GlaxoSmithKline	**Statistical Analysis Plan**
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
eTrack study number and Abbreviated Title	116811 (HBV-322)
Scope:	All data pertaining to the above study.
Date of Statistical Analysis Plan	Final: 12-OCT-2017
Co-ordinating author:	PPD Statistician) PPD (Stat Analyst)
Reviewed by:	PPD (Clinical and Epidemiology Project Lead) PPD (CRDL) PPD (lead Stat) PPD (Lead Stat Analyst) PPD (Lead Scientific writer) PPD (Regulatory Affair) PPD (SERM physician) PPD (Public disclosure representative)
Approved by:	PPD (Clinical and Epidemiology Project Lead) PPD (Lead statistician) PPD (Lead statistical analyst) PPD (Lead Scientific writer)

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

TABLE OF CONTENTS

PAGE

LIST OF FIGURES

PAGE

LIST OF TEMPLATES

LIST OF ABBREVIATIONS

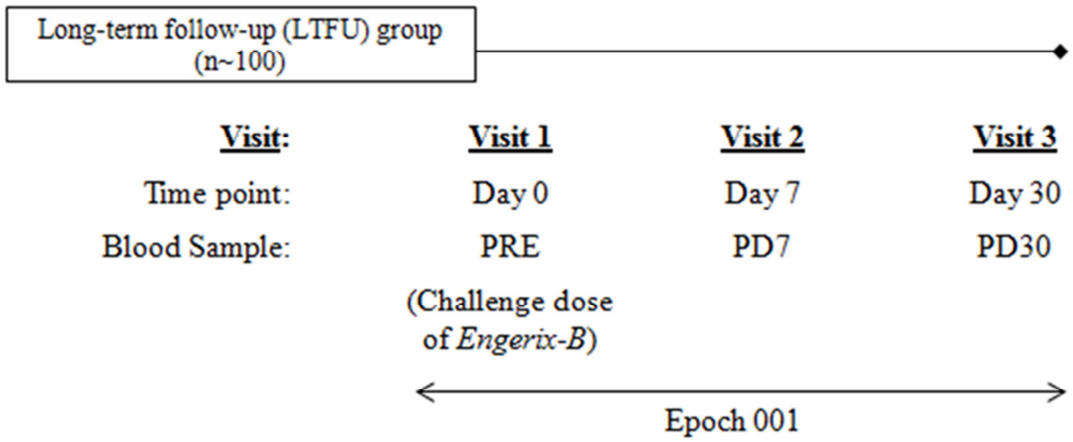
AE	Adverse event
ATP	According to Protocol
CI	Confidence Interval
CRF	Case Report Form
CSR	Clinical Study Report
CTRS	Clinical Trial Registry Summary
EL.U/ml	ELISA unit per milliliter
Eli Type	Internal GSK database code for type of elimination code
ELISA	Enzyme-linked immunosorbent assay
ES	Exposed Set
GMC	Geometric mean antibody concentration
GMT	Geometric mean antibody titer
GSK	GlaxoSmithKline
IU/ml	International units per milliliter
LL	Lower Limit of the confidence interval
MedDRA	Medical Dictionary for Regulatory Activities
N.A.	Not Applicable
PD	Protocol Deviation
PPS	Per Protocol Set
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SBIR	GSK Biological's Internet Randomization System
SD	Standard Deviation
SR	Study Report

TFL	Tables Figures and Listings
TOC	Table of Content
TVC	Total Vaccinated Cohort
UL	Upper Limit of the confidence interval

1. DOCUMENT HISTORY

Date	Description	Protocol Version
12-OCT-2017	first version	Final - 02-FEB-2016

2. STUDY DESIGN



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

- 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.

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

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.
- 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).

The following group names will be used for the statistical analyses:

Group order in tables	Group label in tables	Group definition for footnote
1	HBV	HBV : *Engerix-B* challenge dose

Sub-group order in tables	Sub-group label in tables	Sub-group definition for footnote
1	40-50 Y	40-50 years old subjects
2	51-60 Y	51-60 years old subjects

3. 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)

4. 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 ≥ 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.

5. ANALYSIS SETS

5.1. Definition

Two cohorts are defined for the purpose of analysis

- Total Vaccinated Cohort(TVC) will include all subjects who received the challenge dose
- ATP cohort for analysis of immunogenicity (ATP 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

5.2. Criteria for eliminating data from Analysis Sets

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

5.2.1. Elimination from Total Vaccinated Cohort

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

5.2.2. Elimination from ATP cohort for analyses of Immunogenicity

5.2.2.1. Excluded subjects

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

<table>
<tr><th>Code</th><th>Condition under which the code is used</th></tr>
<tr><td>1030</td><td>Study vaccine not administered at all</td></tr>
<tr><td>1040</td><td>Administration of concomitant vaccine(s) forbidden in the protocol (see also eligibility criteria)</td></tr>
<tr><td>1070</td><td>Administration not according to protocol for reason specified by the investigator, other than side, site and route.</td></tr>
<tr><td>1080</td><td>Vaccine has been administered (effective treatment number) despite a temperature deviation qualified by Status QA GMP NON Use.</td></tr>
<tr><td>1090</td><td>Vaccine has been administered (effective treatment number) out of the expiration date at the time of administration.</td></tr>
<tr><td>2010</td><td>Protocol violation linked to the inclusion/exclusion criteria including age and excluding codes mentioned below.
<table>
<tr><td>DOB</td><td>VAC_1</td><td>40-60 (years)</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>Code</th><th>Condition under which the code is used</th></tr>
<tr><td>2040</td><td>Administration of any medication forbidden by the protocol.</td></tr>
<tr><td>2050</td><td>Underlying medical condition forbidden by the protocol.</td></tr>
<tr><td>2060</td><td>Concomitant infection related to the vaccine which may influence immune response</td></tr>
<tr><td>2070</td><td>Concomitant infection not related to the vaccine which may influence immune response (e.g. Hepatitis infection in a lyme study)</td></tr>
<tr><td>2090</td><td>Blood sample taken but: noncompliance with blood sampling schedules (dates of BS not corresponding to adapted protocol intervals or unknown BS/vaccination dates)
<table>
<tr><td>VAC_1</td><td>SER_2</td><td>5-12(days)</td></tr>
<tr><td>VAC_1</td><td>SER_3</td><td>21-48 (days)</td></tr>
</table></td></tr>
<tr><td>2100</td><td>Serological results not available for antigens POST vaccination (including lost samples, blood sample not done, unable to test, and absence of parallelism).
Please specify the applicable rule:
☒ elimination code if ALL are missing
☐ elimination code if at least ONE is missing
<table>
<tr><th>Schedule

Leave blank if applicable for all schedules</th><th>Activity</th><th>Visit</th><th>Antigen</th><th>Lab variant ID</th><th>Seroprotection level

(if available)</th></tr>
<tr><td></td><td>30</td><td>Visit 3</td><td>Anti-HBS Results not available</td><td></td><td></td></tr>
</table></td></tr>
<tr><td>2120</td><td>Obvious incoherence, abnormal serology evolution or error in data (incoherence between CRF and results, wrong sample labelling)</td></tr>
</table>

5.2.2.2. Right censored Data

-NA-

5.2.2.3. Visit-specific censored Data

-NA-

5.3. Important protocol deviation not leading to elimination from per-protocol analysis set

Protocol Deviations were reviewed by the study team as the new process is not applicable for this study.

6. STATISTICAL ANALYSES

Note that standard data derivation rule and stat methods are described in annex 1 and will not be repeated below. All Confidence Interval (CI) will be two-sided 95% CI.

6.1. Demography

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

- 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

6.1.2. Additional considerations

All demography summaries will be generated for the TVC. The summary of age, height, weight, race and sex will also be provided for the ATP cohort. Summary of important protocol deviations not leading to elimination will be provided for the ATP cohort.

6.2. Exposure

6.2.1. Analysis of exposure planned in the protocol

-NA-

6.2.2. Additional considerations

The number of doses administered will be tabulated.

6.3. Immunogenicity

6.3.1. Analysis of immunogenicity planned in the protocol

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 ≤ 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)

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

6.3.2. Additional considerations

-NA-

6.4. Analysis of safety

6.4.1. Analysis of safety planned in the protocol

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.

6.4.2. Additional considerations

6.4.2.1. Combined Solicited and Unsolicited Adverse Events

For clinicaltrial.gov and EudraCT posting purposes, a summary of combined solicited and unsolicited non-serious adverse events will be produced by System Organ Class and preferred terms and according to occurrence of each event.

A summary of subjects with all combined solicited (regardless of their duration) and unsolicited adverse events will be provided. Solicited adverse events will be coded by MedDRA as per the following codes

Solicited symptom	Lower level term code	Corresponding Lower level term decode
PA	10022086	Injection site pain
RE	10022098	Redness at injection site
SW	10053425	Swelling at injection site
FA	10016256	Fatigue
TE	10016558	Fever
GI	10017944	Gastrointestinal disorder
HE	10019211	Headache

7. ANALYSIS INTERPRETATION

All analyses are descriptive.

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

Description	Analysis ID	Disclosure Purpose (CTRS=public posting, SR=study report, internal)	Dry run review needed (Y/N)	*Study Headline Summary (SHS)*requiring expedited communication to upper management (Yes/No)	Reference for TFL
Analysis of epoch 1	E1_01	SR	Yes	Yes	TFL TOC first version - All TFLs

8.2. Statistical considerations for interim analyses

No interim analysis planned for this study

9. CHANGES FROM PLANNED ANALYSES

-NA-

10. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

The TFL TOC provides the list of tables/listings and figures needed for the study report. It also identifies the tables eligible for each analyses and their role (synopsis, in-text, post-text, SHS, CTRS,). Note that all TFL aimed to be included as post-text are noted as post-text even if these are tabulation of individual data such as listing of SAE. The post-text material contain all source material for the study report and accordingly a post-text table may be redundant with an in-text table.

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

Group order in tables	Group label in tables	Group definition for footnote	Pooled Groups label in tables	Pooled definition for footnote
1	HBV Group	HBV *Engerix-B* challenge dose	HBV Group	HBV Group

The following sub-group names will be used in the TFLs

Sub-group order in tables	Sub-group label in tables	Sub-group definition for footnote
1	40-50 Y	40-50 years old subjects
2	51-60 Y	51-60 years old subjects

11. ANNEX 1 STANDARD DATA DERIVATION RULE AND STATISTICAL METHODS

11.1. Statistical Method References

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

11.2. Standard data derivation

11.2.1. Date derivation

- SAS date derived from a character date: in case day is missing, 15 is used. In case day & month are missing, 30June is used.
- Onset day for an event (ae, medication, vaccination...): the onset day is the number of days between the last study vaccination & the onset/start date of the event. This is 0 for an event starting on the same day as a vaccination. See SAS date derived in case the start date of the event is incomplete.

11.2.2. Dose number

- The study dose number is defined in reference to the number of study visits at which vaccination occurred. Specifically in this study, dose 1 refers to all vaccines administered at the first vaccination visit.
- Relative dose: the relative dose for an event (AE, medication, vaccination) is the most recent study dose given before an event. In case the event takes place on the day a study dose is given, the related dose will be that of the study dose, even if the event actually took place before vaccination. For instance, if an adverse event begins on the day of the study vaccination but prior to administration of the vaccine, it will be assigned to this dose.
- The number of doses for a product is the number of time the product was administered to a subject.

11.2.3. Demography

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

11.2.4. Immunogenicity

- 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. ≥) 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.
- The Geometric mean antibody concentrations (GMCs) calculations will be performed by taking the anti-log of the mean of the $\log_{10}$ concentration transformations. All subjects will be considered. Subjects whose antibody concentrations are below the cut-off of the assay will be given an arbitrary value of half the cut-off for the purpose of GMC calculation Note that as per assay specification, results between the assay cut-off of 6.2 mIU/ml and 7.65 mIU/ml (= Lower limit of Quantification) will be quantified as 6.2 mIU/ml.

- For a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, an analysis will exclude subjects with missing or non-evaluable measurements
- All CI computed will be two-sided 95% CI

11.2.5. Safety/Reactogenicity

- For the analyses of solicited symptoms, missing or non-evaluable measurements will not be replaced. Therefore, the analyses of the solicited symptoms based on the TVC will include only subjects with documented safety data (i.e. symptom screen completed).
- For the analyses of unsolicited adverse events/concomitant medication, all vaccinated subjects will be considered and subjects who did not report an event will be considered as subjects without an event.
- The maximum intensity of local injection site redness/swelling will be scored at GSK Biologicals as follows:

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

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

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

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

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

11.2.6. Number of decimals displayed:

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

Display Table	Parameters	Number of decimal digits
Demographic characteristics	Mean, median age	1
Demographic characteristics	SD (age)	1
Immunogenicity	Anti-HBs GMC, including LL & UL of CI	1
All summaries	% of count, including LL & UL of CI	1

12. ANNEX 2: SUMMARY ON ELIMINATION CODES

Refer to Section 5.2.1

13. ANNEX 3: STUDY SPECIFIC MOCK TFL

The following draft study specific mock TFLs will be used.

The data display, title and footnote are for illustration purpose and will be adapted to the study specificity as indicated in the TFL TOC.

These templates were copied from HBV 319(116722) and additional tables required for public disclosure were added. Note that there may be few changes between the study specific SAP mock TFL and the final TFLs. These editorial/minor changes will not lead to a SAP amendment.

Template 1 Minimum and maximum activity dates (Total vaccinated cohort)

Activity number	Activity Description	Minimum date	Maximum date
10	VISIT 1		
20	VISIT 2		
30	VISIT 3		

Template 2 Number of subjects by center (Total Vaccinated Cohort)

	HBV group	
Center	N	%
PPD		
PPD P		
P		
D		
All		

HBV : Engerix-B challenge dose
n = number of subjects included in each group or in total for a given center or for all centers
All = sum of all subjects in each group or in total (sum of all groups)
% = n/All x 100
Center = GSK Biologicals assigned center number

Template 3 Number of subjects vaccinated, completed and withdrawn with reason for withdrawal (Total Vaccinated Cohort)

	HBV group
Number of subjects vaccinated	
Number of subjects completed	
Number of subjects withdrawn	
Reasons for withdrawal:	
Subject died	
Serious Adverse Event	
Non-serious adverse event	
Eligibility criteria not fulfilled (inclusion and exclusion criteria)	
Protocol violation	
Consent withdrawal (not due to an adverse event)	
Migrated/moved from study area	
Lost to follow-up (subjects with incomplete vaccination course)	
Lost to follow-up (subjects with complete vaccination course)	
Sponsor study termination	
Others	

HBV : Engerix-B challenge dose
Vaccinated = number of subjects who were vaccinated in the study
Completed = number of subjects who completed last study visit
Withdrawn = number of subjects who did not come for the last visit

Template 4 Number of subjects enrolled into the study as well as the number of subjects excluded from ATP analyses with reasons for exclusion

Title	HBV Group		
	n	s	%
Total cohort			
Invalid informed consent or fraud data (code 900)			
Study vaccine dose not administered AT ALL but subject number allocated (code 1030)			
Total vaccinated cohort			
Administration of vaccine(s) forbidden in the protocol (code 1040)			
Study vaccine dose not administered according to protocol (code 1070)			
Non compliance with blood sampling schedule (including wrong and unknown dates (code 2090)			
Essential serological data missing (code 2100)			
Obvious incoherence or abnormality or error in data (code 2120)			
ATP cohort for analysis of immunogenicity			

HBV : Engerix-B challenge dose
Note: Subjects may have more than one elimination code assigned
n = number of subjects with the elimination code assigned excluding subjects who have been assigned a lower elimination code number
s = number of subjects with the elimination code assigned
% = percentage of subjects in the considered ATP cohort relative to Total vaccinated cohort
Codes are listed based on a ranking order and actual codes listed in the final will be only those applicable.

Template 5 Deviations from specifications for age and intervals between study visits (Total Vaccinated Cohort)

		Age	VAC_1 – SER: 2	VAC_1 – SER: 2
GROUP		**Protocol**	**Protocol**	**Protocol**
		from 40 to 60 years	from 5 to 12 days	from 21 to 48 days
HBV group	N			
	n			
	%			
	range			

HBV : Engerix-B challenge dose
N = total number of subjects with available results
n/% = number / percentage of subjects with results outside of the interval
range = minimum-maximum for age and intervals
Age = Age computed at challenge dose
VAC: 1= Vaccination at visit 1
SER-2 = Blood sample collected 7 days after vaccination, SER-3 = Blood sample collected 30 days after vaccination.

Template 6 Number of subjects at each visit and list of withdrawn subjects (Total Vaccinated Cohort)

Group	VISIT	N	Withdrawn Subject numbers	Reason for withdrawal
HBV group	VISIT 1			
	VISIT 2			
	VISIT 3			

HBV : Engerix-B challenge dose
N = Number of subjects who completed the visit, Withdrawn = Subjects who did not return after the visit

Template 7 Summary of demographic characteristics (Total vaccinated cohort)

Characteristics	Parameters or Categories	HBV group N = XXX	
		Value or n	%
Age (years) at challenge dose	Mean		
	SD		
	Median		
	Minimum		
	Maximum		
Gender	Female		
	Male		
Geographic Ancestry	White - Caucasian / European Heritage		

HBV : Engerix-B challenge dose
N = total number of subjects
n/% = number / percentage of subjects in a given category
Value = value of the considered parameter
SD = standard deviation

Template 8 Summary of vital signs characteristics (Total Vaccinated Cohort)

		HBV group (N = XXX)
Characteristics	**Parameters**	**Value**
Height (cm)	Mean	
	SD	
	Median	
	Minimum	
	Maximum	
	Unknown	
Weight (kg)	Mean	
	SD	
	Median	
	Minimum	
	Maximum	
	Unknown	
BMI (kg/m²)	Mean	
	SD	
	Median	
	Minimum	
	Maximum	
	Unknown	

HBV : Engerix-B challenge dose
N = total number of subjects
Value = value of the considered parameter
SD = standard deviation
Height (cm) =Height expressed in centimetres
Weight (kg) =Weight expressed in kilograms
BMI (kg/m²) = Body Mass Index in kilograms per meter square

Template 9 Distribution of subjects based on age category (Total vaccinated cohort)

		HBV group N = XXX	
Characteristics	**Categories**	**n**	**%**
Age category	40-50 Years		
	51-60 Years		

HBV : Engerix-B challenge dose
N = number of subjects
n = number of subjects in a given category
% = n / Number of subjects with available results x 100

Template 10 Number of subjects by country (Total Vaccinated cohort)

	HBV Group N = xxx
Country	**n**
xxxxx	
xxxxx	
xxx	

HBV : Engerix-B challenge dose
N = number of subjects
n= number of enrolled subjects included in each group

Template 11 Number of enrolled subjects by age category

		HBV Group N =
Characteristics	**Categories**	**n**
Age category	In utero	
	Preterm newborn infants (gestational age < 37 wks)	
	Newborns (0-27 days)	
	Infants and toddlers (28 days-23 months)	
	Children (2-11 years)	
	Adolescents (12-17 years)	
	Adults (18-64 years)	
	From 65-84 years	
	85 years and over	
	Missing	

HBV : Engerix-B challenge dose
N = Number of enrolled subjects
n= number of enrolled subjects included in each group or in total for a given age category or for all age categories
Missing = <describe missing>

Template 12 Percentage of subjects with anti-HBs antibody concentrations ≥ 6.2 mIU per ml, ≥ 10 mIU per ml, ≥ 100 mIU per ml, ≥ 1000 mIU per ml and GMCs at pre and post challenge dose time points (ATP cohort for immunogenicity)

				≥ 6.2 mIU/ml			≥ 10 mIU/ml				≥ 100 mIU/ml				≥ 1000 mIU/ml				GMC		
						95%CI			95% CI				95% CI				95% CI				95% CI	
Antibody	Group	Timing	N	n	%	LL	UL	n	%	LL	UL	n	%	LL	UL	n	%	LL	UL	Value	LL	UL
anti-HBs antibody	HBV group	PRE																				
		Day 7																				
		Day 30																				

HBV : Engerix-B challenge dose
Seropositive=anti-HBs antibody concentration ≥6.2 mIU/mL
Seroprotection= anti-HBs antibody concentration ≥10 mIU/mL
GMC = geometric mean antibody concentration calculated on all subjects
N = number of subjects with available results
n/% = number/percentage of subjects with concentration equal to or above specified cut-off
95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

Template 13 Percentage of subjects with antibody concentrations ≥ 6.2 mIU/mL, ≥ 10 mIU/mL, ≥ 100mIU/mL and GMCs for anti-HBs antibody concentrations stratified based on the pre-challenge dose status (ATP cohort for analysis of immunogenicity)

				≥ 6.2 mIU/mL				≥ 10 mIU/mL				≥ 100 mIU/mL				GMC		
						95% CI				95% CI				95% CI			95% CI	
Group	Pre-vaccination status	Timing	N	n	%	LL	UL	n	%	LL	UL	n	%	LL	UL	value	LL	UL
HBV Group	< 6.2 mIU/mL	PRE																
		Day 7																
		Day 30																
	≥ 6.2 mIU/mL - <10 mIU/mL	PRE																
		Day 7																
		Day 30																
	≥ 10mIU/mL	PRE																
		Day 7																
		Day 30																
	Overall	PRE																
		Day 7																
		Day 30																

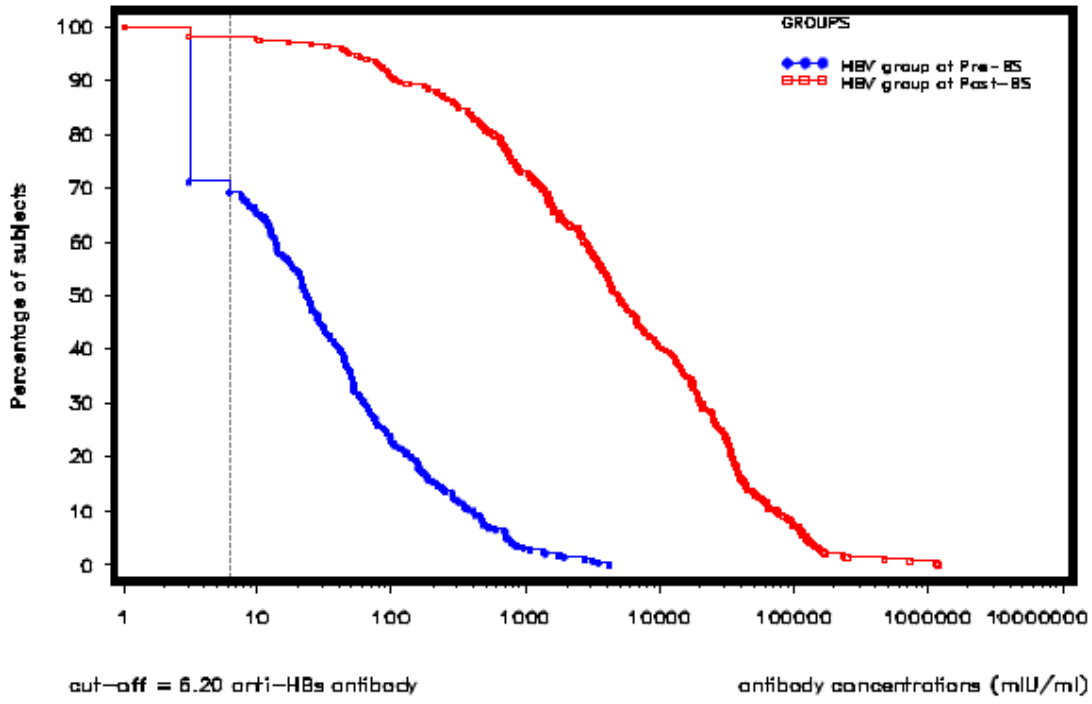
HBV : Engerix-B challenge dose
GMC = geometric mean antibody concentration calculated on all subjects
N = number of subjects with available results
n/% = number/percentage of subjects with concentration within the specified range
95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit
Pre: Blood sampling at pre-challenge dose time point,Day7: Blood sampling at visit2 ,Day30: Blood sampling at visit 3

Template 14 Anamnestic response to the HBV challenge dose stratified based on the last available time point before the challenge dose (ATP cohort for immunogenicity)

			Anamnestic response to the challenge dose			
					95% CI	
Group	**Pre-vaccination status**	**N**	**n**	**%**	**LL**	**UL**
HBV Group	S-					
	S+					
	Total					

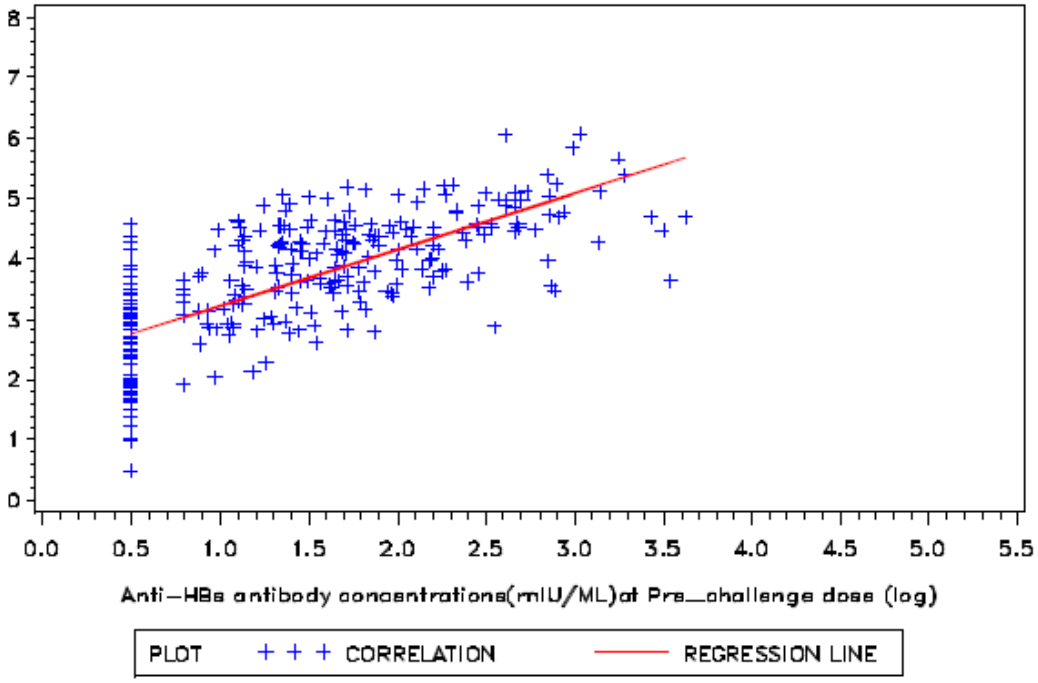
HBV : Engerix-B challenge dose
S- = seronegative subjects (antibody concentration < 6.2mIU/mL for anti-HBV) prior to vaccination
S+ = seropositive subjects (antibody concentration ≥ 6.2mIU/mL for anti-HBV) prior to vaccination
Total = subjects either seropositive or seronegative at pre-vaccination
Challenge Dose response is defined as:
For initially seronegative subjects, antibody concentration greater than or equal to 10mIU/mL (≥10mIU/mL)
For initially seropositive subjects: antibody concentration at least four times the pre-challenge antibody concentration
N = number of subjects with both pre- and post-vaccination results available
n(%) = number(percentage) of responders
95% CI = exact 95% confidence interval; LL = lower limit, UL = upper limit

Figure 1 Reverse cumulative curve of Anti-HBs antibody concentration at the pre-challenge dose and Day 30 post-challenge dose (ATP cohort for immunogenicity)



HBV : Engerix-B challenge dose

Figure 2 **Anti-HBs antibody concentrations post challenge as a function of pre-challenge concentrations, with regression line (ATP cohort for immunogenicity)**



Regression equation and R2 is given by
y=2.2815+0.935x; R2=0.4976
Where,
y=post challenge dose (log)
x=pre challenge dose (log)
R2=proportion of variation in post challenge dose (log) that is predictable from pre challenge dose (log)
50% of the variation in the post challenge dose is predicted from pre-challenge dose results.

Template 15 CD4+ T- lymphocytes response by Cytokine Flow Cytometry (CFC) by overall for HBV02 (ATP cohort for immunogenicity)

Test description	Stimulant	Timing	Parameters or Categories	HBV group Value or n
<each test>	*<each stimulant>*	*<each timing>*	N	
			Missing	
			Minimum	
			Q1	
			Median	
			Q3	
			Maximum	
			Gmean	

HBV : Engerix-B challenge dose
<each test>: Cells CD4.CD40L(+)+Interleukin-2(-)+Tumor Necrosis Factor alpha(-)+Interferon gamma(-)
<each stimulant>: HBV02
<each timing>= PRE, PD7, PD30
PRE = Blood sample collected before vaccination
PD7 = Blood sample collected 7 days after vaccination
PD30 = Blood sample collected 30 days after vaccination.
N = number of subjects
Q1 = 25% percentile; Q3 = 75% percentile
Gmean = Geometric mean
Value = value of the considered parameter

Template 16 B-cell Elispot response to HBV03 by overall (ATP cohort for immunogenicity)

Stimulant	Timing	Parameters or Categories	HBV group Value or n
<each stimulant>	<each timing>	N	
		Missing	
		Minimum	
		Q1	
		Median	
		Q3	
		Maximum	
		Gmean	

HBV : Engerix-B challenge dose
<each stimulant>: HBV03
<each timing>= PRE, PD7, PD30
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.
N = number of subjects
Q1 = 25% percentile; Q3 = 75% percentile
Gmean = Geometric mean (Values of 0 will be given an arbitrary value of 0.5 for the purpose of geometric mean calculation)
Value = value of the considered parameter

Template 17 Anamnestic Response for Anti-HBs antibody concentrations in relation to their pre vaccination status (ATP cohort for immunogenicity)

			Anamnestic response to the challenge dose			
					95% CI	
Group	Pre-vaccination status	N	n	%	LL	UL
HBV Group	< 6.2 mIU/mL					
	≥ 6.2 mIU/mL - <10 mIU/mL					
	≥ 10mIU/mL					
	Total					

HBV : Engerix-B challenge dose
S- = seronegative subjects (antibody concentration < 6.2mIU/mL for anti-HBs) prior to vaccination
S+ = seropositive subjects (antibody concentration ≥ 6.2mIU/mL for anti-HBs) prior to vaccination
Total = subjects either seropositive or seronegative at pre-vaccination
Challenge Dose response defined as:
For initially seronegative subjects, antibody concentration greater than or equal 10mIU/mL (≥10mIU/mL)
For initially seropositive: antibody concentration at least four times the pre-vaccination antibody concentration
N = number of subjects with both pre- and post-vaccination results available
n(%) = number(percentage) of responders
95% CI = exact 95% confidence interval; LL = lower limit, UL = upper limit

Template 18 Percentage of subjects with positive anti-HBc antibody concentrations at baseline (ATP cohort for immunogenicity)

					95%CI		
Antibody	Group	Timing	N	n	%	LL	UL
Positive Anti-HBc antibody	HBV group	Baseline					

HBV : Engerix-B challenge dose
GMC = geometric mean antibody concentration calculated on all subjects
N = number of subjects with available results
n/% = number/percentage of subjects with concentration within the specified range
95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

Template 19 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 by Pearson's correlation coefficient (ATP cohort for immunogenicity)

		95% CI	
Stimulant	Pearson's correlation coefficient	LL	UL

Stimulant: anti-HBs specific T and memory B cells
95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

Template 20 Estimated coefficient of the logistic regression analysis on anti-HBs antibody Seroprotection status (ATP cohort for Immunogenicity)

				95% CI	
Model	**Characteristics**	**P -value**	**Odds Ratio**	**LL**	**UL**
Saturated Model	Treatment Group				
	BMI				
	Gender				
	Age				
	Geographic Ancestry				
	pre-vaccination seroprotected status				
Final model	BMI				
	Age				

Gender was coded in the following order
- Female was coded as 1
-Male was coded as 0
Age (per 10 Years), BMI (per 10 kg/m²), eGFR (mL/min/1.73m²), HbA1c (%) at screening visit
Geographic Ancestry was coded in the following order
- White - Caucasian / European Heritage was coded as 1
- Not White - Caucasian / European Heritage was coded as 0
Odds ratio: for binary co variable this represents the ratio of odds between the category coded 1 over the category code 0. For continuous co variable this represents the ratio of odds associated to a co variable increase by one unit. A value above 1 is associated to an increase in seroprotection.
The p-value for each term tests the null hypothesis that the coefficient is equal to zero (no effect).
Note: Saturated model is without considering stepwise elimination strategy and final model is after consideration of stepwise elimination strategy. P-value below 10% was used as criteria for retaining/adding factors in the final model.

Template 21 Number and percentage of subjects who received the study vaccine dose (Total vaccinated cohort)

	HBV group N = XXX	
Total number of doses received	**N**	**%**
1		

HBV : Engerix-B challenge dose
N = number of subjects included in the HBV cohort
n/% = number/percentage of subjects receiving the specified total number of doses

Template 22 Compliance in returning symptom sheets (Total vaccinated cohort)

Group	**Number of doses**	**Doses NOT according to protocol**	**Number of general SS**	**Compliance % general SS**	**Number of local SS**	**Compliance % local SS**
HBV group						

HBV : Engerix-B challenge dose
SS = Symptom screens/sheets used for the collection of local and general solicited AEs
Compliance % = (number of doses with symptom screen/sheet return / number of administered doses) X 100

Template 23 Incidence and nature of symptoms (solicited and unsolicited) reported during the 4-day (Days 0-3) post-vaccination period (Total vaccinated cohort)

		Any symptom					General symptoms					Local symptoms				
					95% CI					95% CI					95% CI	
	Group	N	N	%	LL	UL	N	n	%	LL	UL	N	n	%	LL	UL
Dose 1	HBV group															

HBV : Engerix-B challenge dose
N= number of subjects with at least one documented dose
n/%= number/percentage of doses followed by at least one type of symptom
95% CI = exact 95% confidence interval, LL = Lower Limit, UL = Upper Limit

Template 24 Incidence of solicited local symptoms reported during the 4-day (Days 0-3) post-vaccination period (Total Vaccinated Cohort)

		HBV Group				
					95 % CI	
Symptom	Type	N	n	%	LL	UL
Pain	All					
	Grade 3					
	Medical advice					
Redness	All					
	> 50 mm					
	Medical advice					
Swelling	All					
	> 50 mm					
	Medical advice					

HBV : Engerix-B challenge dose
N = number of subjects with documented dose
n/% = number/percentage of subjects reporting the symptom at least once
95%CI = Exact 95% confidence interval; LL = lower limit, UL = upper limit

Template 25 Incidence of solicited general symptoms reported during the 4-day (Days 0-3) post-vaccination period (Total Vaccinated Cohort)

		HBV Group				
					95 % CI	
Symptom	**Type**	**N**	**n**	**%**	**LL**	**UL**
Fatigue	All					
	Grade 3					
	Related					
	Medical advice					
Gastrointestinal symptoms	All					
	Grade 3					
	Related					
	Medical advice					
Headache	All					
	Grade 3					
	Related					
	Medical advice					
Temperature/(Oral) (°C)	All					
	≥37.5					
	>38.0					
	>38.5					
	>39.0					
	Related					
	>39.0 Related					
	Medical advice					

HBV : Engerix-B challenge dose
N = number of subjects with documented dose
n/% = number/percentage of subjects reporting the symptom at least once
95%CI = Exact 95% confidence interval; LL = lower limit, UL = upper limit

Template 26 Percentage of subjects reporting the occurrence of unsolicited symptoms classified by MedDRA Primary System Organ Class and Preferred Term within the 31-day (Days 0-30) post-vaccination period (Total Vaccinated Cohort)

		HBV group N =XXX			
				95% CI	
Primary System Organ Class (CODE)	**Preferred Term (CODE)**	**n**	**%**	**LL**	**UL**
At least one symptom					
Blood and lymphatic system disorders (10005329)	Anaemia (10002034)				
	Hypochromic anaemia (10020969)				
	Thrombocytopenia (10043554)				
Immune system disorders (10021428)	Milk allergy (10027633)				
Infections and infestations (10021881)	Bronchiolitis (10006448)				
	Bronchitis (10006451)				
	Gastroenteritis (10017888)				
	Rash (10037844)				

HBV : Engerix-B challenge dose
At least one symptom = at least one symptom experienced (regardless of the MedDRA Preferred Term)
N = number of subjects with at least one administered dose
n/% = number/percentage of subjects reporting the symptom at least once
95% CI= exact 95% confidence interval; LL = Lower Limit, UL = Upper Limit

Template 27 Incidence of concomitant medication during the 4-day (Days 0-3) post-vaccination period (Total vaccinated cohort)

	HBV group				
				95% CI	
	N	n	%	LL	UL
Any					
Any antipyretic					
Prophylactic antipyretic					

HBV : Engerix-B challenge dose
95% CI = exact 95% confidence interval, LL = Lower Limit, UL = Upper Limit

Template 28 Number (%) of subjects reporting serious adverse events during the whole study period including number of events reported (Total vaccinated cohort)

			HBV group N =		
Type of Event	Primary System Organ Class	Preferred Term (CODE)	n*	n	%
SAE	At least one symptom				
	<each SOC>	<each PT term>			
Related SAE	At least one symptom				
	<each SOC>	<each PT term>			
Fatal SAE	At least one symptom				
	<each SOC>	<each PT term>			
Related fatal SAE	At least one symptom				
	<each SOC>	<each PT term>			

HBV : Engerix-B challenge dose
At least one symptom = at least one symptom experienced (regardless of the MedDRA Preferred Term)
N = number of subjects with the administered dose
n* = number of events reported
n/% = number/percentage of subjects reporting the symptom at least once

Template 29 Listing of SAEs (Total Vaccinated Cohort)

Group	Sub.	Case Id	Age at onset (Year)	Sex	Verbatim	Preferred term	System Organ Class	MED type	Dose	Day of onset	Duration	Intensity	Causality	Outcome
HBV group														

HBV : Engerix-B challenge dose
Dose = dose given prior to the serious adverse event
N/Y = No/Yes

Template 30 Listing of dropouts due to AEs, SAEs and solicited symptoms during the study period (Total vaccinated cohort)

Study-Subject No.	Country	Gender	Race	AE Description	SAE	Causality	Outcome	Type of discontinuation

HBV : Engerix-B challenge dose

Template 31 Solicited and Unsolicited symptoms experienced by at least 5 % of subjects classified by MedDRA Primary System Organ Class and Preferred Term within the 31-day (Days 0-30) post-vaccination - SAE excluded (Total Vaccinated cohort)

		HBV Group N = XXX				
					95% CI	
Primary System Organ Class (CODE)	Preferred Term (CODE)	n*	n	%	LL	UL
At least one symptom						
Blood and lymphatic system disorders (10005329)	Leukocytosis (10024378)					
Cardiac disorders (10007541)	Angina unstable (10002388)					
	Mitral valve disease (10061532)					
	Myocardial infarction (10028596)					
Respiratory, thoracic and mediastinal disorders (10038738)	Pleural effusion (10035598)					
	Pneumothorax (10035759)					

HBV : Engerix-B challenge dose
At least one symptom = at least one symptom experienced (regardless of the MedDRA Preferred Term)
N = number of subjects with the administered dose
n* = number of events reported
n/%,= number/percentage of subjects reporting the symptom at least once
95% CI = exact 95% confidence interval; LL = Lower Limit, UL = Upper Limit