (Days 0-30) follow-up period after the challenge dose.
- Serious adverse events.
 - Occurrence of SAEs after the challenge dose, up to the study end.

9.3. Determination of sample size

The primary objective of the study is to assess the anamnestic response to a challenge dose of *Engerix-B* in adults subjects vaccinated 20 to 30 years ago with 3 or 4 doses of *Engerix-B*. Since the objectives of the study are descriptive, the sample size for this study was not estimated using any power based computations. Considering at least 20% of enrolled subjects would be non-evaluable at the time of the analysis (e.g. drop-outs, non-compliance with protocol, etc.), the approximate number of subjects to be enrolled in the study is 100.

The precision achieved with a sample size of 80 evaluable subjects (after accounting for 20% drop-outs from 100 enrolled subjects), for anamnestic response to the challenge dose of *Engerix-B* in terms of exact 95% confidence interval (CI) is presented in [Table 18](#).

Table 18 Exact two-sided 95% CI for a sample size of 80 evaluable subjects according to the anamnestic response to the challenge dose of *Engerix-B*

No. of subjects to be enrolled	No. of evaluable subjects to be expected (N) considering 20% drop out rate	Subjects who mounted an anamnestic response as defined in primary endpoint			
		n	%	Exact 95% CI	
				LL	UL
100	80	72	90.0	81.2	95.6
		72	91.0	82.4	96.3
		73	92.0	83.6	97.0
		74	93.0	85.0	97.5
		75	94.0	86.2	98.2
		76	95.0	87.7	98.6
		76	96.0	88.9	99.2
		77	97.0	90.2	99.6
		78	98.0	91.9	99.9
		79	99.0	93.2	100.0
		80	100.0	95.5	100.0

n = Number of subjects who mounted an anamnestic response.

% = (n/N)*100; LL = Lower limit; UL = Upper limit

Reference data: Please refer [Table 19](#).

The reference values for the sample size computation mentioned above have been presented in [Table 19](#).

Table 19 Reference values

Study	Country	Population (Age in Years)	N	N ATP	Anamnestic response % (CI)
108984 (HBV-313)	Thailand	19-21	76	72	95.8 (88.3,99.1)
110071 (HBV-316 BST:115)	Thailand	19-20	29	29	96.6 (82.2,99.9)
110073 (HBV-317 BST: 143) (CLIA)	Thailand	19-20	49	43	92.1 (78.6,98.3)
110073 (HBV-317 BST: 143) (ELISA)	Thailand	19-20	49	43	95.3 (84.2,99.4)

N = number of subjects included in the Total vaccinated cohort

N ATP=number of subjects included in the ATP cohort for immunogenicity

Response defined as:

For initially seronegative subjects, antibody concentration ≥ 10 mIU/ml one month after the post-challenge dose

For initially seropositive subjects: antibody concentration at one month after the post-challenge dose ≥ 4 fold the pre-vaccination antibody concentration.

9.4. Cohorts for Analyses

The two cohorts defined for the purpose of analysis are:

- Total vaccinated cohort,
- ATP cohort for analysis of immunogenicity.

9.4.1. Total vaccinated cohort

The TVC will include all subjects who received the challenge dose.

9.4.2. According-to-protocol cohort for analysis of immunogenicity

The ATP cohort for analysis of immunogenicity will include all evaluable subjects (i.e. those meeting all eligibility criteria, complying with the procedures defined in the protocol, with no elimination criteria during the study) who have received the challenge dose and for whom data concerning immunogenicity endpoint measures at pre-challenge (Visit 1) and one month post-challenge (V3) are available. The interval between Visit 1 and Visit 3, considered for inclusion of a subject in the ATP cohort for analysis of immunogenicity will be 21-48 days.

9.5. Derived and transformed data

- Seronegative subjects:
 - Subjects with anti-HBs antibody concentration < 6.2 mIU/ml
- Seropositive subjects:
 - Subjects with anti-HBs antibody concentration ≥ 6.2 mIU/ml
- Seroprotected subjects:
 - Subjects with anti-HBs antibody concentration ≥ 10 mIU/ml

- Anamnestic response to the challenge dose is defined as:
 - At least (i.e. \geq) 4-fold rise in one month post-vaccination anti-HBs antibody concentrations in previously seropositive subjects.
 - In previously seronegative subjects, anti-HBs antibody concentrations ≥ 10 mIU/ml at one month post-challenge dose time-point.
- Amplitude of anamnestic response is the change in fold value from pre-challenge dose time-point to one month post-challenge dose.
- GMC calculations are performed by taking the anti-log of the mean of the \log_{10} concentration transformations. GMC will be calculated on all subjects. Antibody concentrations below the cut-off of the assay will be given an arbitrary value of half the cut-off for the purpose of GMC calculation.
- 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 analysis of solicited symptoms within 4 days (Days 0-3) post-challenge dose, missing or non-evaluable measurements will not be replaced. Therefore, the analysis of the solicited symptoms based on the TVC will include only subjects with documented safety data (i.e. symptom screen completed).

More specifically, the following rules will be used:

- Subjects who documented the absence of a solicited symptom after challenge dose will be considered not having that symptom after that dose.
- Subjects who documented the presence of a solicited symptom and fully or partially recorded daily measurement over the solicited period will be included in the summaries at the challenge dose and classified according to their maximum observed daily recording over the solicited period.
- Subjects who documented the presence of a solicited symptom after the challenge dose without having recorded any daily measurement will be assigned to the lowest intensity category at that dose (i.e. 37.5°C for fever or grade 1 for other symptoms).
- Doses without symptom sheets documented will be excluded.
- For the analysis 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.

9.6. Analysis of demographics

- The demographic characteristics (age in years at challenge dose, gender, geographic ancestry, height, weight and BMI) at Visit 1, cohort description and withdrawal status will be summarised using descriptive statistics. The same analysis will be performed stratified by age. The age stratification will be 40-50 and 51-60 years.

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

9.7. Analysis of immunogenicity

The primary analysis on the response to the challenge dose will be performed on the ATP cohort for analysis of immunogenicity. If the percentage of subjects excluded from the ATP cohort for immunogenicity is more than 5%, a second analysis based on the TVC will be performed to complement the ATP analysis of immunogenicity. The immunogenicity analysis based on the TVC will include subjects for whom immunogenicity data are available. All the immunogenicity analysis will be performed as a whole group and by age stratification. The age stratification will be 40-50 years and 51-60 years.

The following analyses will be performed:

- The percentage of subjects with anti-HBs antibody concentrations ≥ 6.2 mIU/ml, ≥ 10 mIU/ml, ≥ 100 mIU/ml, ≥ 1000 mIU/ml with exact 95% CIs will be calculated at the pre-challenge dose time-point, Day 7 and Day 30 post-challenge dose time-points.
- The percentage of subjects who mount an anamnestic response to the challenge dose, one month after vaccination, will be tabulated with exact 95% CI as a whole and according to their seroprotection status at pre-challenge time-point (anti-HBs antibody concentrations \leq or ≥ 10 mIU/ml).
- GMCs with 95% CI will be calculated for anti-HBs antibodies at the pre-challenge dose time-point, Day 7 and Day 30 post-challenge dose time-points.
- The distribution of anti-HBs antibody concentrations will be displayed using reverse cumulative distribution curve (RCC) at the pre-challenge dose time-point and Day 30 post-challenge dose time-point.
- Relationship between pre-challenge dose time-point and Day 30 post-challenge dose time-point results will be presented by regression line graph.
- CMI responses in terms of frequency of HBs-specific CD4 $^{+}$ T-lymphocytes and frequency of HBs-specific memory B cells at Day 0, Day 7 and Day 30 (for subjects who received a challenge dose) will be evaluated.
- The percentage of subjects with anti-HBs concentrations ≥ 6.2 mIU/ml, ≥ 10 mIU/ml and ≥ 100 mIU/ml (with 95% CI) at the Day 7 and Day 30 post-challenge dose time-points will be tabulated in relation to their pre-challenge dose status (overall, < 10 mIU/ml and ≥ 10 mIU/ml).
- The percentage of subjects (with 95% CI) who mount an anamnestic response will be calculated and in relation to their pre-challenge dose status (< 10 mIU/ml and ≥ 10 mIU/ml).

9.8. Exploratory Analysis:

- Correlation between the anti-HBs specific T and memory B cells (frequency of cytokine-positive CD4⁺ or T-lymphocytes and frequency of memory B cells) with the amplitude of anamnestic response one month after the challenge dose will be analysed by Pearson's correlation coefficient.
- Further logistic regression modelling will be used to assess the impact of prognosis factors like age, gender, geographic ancestry, BMI and pre-vaccination status (seroprotected or not) on the seroprotection rate after the challenge dose. Actual age and BMI at the time of screening will be considered in the model.

9.9. Analysis of safety

The primary analysis will be performed on the TVC.

- The percentage of subjects who reported 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 (Days 0-3) follow-up period after the vaccination will be tabulated with exact 95% CI. The same calculations will be performed for any Grade 3 (solicited or unsolicited) symptoms and any symptoms requiring medical attention.
- The percentage of subjects reporting each individual solicited symptom during the 4-day (Days 0-3) follow-up period with exact 95% CI, by type of AE; 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 reporting at least one report of unsolicited AE classified by the Medical Dictionary for Regulatory Activities (MedDRA) and reported within the 31-day (Days 0-30) follow-up period after vaccination will be tabulated with exact 95% CI. The same tabulation will be performed for Grade 3 unsolicited AEs and for unsolicited AEs with a causal relationship to vaccination.
- SAEs during the entire study period and withdrawals due to AEs and SAEs reported during the 31-day follow-up period after the challenge dose will be described in detail.

9.10. Interpretation of analyses

The analyses will be detailed in a descriptive manner.

9.11. 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.

9.11.1. Sequence of analyses

The demographic, immunogenicity and safety analyses of the antibodies and the challenge dose will be performed at the end of the study when all data are available. These analyses will be the basis of a Clinical Study Report and will be made available to the investigator.

9.11.2. Statistical considerations for interim analyses

Interim analyses are not planned for this study.

10. ADMINISTRATIVE MATTERS

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

10.1. Electronic Case Report Form instructions

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

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

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

10.2. 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.

10.3. 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.

10.4. 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.

10.5. 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 12 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.

10.6. Provision of study results to investigators

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

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

11. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

12. REFERENCES

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APPENDIX A CLINICAL LABORATORIES**Table 20 GSK Biologicals' laboratories**

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

Table 21 Outsourced laboratories

Laboratory	Address
CEVAC - University of Gent	De Pintelaan, 185 Gent Belgium

CONFIDENTIAL

116811 (HBV-322)
Protocol Final Version 1

Protocol Sponsor Signatory Approval

**eTrack study number and
Abbreviated Title**

EudraCT number 2015-004099-31

Date of protocol Final Version 1: 02 February 2016

Detailed Title A phase IV, open-label, non-randomised, multicentre study to assess the long-term persistence of immunity to hepatitis B in adults vaccinated 20 to 30 years ago with 3 or 4 doses of GSK Biologicals' hepatitis B vaccine, EngerixTM-B.

Sponsor signatory Dominique Descamps,
Vice President, Head of Clinical Research and
Development
GlaxoSmithKline Vaccines

Signature

PPD  

Date

10 MAR 2016

For internal use only

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