



**eTrack study number and
Abbreviated Title**

201630 (EPI-HAV-007 BOD PA)

Study Protocol

Sponsor:

GlaxoSmithKline Biologicals

Rue de l'Institut 89

1330 Rixensart, Belgium

Date of protocol

Final Version 3: 02 October 2015

**Date of protocol
amendment**

Amendment 1 Final: 05 April 2016

Amendment 2 Final: 24 July 2017

Title

Long term hepatitis A virus (HAV) antibody persistence in children vaccinated with 1 dose and those vaccinated with 2 doses of Havrix® in Panama.

Detailed Title

Epidemiological, prospective, interventional, multi-centre, long term HAV antibody persistence in children vaccinated with 1 dose and those vaccinated with 2 doses of Havrix® in Panama.

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(Amended: 24 July 2017)**

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Epidemiological, prospective, interventional, multi-centre, long term HAV antibody persistence in children vaccinated with 1 dose and those vaccinated with 2 doses of Havrix® in Panama.

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GSK Biologicals' protocol template for observational studies and interventional studies without administration of medicinal products as described in a research protocol based on the Protocol Document Standard version 14.1.2

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Protocol Amendment 2 Sponsor Signatory Approval

eTrack study number and Abbreviated Title 201630 (EPI-HAV-007 BOD PA)

Date of protocol amendment Amendment 2 Final: 24 July 2017

Detailed Title Epidemiological, prospective, interventional, multi-centre, long term HAV antibody persistence in children vaccinated with 1 dose and those vaccinated with 2 doses of Havrix® in Panama.

Sponsor signatory Frank Struyf,
Clinical and Epidemiology Project Leader
Hepatitis/HPV

Signature _____

Date _____

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201630 (EPI-HAV-007 BOD PA)
Protocol Amendment 2 Final

Protocol Amendment 2 Sponsor Signatory Approval

eTrack study number and Abbreviated Title 201630 (EPI-HAV-007 BOD PA)

Date of protocol amendment Amendment 2 Final: 24 July 2017

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Clinical and Epidemiology Project Leader
Hepatitis/HPV

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05 SEP 2017

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Protocol Amendment 2 Rationale

Amendment number:	Amendment 2
Rationale/background for changes:	
<ul style="list-style-type: none">• The date of birth requirement has been eliminated from the study as it is not necessary for the design or the objectives of the study and will ease the recruitment constraint observed in the study.• The post last dose interval will be updated due to low number of eligible subjects identified within the existing intervals which puts at risk achieving adequate sample size.<ul style="list-style-type: none">– For the Year 8 survey: The new post last dose time interval may include children with ≥ 7 years and < 10 years between last dose.– For the Year 10 survey: The new post last dose time interval may include children with ≥ 10 years and < 13 years between last dose.– This post last dose time criteria has been added to inclusion criteria section for clarification.	

Protocol Amendment 2 Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any mutually agreed future protocol amendments or protocol administrative changes, with the terms of the study 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) or other applicable guidelines such as International Society for Pharmacoepidemiology (ISPE) guidelines for Good Pharmacoepidemiology Practices (GPP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about 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 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 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, 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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201630 (EPI-HAV-007 BOD PA)

Protocol Amendment 2 Final

**eTrack study number and
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201630 (EPI-HAV-007 BOD PA)

Date of protocol amendment Amendment 2 Final: 24 July 2017

Detailed Title

Epidemiological, prospective, interventional, multi-centre, long term HAV antibody persistence in children vaccinated with 1 dose and those vaccinated with 2 doses of Havrix® in Panama.

Investigator name _____

Signature _____

Date _____

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Sponsor Information

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Rue de l'Institut 89
1330 Rixensart, Belgium

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Refer to the local study contact information document.

Sponsor Study Monitor

Refer to the local study contact information document.

Study Contact for Reporting of a Serious Adverse Event (SAE)

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to protocol Section [7.3.2](#).

SYNOPSIS

Detailed Title Epidemiological, prospective, interventional, multi-centre, long term HAV antibody persistence in children vaccinated with 1 dose and those vaccinated with 2 doses of Havrix® in Panama.

Objectives **Primary**

- To assess the persistence of anti-hepatitis A virus (HAV) antibodies, approximately 8 years and 10 years post vaccination with the last received vaccine dose of the complete series of *Havrix* (2 doses) and the partial series completion (1 dose) in Panama.

Secondary

- To assess geometric mean concentration (GMC) of anti-HAV antibodies, approximately 8 years and 10 years post vaccination with the last received dose of the complete series of *Havrix* (2 doses) and the partial series completion (1 dose) in Panama.
- To explore the non-inferiority of the 1-dose schedule of *Havrix* when compared to the 2-dose schedule in terms of the percentage of subjects with anti-HAV antibody concentrations ≥ 15 mIU/mL, approximately 8 years and 10 years after the last received vaccination dose.

Criteria for evaluation: The lower limit of the 2-sided 95% CI for the difference (1-dose group minus the 2-dose group) of percentage of subjects with anti-HAV antibody concentrations ≥ 15 mIU/mL is greater than or equal to the pre-defined clinical non-inferiority limit of -10%.

Rationale for the study Due to limited financial resources, most countries in Latin America (LatAm) have not included *Havrix* as part of the Universal Mass Vaccination (UMV) program. A possible solution to this problem is to offer a one dose series which would be more economical and logically feasible in these resource limited countries. Many countries within LatAm have shown interest in this possible new vaccination schedule but evidence is limited on the long term protection of 1-dose schedule of *Havrix* compared to the demonstrated long term protection of the recommended 2-dose schedule, especially in the paediatric population.

In LatAm, Argentina was the first country that introduced an off label schedule for *Havrix*. In Argentina, there was a nationwide outbreak of hepatitis and the rate of infection

increased from 55.3 cases per 100,000 inhabitants in 2002 to 107.5 and 113.3 cases per 100,000 inhabitants in 2003 and 2004, respectively. Additionally, hepatitis A virus (HAV) was the leading cause of fulminant hepatic failure (FHF) and liver transplantation in children. To reduce the health burden associated with HAV disease, the Ministry of Health decided to implement a mandatory one-dose vaccination strategy into the Argentinean regular immunisation schedule for all children aged 12 months in June 2005.

Recent published data show that since 2006, the mean Argentinean hepatitis A incidence rate has dropped to 7.9 per 100,000 inhabitants in the post-vaccination period (2006-2011), which represents an 88.1% reduction compared to the pre-vaccination period (2000-2002), with mean incidence rate of 66.5 per 100,000 inhabitants. There was a striking decrease in HAV-related FHF and resulting liver failure, with no new HAV-related FHF/liver failure cases recorded after end of 2006 / March 2007.

Universal hepatitis A vaccination was introduced in Panama in April 2007. Two doses of the vaccine were given to children at 12 and 18 months of age. The Expanded Program of Immunisation (EPI) of Panama had reportedly provided 53,417 and 7,104 doses of *Havrix* in Panama (first and second dose) during 2007 for an estimated coverage of 81% of the population receiving at least one dose of *Havrix* and 11% receiving both doses (complete series). In 2008, vaccination coverage rose as 99% of the eligible population received at least one dose of *Havrix* and 65% received the complete vaccination series (two doses).

Panama is an ideal setting to conduct the present study since it consists of an adequate size cohort of children who had received both doses of *Havrix* as well as children who had received one dose of *Havrix*. In addition, this population provides an adequate time interval to measure long term antibody persistence since the children were vaccinated approximately 8 years ago.

The present study will be conducted to evaluate the persistence of hepatitis A antibodies, approximately 8 years and 10 years post vaccination with the complete series of *Havrix* (2 doses) and the partial series completion (1 dose).

**Study design
(Amended: 24 July
2017)**

- Type of design: Epidemiological, serial, cross-sectional, interventional, multi-centre study in Panama.
- Study population comprises of children who had received

Havrix at selected health centres of Panama.

- Eligible children affiliated with the study sites will be enrolled in the study.
- Biological samples: A blood sample (~5mL) will be collected from all subjects at each cross-sectional survey (Year 8 and Year 10).
- Type of study: self-contained.
- Data collection: Electronic Case Report Form (eCRF).
- Duration of the study: This study involves only one study visit per subject. However, since the study comprises of two independent cross-sectional surveys (Year 8 and Year 10), the same subject who participated in the Year 8 cross-sectional survey may be randomly enrolled for the Year 10 cross-sectional survey. In that case, the subject will be enrolled as a new subject and will not be associated to his/her participation in the Year 8 cross-sectional survey. The overall duration of the study is estimated to be approximately 2 years.
- Epochs foreseen in the study:
 - Epoch 001: Persistence Visit 1 (Year 8)
 - Epoch 002: Persistence Visit 1' (Year 10)
- Definition of the cohorts foreseen in the study:
 - Year 8 cohort: All subjects participating in the Year 8 cross-sectional survey (Visit 1 Epoch 001) - May include children with ≥ 7 years and < 10 years between last Havrix dose and Persistence Visit 1 (Year 8)
 - Year 10 cohort: All subjects participating in the Year 10 cross-sectional survey (Visit 1' Epoch 002) - May include children with ≥ 10 years and < 13 years between last Havrix dose and Persistence Visit 1' (Year 10)

Synopsis Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Epochs	
		Epoch 001	Epoch 002
Yr 8 Havrix_1 dose	~300	x	
Yr 8 Havrix_2 dose	~300	X	
Yr 10 Havrix_1 dose	~300		x
Yr 10 Havrix_2 dose	~300		x

Discussion of study design

A serial, cross-sectional, serological study will allow evaluating the long term persistence of anti-HAV antibodies and GMCs in children who had received 1 dose of *Havrix* vaccine (partial series) and children who had received 2 doses of *Havrix* vaccine (complete series) during the same time point.

The first cross-sectional serousurvey will evaluate the long term persistence of immunity approximately 8 years post vaccine administration and provide preliminary data on the non-inferiority of 1 dose versus 2 doses, in terms of long term persistence. The second cross-sectional study will evaluate long term persistence, approximately 10 years post vaccine administration, since the duration of protection beyond 10 years remains unknown and provide preliminary data on the non-inferiority of 1 dose versus 2 doses, in terms of long term persistence. However, the sampling and testing for Year 8 and Year 10 cross-sectional surveys will be independent of each other.

Persistence of immunity to HAV will be assessed by measuring the persisting, circulating anti-HAV antibodies by Enzyme-Linked Immunosorbent Assay (ELISA). Since only subjects who were previously vaccinated with *Havrix* are being followed up in this long term persistence study, a control group will not be available for comparison. No vaccine will be administered during the study period.

Number of subjects (Amended: 24 July 2017)

Approximately 600 subjects (about 300 subjects vaccinated with one dose of *Havrix* and about 300 subjects vaccinated with two doses of *Havrix*) are planned to be enrolled at each cross-sectional survey time points (Year 8 and Year 10). Two different cohorts of subjects will be enrolled at each epoch, therefore about 600 subjects will be enrolled at Epoch 001 (Year 8 cohort) and another cohort of about 600 subjects will be enrolled at Epoch 002 (Year 10 cohort). In total, approximately 1200 subjects will be enrolled in the study. Subjects in the Year 8 cross-sectional survey may be randomly enrolled for the Year 10 cross-sectional survey. In this case, the subject will not be associated to his / her previous participation and a new subject number will be re-assigned.

Endpoints**Primary**

- Anti-HAV seropositivity status at approximately 8 years and 10 years after administration with the last received dose of *Havrix*.

Secondary

- Anti-HAV concentrations (GMC) at approximately 8 years and 10 years after administration with the last received dose of Havrix
- Anti-HAV seropositivity status at approximately 8 years and 10 years after administration with the last received dose of *Havrix*.

Subjects are defined as being seropositive if their anti-HAV antibody concentration is $\geq 15 \text{ mIU/mL}$.

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LIST OF ABBREVIATIONS

ANOVA:	Analysis of Variance
ATP:	According-To-Protocol
CI:	Confidence Interval
eCRF:	electronic Case Report Form
ELISA:	Enzyme-Linked Immunosorbent Assay
EPI:	Expanded Program of Immunisation
FHF:	Fulminant Hepatic Fever
GCP:	Good Clinical Practice
GMC:	Geometric Mean Concentration
GPP:	Good Pharmacoepidemiology Practices
GSK:	GlaxoSmithKline
HAV:	Hepatitis A Virus
ICF:	Informed Consent Form
ICH:	International Conference on Harmonisation
IEC:	Independent Ethics Committee
IRB:	Institutional Review Board
ISPE:	International Society for Pharmacoepidemiology
LAR:	Legally Acceptable Representative
LatAm:	Latin America
SAE:	Serious Adverse Event
SAP:	Statistical Analysis Plan
SBIR:	GSK Biologicals' Central randomisation system on Internet
SDV:	Source Document Verification
SPM:	Study Procedures Manual
UMV:	Universal Mass Vaccination

GLOSSARY OF TERMS

Child in care:	A child who has been placed under the control or protection of an agency, organisation, institution or entity by the courts, the government or a government body, acting in accordance with powers conferred on them by law or regulation. The definition of a child in care can include a child cared for by foster parents or living in a care home or institution, provided that the arrangement falls within the definition above. The definition of a child in care does not include a child who is adopted or has an appointed legal guardian.
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
Enrolled subject:	Enrolled subjects are subjects that have signed an ICF (valid or not) and have data entered in the database.
Epidemiological study:	An observational or interventional study without administration of medicinal product(s) as described in a research protocol.
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. Typical examples of epochs are retrospective data collection and prospective data collection, etc.
eTrack:	GSK Biologicals' tracking tool for clinical/epidemiological 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 Section 8.3 for details on criteria for evaluability).
Interventional Human Subject Research:	Studies in which participants are administered medical care, medicinal products and/or medical/scientific procedures as described in a research protocol.
Prospective study:	A study in which the subjects/cases are identified and then followed forward in time in order to address one or more study objectives.

Research protocol:	A document that describes the objective(s), design, methodology, statistical considerations, and organisation of a study. The protocol usually also gives the background and rationale for the study, but these could be provided in other protocol referenced documents.
Self-contained study:	Study with objectives not linked to the data of another study.
Study population:	Sample of population of interest.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the epidemiological study or a person about whom some medical information has been recorded in a database.
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.

TRADEMARKS

The following trademark is used in the present protocol.

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

Trademarks of the GlaxoSmithKline group of companies	Generic description
Havrix®	Hepatitis A vaccine

1. INTRODUCTION

1.1. Background

Due to limited financial resources, most countries in Latin America (LatAm) have not included *Havrix* as part of the Universal Mass Vaccination (UMV) program. A possible solution to this problem is to offer a one dose series which would be more economical and logically feasible in these resource limited countries. Many countries within LatAm have shown interest in this possible new vaccination schedule but evidence is limited on the long term protection of 1-dose schedule of *Havrix* compared to the demonstrated long term protection of the recommended 2-dose schedule, especially in the paediatric population [Ott, 2013; WHO, 2011].

In LatAm, Argentina was the first country that introduced an off label schedule for *Havrix*. In Argentina, there was a nationwide outbreak of hepatitis and the rate of infection increased from 55.3 cases per 100,000 inhabitants in 2002 to 107.5 and 113.3 cases per 100,000 inhabitants in 2003 and 2004, respectively [Vizzotti, 2014].

Additionally, hepatitis A virus (HAV) was the leading cause of fulminant hepatic failure (FHF) and liver transplantation in children [Ciocca, 2004; Ciocca, 2007; Ciocca, 2008]. To reduce the health burden associated with HAV disease, the Ministry of Health decided to implement a mandatory one-dose vaccination strategy into the Argentinean regular immunisation schedule for all children aged 12 months in June 2005 [Hendrickx, 2008].

Recent published data show that since 2006, the mean Argentinean hepatitis A incidence rate has dropped to 7.9 per 100,000 inhabitants in the post-vaccination period (2006-2011), which represents an 88.1% reduction compared to the pre-vaccination period (2000-2002), with mean incidence rate of 66.5 per 100,000 inhabitants [Vizzotti, 2014]. There was a striking decrease in HAV-related FHF and resulting liver failure, with no new HAV-related FHF/liver failure cases recorded after end of 2006 / March 2007 [Cervio, 2011; Vizzotti, 2014].

Universal hepatitis A vaccination was introduced in Panama in April 2007. Two doses of the vaccine were given to children at 12 and 18 months of age. The Expanded Program of Immunisation (EPI) of Panama had reportedly provided 53,417 and 7,104 doses of *Havrix* in Panama (first and second dose) during 2007 for an estimated coverage of 81% of the population receiving at least one dose of *Havrix* and 11% receiving both doses (complete series). In 2008, vaccination coverage rose as 99% of the eligible population received at least one dose of *Havrix* and 65% received the complete vaccination series (two doses) [GlaxoSmithKline Biologicals Clinical Study Report 112263].

1.2. Rationale for the study

Panama is an ideal setting to conduct the present study since it consists of an adequate size cohort of children who had received both doses of *Havrix* as well as children who had received one dose of *Havrix*. In addition, this population provides an adequate time interval to measure long term antibody persistence since the children were vaccinated approximately 8 years ago.

The present study will be conducted to evaluate the persistence of hepatitis A antibody, approximately 8 years and 10 years post vaccination with the complete series of *Havrix* (2 doses) and the partial series completion (1 dose).

2. BENEFIT: RISK ASSESSMENT

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

2.1. Risk Assessment

Risks associated with blood collection (such as pain at blood sampling site, haematoma or thrombus, vasovagal reaction, syncope or fainting) can be reduced by following best practices listed in the [\[WHO guidelines\]](#) for drawing blood (2010). Some examples are provided in the table below.

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Study procedure: Blood sample collection.	Pain at blood sampling site	<ul style="list-style-type: none"> Well-trained person should take the blood sample Use needle of smaller gauge than the vein
	Haematoma or thrombus	<ul style="list-style-type: none"> Enter vessel at an angle of 30 degrees or less Use gauge of needle smaller than the vein Apply pressure to a straight arm for 3–5 minutes after drawing blood
	Vasovagal reaction, Syncope, fainting	<ul style="list-style-type: none"> Hydrate patient, take postural blood pressure if dehydrated Reduce anxiety Have patient lie down if the person expresses concern Provide audio-visual distraction

Please refer to [WHO guidelines](#) for drawing blood (2010): Best practices in phlebotomy, in particular section 8.5.3 on risk assessment and risk reduction strategies.

2.2. Benefit Assessment

Most people vaccinated against hepatitis A are protected against the disease. By taking part in this study, the subject will know his/her anti-hepatitis A antibody levels. If the subject is not protected, the subject maybe offered hepatitis A vaccination as per routine practice in Panama.

This study will provide information about the duration of protection offered by *Havrix* against hepatitis A infection.

2.3. Overall Benefit: Risk Conclusion

Taking into account the measures taken to minimise the risk to subjects participating in this study, the potential or identified risks identified are justified by the potential benefits that will be provided to the subjects.

3. OBJECTIVES

3.1. Primary objective

- To assess the persistence of anti-hepatitis A virus (HAV) antibodies, approximately 8 years and 10 years post vaccination with the last received vaccine dose of the complete series of *Havrix* (2 doses) and the partial series completion (1 dose) in Panama.

Refer to Section 8.1.1 for the definition of the primary endpoint.

3.2. Secondary objectives

- To assess geometric mean concentration (GMC) of anti-HAV antibodies, approximately 8 years and 10 years post vaccination with the last received dose of the complete series of *Havrix* (2 doses) and the partial series completion (1 dose) in Panama.
- To explore the non-inferiority of the 1-dose schedule of *Havrix* when compared to the 2-dose schedule in terms of the percentage of subjects with anti-HAV antibody concentrations ≥ 15 mIU/mL, approximately 8 years and 10 years after the last received vaccination dose.

Criteria for evaluation: The lower limit of the 2-sided 95% CI for the difference (1-dose group minus the 2-dose group) of percentage of subjects with anti-HAV antibody concentrations ≥ 15 mIU/mL is greater than or equal to the pre-defined clinical non-inferiority limit of -10%.

Refer to Section 8.1.2 for the definition of the secondary endpoints.

4. STUDY DESIGN OVERVIEW (AMENDED: 24 JULY 2017)

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 6.4), are essential and required for study conduct.

- Type of design: Epidemiological, serial, cross-sectional, interventional, multi-centre study in Panama.
- Study population comprises of children who had received *Havrix* at selected health centres of Panama.
- Eligible children affiliated with the study sites will be enrolled in the study.
- Biological samples: A blood sample (~5mL) will be collected from all subjects at each cross-sectional survey (Year 8 and Year 10).
- Type of study: self-contained.
- Data collection: Electronic Case Report Form (eCRF).
- Duration of the study: This study involves only one study visit per subject. However, since the study comprises of two independent cross-sectional surveys (Year 8 and Year 10), the same subject who participated in the Year 8 cross-sectional survey may be randomly enrolled for the Year 10 cross-sectional survey. In that case, the subject will be enrolled as a new subject and will not be associated to his/her participation in the Year 8 cross-sectional survey. The overall duration of the study is estimated to be approximately 2 years.
- Epochs foreseen in the study:
 - Epoch 001: Persistence Visit 1 (Year 8)
 - Epoch 002: Persistence Visit 1' (Year 10)
- Definition of the cohorts foreseen in the study:
 - Year 8 cohort: All subjects participating in the Year 8 cross-sectional survey (Visit 1 Epoch 001) - May include children with ≥ 7 years and < 10 years between last *Havrix* dose and Persistence Visit 1 (Year 8)
 - Year 10 cohort: All subjects participating in the Year 10 cross-sectional survey (Visit 1' Epoch 002) - May include children with ≥ 10 years and < 13 years between last *Havrix* dose and Persistence Visit 1' (Year 10)

Table 1 presents the study groups and epochs foreseen in the study.

Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Epochs	
		Epoch 001	Epoch 002
Yr 8 Havrix_1 dose	~300	x	
Yr 8 Havrix_2 dose	~300	x	
Yr 10 Havrix_1 dose	~300		x
Yr 10 Havrix_2 dose	~300		x

4.1. Discussion of study design

A serial, cross-sectional, serological study will allow evaluating the long term persistence of anti-HAV antibodies and GMCs in children who had received 1 dose of *Havrix* vaccine (partial series) and children who had received 2 doses of *Havrix* vaccine (complete series) during the same time point.

The first cross-sectional serosurvey will evaluate the long term persistence of immunity approximately 8 years post vaccine administration and provide preliminary data on the non-inferiority of 1 dose versus 2 doses, in terms of long term persistence. The second cross-sectional study will evaluate long term persistence, approximately 10 years post vaccine administration, since the duration of protection beyond 10 years remains unknown and provide preliminary data on the non-inferiority of 1 dose versus 2 doses, in terms of long term persistence. However, the sampling and testing for Year 8 and Year 10 cross-sectional surveys will be independent of each other.

Persistence of immunity to HAV will be assessed by measuring the persisting, circulating anti-HAV antibodies by Enzyme-Linked Immunosorbent Assay (ELISA). Since only subjects who were previously vaccinated with *Havrix* are being followed up in this long term persistence study, a control group will not be available for comparison. No vaccine will be administered during the study period.

5. STUDY POPULATION

5.1. Number of subjects/centres (Amended: 24 July 2017)

Approximately 600 subjects (about 300 subjects vaccinated with one dose of *Havrix* and about 300 subjects vaccinated with two doses of *Havrix*) are planned to be enrolled at each cross-sectional survey time points (Year 8 and Year 10). Two different cohorts of subjects will be enrolled at each epoch, therefore about 600 subjects will be enrolled at Epoch 001 (Year 8 cohort) and another cohort of about 600 subjects will be enrolled at Epoch 002 (Year 10 cohort). In total, approximately 1200 subjects will be enrolled in the study. Subjects in the Year 8 cross-sectional survey may be randomly enrolled for the Year 10 cross-sectional survey. A new subject number will be re-assigned.

If needed, the recruitment period for each cross-sectional survey may be extended to ensure the target sample size is achieved.

Overview of the recruitment plan:

- The parent(s)/legally acceptable representative (s) [LAR(s)] of eligible children attending the schools will be contacted by the school management and informed of the study rationale and procedures.
- The parent(s) or LAR(s) along with the preselected children will be asked to visit the study site at a scheduled appointment where the informed consent process will be initiated and the children will be formally enrolled into the study.
- The parent(s) or LAR(s) will need to sign the informed consent form (ICF) and all children-as they are above 7 years of age will be asked to provide informed assent.
- However, the recruitment of subjects will not be limited to schools. Some subjects maybe enrolled directly at the study site also.
- The recruitment will be performed using the SBIR application. The aim is to control the enrolment per group at each epoch and stop the enrolment once the defined target has been reached. After having checked the eligibility of the subject and obtaining the ICF/informed assent form, the study site staff will access SBIR. 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 300 subjects per study group per epoch has been reached, the enrolment will be frozen.

5.2. Inclusion criteria for enrolment (Amended: 24 July 2017)

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 whose parent(s)/ LAR(s), in the opinion of the investigator, can and will comply with the requirements of the protocol.
- Written informed assent/consent obtained from the subject or subject's parent(s)/ LAR(s) of the subject.
- Available HAV vaccination records.
- Children who have received either 1 or two doses of Havrix at selected health centres of Panama.
- Children with ≥ 7 years and < 10 years between last dose and Persistence Visit 1 (Year 8) and children ≥ 10 years and < 13 years between last dose and Persistence Visit 1' (Year 10).

5.3. Exclusion criteria/criterion 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:

- Child in care
Please refer to the [glossary of terms](#) for the definition of child in care.
- Subjects with history of vaccination with other hepatitis A vaccines other than *Havrix*.
- Subjects with known past history of hepatitis A infection, both without vaccination and after they received the last dose of *Havrix* (1 dose or the complete 2 dose schedule).

6. CONDUCT OF THE STUDY

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

The study will be conducted in accordance with the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice (GCP) or other applicable guidelines, such as Guidelines for Good Pharmacoepidemiology Practices (GPP) [[International Society for Pharmacoepidemiology \(ISPE\)](#), 2007], all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

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

GSK will obtain favourable opinion/approval to conduct the study prior to a site initiating the study in that country or will document that neither a favourable opinion nor an approval to conduct the study is needed.

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

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

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

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

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

In accordance with the ICH Harmonised Tripartite Guidelines for Good Clinical Practice, a subject who can only be enrolled in the study with the consent of his/her parent(s) or legally acceptable representative (e.g., minors), should be informed about the study to the extent compatible with the subject's understanding and, if capable, the subject should sign and personally date a written informed assent form. It is required that the assent be signed by each subject, if capable, in addition to the informed consent that is to be signed by his/her parent or legal representative. It should be assessed whether an assent is required depending on the age of the study population and the local requirements.

GSK Biologicals strongly recommends that if the subject reaches the age of consent during the study they will be asked to provide consent at the next study visit (if applicable). This procedure should be applied according to local laws and regulations.

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.

6.2. Subject identification

Subject numbers will be assigned sequentially to subjects to be included in the study, according to the range of subject numbers allocated to each study centre.

6.3. 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.

6.4. Outline of study procedures (Amended: 24 July 2017)

Table 2 List of study procedures

Epochs	001 and 002
Visits	Visit 1 – Year 8 and Visit 1' – Year 10
Time points	Day 0 and Year 2
Reporting of subjects in the individual screening log sheet	○
Informed consent/assent	●
Check inclusion/exclusion criteria	●
Subject number attribution	○
Collect socio-demographic data	●
Study group allocation	○
Record medical history including hepatitis A disease/ vaccination history	●
Blood sampling for antibody determination (~5mL)	●
Recording of SAEs related to study participation	●
Study conclusion*	●

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

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

*The study conclusion is applicable to both epochs, Epoch 001 and Epoch 002. Different set of subjects will visit the study centre at Year 8 (Epoch 001) and Year 10 (Epoch 002). However, the study conclusion page will be filled for all the subjects in the eCRF.

Table 3 Intervals between study visits/contacts/observations (Amended: 24 July 2017)

Interval	Allowed interval ¹
Last Havrix dose vaccination date → Visit 1 Epoch 001 (Year 8 Survey)	≥ 2555 days to < 3650 days (7.0-9.9 years)
Last Havrix dose vaccination date → Visit 1' Epoch 002 (Year 10 Survey)	≥ 3650 days to < 4745 days (10.0-12.9 years)

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

6.5. Detailed description of study procedures

6.5.1. Reporting of subjects in the individual screening log sheet

All subjects who are approached for the trial will be reported in this sheet (including non-eligible subjects). This is a log sheet to be maintained only by the sites for their reference.

6.5.2. Informed consent/assent

The signed/witnessed/thumb printed informed consent of the subject's parent(s)/LAR(s) must be obtained before study participation. The signed informed assent of a subject below the age of consent (i.e., minor) should be obtained in addition to the signed informed consent by his/her parent(s)/LAR(s) according to local rules and regulations. Refer to Section 6.1 for the requirements on how to obtain informed consent and assent, as appropriate.

6.5.3. Check inclusion and exclusion criteria

Check all applicable inclusion and exclusion criteria as described in Sections [5.2](#) and [5.3](#) before enrolment.

6.5.4. Subject number attribution

Subject numbers will be assigned sequentially to subjects to be included in the study, according to the range of subject numbers allocated to each study centre.

Two different cohorts of subjects will be enrolled at each epoch. About 600 subjects will be enrolled at Epoch 001 and another cohort of about 600 subjects will be enrolled at Epoch 002. Subjects in the Year 8 cross-sectional survey may be randomly enrolled for the Year 10 cross-sectional survey. In this case, the subject will not be associated to his / her previous participation and a new subject number will be re-assigned.

6.5.5. Collect socio-demographic data

Record demographic data such as date of birth, gender, geographic ancestry, urban or rural residency in the subject's eCRF.

6.5.6. Study group allocation

After obtaining the ICF and informed assent form (as applicable) and confirming eligibility, the subject will be entered in to the SBIR enrolment module. SBIR will keep track of the enrolment status in each group during the enrolment in an epoch and freeze enrolment once the defined target per group is reached.

6.5.7. Record medical history including hepatitis A disease or vaccination 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 participation in the study in the eCRF. Record any previous history of hepatitis A disease or vaccination. The investigator will also record if the subject received 1 dose or 2 doses of *Havrix* previously along with the vaccination dates, in order to assign the subject to the appropriate study group.

6.5.8. Blood sampling for antibody determination

About 5 mL of blood samples will be collected at each serosurvey for the determination of antibodies against HAV. Refer to the Module on Biospecimen Management in the SPM for general handling of blood samples.

6.5.9. Recording of SAEs related to study participation

- Refer to Section 7.2 for procedures for the investigator to record SAEs related to study participation. Refer to Section 7.3 for guidelines on how to submit SAE reports to GSK Biologicals.
- The subjects' parent(s)/LAR(s) will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

6.5.10. Study conclusion

The investigator will:

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

The study conclusion is applicable to both epochs, Epoch 001 and Epoch 002. Different set of subjects will visit the study centre at Year 8 (Epoch 001) and Year 10 (Epoch 002). However, the study conclusion will be filled by all the subjects in the eCRF.

6.6. Biological sample handling and analysis

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

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

- Collected samples will be used for protocol mandated research. In addition, these samples may be used to perform research 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 invited to give another specific consent to allow GSK or a contracted partner use the samples for future research including development of tests and their quality assurance. Future research will be subject to the laws and regulations in the respective countries and will only be performed once an independent Ethics Committee or Review Board has approved this research.

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

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

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

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.

6.6.1. Use of specified study materials

When materials are provided by GSK Biologicals, it is MANDATORY that all 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 [8.3](#) for the definition of study cohort's/data sets 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 samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM.

6.6.2. Biological samples

Table 4 Biological samples

Sample type	Quantity	Unit	Time point
Blood	~ 5	ml	Visit 1 – Year 8 and Visit 1' – Year 10

6.6.3. Laboratory assays

Please refer to [APPENDIX A](#) for a detailed description of the assays performed in the study. Please refer to [APPENDIX B](#) for the address of the clinical laboratories used for sample analysis.

Serological assays for the determination of antibodies against HAV will be performed by ELISA using standardised and validated procedures at GSK Biologicals' laboratory or in a laboratory designated by GSK Biologicals. Subjects will be defined as being seropositive if their anti-HAV antibody concentration is ≥ 15 mIU/mL

Table 5 Humoral immunity (antibody determination)

System	Component	Method	Kit / Manufacturer	Cut-off	Unit	Laboratory*
SER	Hepatitis A Virus Antibodies	ELI	Enzygnost (Siemens Healthcare)	15	mIU/mL	GSK Biologicals**

SER = Serum

ELI = Enzyme-linked immunosorbent assay

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

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

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.

6.6.4. Biological samples evaluation

6.6.4.1. Immunological read-outs

Table 6 Immunological read-outs

Blood sampling time point	No. subjects	Component	Components priority rank
Year 8 – Visit 1	All (Year 8 cohort)	Anti-HAV antibodies	Not applicable
Year 10 – Visit 1 ¹	All (Year 10 cohort)	Anti-HAV antibodies	Not applicable

Investigators will be provided with the immunogenicity results for individual subjects enrolled at their site after the statistical analyses of each epoch have been completed.

If subjects have anti-HAV levels below the seropositivity cut-off of 15 mIU/ml (anti-HAV antibody concentration is < 15 mIU/mL), the investigator will refer them to the appropriate point of care to assess if an extra dose of the *Havrix* vaccine needs be administered.

7. SAFETY

All SAEs related to the study procedure (blood sample collection) will be collected from the time of sample collection up to study conclusion.

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

Each subject's parent(s)/ LAR(s) will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious/ of concern or indicating a change in their health status.

7.1. Safety definitions

7.1.1. Definition of a serious adverse event

An SAE is any untoward medical occurrence that:

- a. Results in death,
- b. Is life-threatening,

NB: 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 an existing hospitalisation,

NB: 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 SAE.

- d. Results in disability/incapacity,

NB: 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.

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.

7.1.2. Clinical laboratory parameters and other abnormal assessments qualifying as SAEs

In absence of diagnosis, abnormal laboratory findings (e.g., clinical chemistry, haematology, urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as SAEs if they meet the

definition of an SAE (refer to Section 7.1.1). 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 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. Detecting and recording SAEs

7.2.1. Time periods for detecting and recording SAEs

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) will be collected and recorded from the time the study start until she/he is discharged from the study.

An overview of the protocol-required reporting periods for SAEs is given in [Table 7](#).

Table 7 Reporting periods for SAEs

Epoch 001:

Study activity	Year 8 (Visit 1)	Study Conclusion
SAEs related to study procedure		

Epoch 002:

Study activity	Year 10 (Visit 1')	Study Conclusion
SAEs related to study procedure		

7.2.2. Evaluation of SAEs

7.2.2.1. Active questioning to detect SAEs

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

All SAEs either observed by the investigator or his/ her staff or reported by the subject's parents/ LAR(s) spontaneously or in response to a direct question will be evaluated by the investigator. The nature of each event, date and time of onset, outcome, intensity and possible relationship to the study procedures should be established.

When an 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 the 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 SAE and not the individual signs/symptoms.

The investigator will assess the maximum intensity that occurred over the duration of the event for all 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 SAE which is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
2 (moderate)	=	An SAE which is sufficiently discomforting to interfere with normal everyday activities.
3 (severe)	=	An SAE which prevents normal, everyday activities

An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 7.1.1.

7.2.2.2. Assessment of causality

The investigator should assess the causality of each SAE. The investigator will use clinical judgement to determine the relationship between the SAEs and study participation. Alternative causes, such as natural history of the underlying diseases, other concomitant therapy and other risk factors will be considered and investigated.

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 SAE 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.

If an event meets the criteria to be considered as 'serious' (see Section 7.1.1), additional examinations/tests will be performed by the investigator in order to determine ALL possibly contributing factors to each SAE.

Possibly contributing factors include:

- Medical history.
- Concomitant medication.
- Protocol required procedure.

- Other procedure not required by the protocol.
- Lack of efficacy of the vaccine

7.2.2.3. Assessment of outcomes

The investigator will assess the outcome of all 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. Reporting of SAEs

7.3.1. Prompt reporting of SAEs related to study participation

SAEs that occur in the time period defined in Section 7.2.1 will be reported promptly to GSK within the timeframes described in Table 8 once the investigator determines that the event meets the protocol definition of an SAE.

Table 8 Timeframes for submitting SAEs related to study participation

Type of event	Initial reports		Follow-up of relevant information on a previous report	
	Timeframe	Documents	Timeframe	Documents
SAEs related to study participation	24 hours*‡	electronic Expedited Adverse Event Report	24 hours*	electronic Expedited Adverse Event 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

7.3.2. Contact information for reporting SAEs

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

7.3.3. Completion and transmission of SAEs reports related to study participation

Once an investigator becomes aware that an SAE has occurred in a study subject, the investigator (or designee) must complete the information in the electronic Expedited Adverse Event 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 an SAE, the report should still be completed within 24 hours. Once additional 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.

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

If the electronic reporting system does not work, the investigator (or designee) must complete, then date and sign a paper Expedited Adverse Event Report and fax it to the 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 designee) must complete the electronic Expedited Adverse Event Report within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic reporting system.

7.3.4. Updating of SAE information after freezing of the subject's eCRF

When additional SAE information is received after freezing of the subject's eCRF, new or updated information should be recorded on a paper Expedited Adverse Event Report, with all changes signed and dated by the investigator. The updated report should be faxed to the GSK Biologicals Clinical Safety and Pharmacovigilance department or to the Study Contact for Reporting SAEs (see the [Sponsor Information](#)) within the designated reporting time frames specified in [Table 8](#).

7.3.5. Regulatory reporting requirements for SAEs

The investigator will promptly report all SAEs to GSK Biologicals in accordance with the procedures detailed in Section [7.3.1](#). GSK Biologicals has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under epidemiological 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.

7.4. Follow-up of SAEs

7.4.1. Follow-up during the study

After the initial SAE report, the investigator is required to proactively follow each subject and provide further relevant information on the subject's condition to GSK Biologicals (within 24 hours for SAEs, refer to [Table 8](#))

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

The investigator will follow-up subjects:

- With SAEs 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 Expedited Adverse Event Report.

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

8. STATISTICAL METHODS

8.1. Endpoints

8.1.1. Primary endpoint

- Anti-HAV seropositivity status at approximately 8 years and 10 years after administration with the last received dose of *Havrix*.

Subjects are defined as being seropositive if their anti-HAV antibody concentration is $\geq 15 \text{ mIU/mL}$.

8.1.2. Secondary endpoints

- Anti-HAV concentrations (GMC) at approximately 8 years and 10 years after administration with the last received dose of *Havrix*.
- Anti-HAV seropositivity status at approximately 8 years and 10 years after administration with the last received dose of *Havrix*.

Subjects are defined as being seropositive if their anti-HAV antibody concentration is $\geq 15 \text{ mIU/mL}$.

8.2. Determination of sample size

The primary objective of this study is to evaluate the persistence of anti-HAV antibodies, approximately 8 years and 10 years post vaccination with the last vaccination of the complete series of *Havrix* (2 doses) and the partial series (1 dose). Around 300 subjects will be enrolled in each group at Year 8 and Year 10 visits. Considering that 10-15% of the subjects will be non-evaluable or will not have any available immunogenicity result, it will be assumed that around 260 subjects per group will be included in the ATP cohort of persistence at Year 8 and at Year 10 for the analysis of the primary endpoint.

Table 9 illustrates the accuracy that can be expected from sample size of 260 evaluable subjects in each group at Year 8 and Year 10 visits for evaluating the percentage of subjects with anti-HAV concentrations ≥ 15 mIU/mL. With 260 subjects per group, the width of the 95% CI giving a precision of approximately 5% around the point estimate is considered meaningful.

Table 9 Exact 95% confidence intervals of the percentage of subjects with anti-HAV concentrations greater than or equal to 15 mIU/mL

	Percentage (%)	N=260
At Year 8 and Year 10	20	[15.3; 25.4]
	30	[24.5; 36.0]
	40	[34.0; 46.2]
	50	[43.8; 56.2]
	60	[53.8; 66.0]
	70	[64.0; 75.5]
	80	[74.6; 84.7]
	90	[85.7; 93.4]
	100	[98.6; 100.0]

N: Total number of eligible subjects with available immunogenicity results

Table 10 illustrates the sample size and the power associated with the objective below at each epoch. The power to meet the criteria associated the objective below is at least 80% with 260 evaluable subjects in each group at each cross-sectional survey time period (Year 8 and Year 10 visits).

In terms of seropositivity rates: To explore the non-inferiority of the 1-dose of *Havrix* when compared to 2-doses in terms of the percentage of subjects with anti-HAV antibody concentrations ≥ 15 mIU/mL, approximately 8 years and 10 years after the last vaccination dose.

Criteria for evaluation: *The lower limit of the 2-sided 95% CI for the difference (1-dose group minus the 2-dose group) of percentage of subjects with anti-HAV antibody concentrations ≥ 15 mIU/mL is greater than or equal to the pre-defined clinical non-inferiority limit of -10%.*

Table 10 Power to show non-inferiority of the immunogenicity of 1-dose compared to 2-doses of Havrix

Endpoint	% in the two group**	δ	Power* N=260/group
Anti-HAV \geq 15 mIU/mL	80%	-10%	80.9%

* Pass 2005, one-sided equivalence test of proportions (non-inferiority), alpha = 2.5%, power under equal proportions.

H0: group difference in expected % of subjects with anti-HAV concentrations $< \delta$ (P1-dose – P2-dose $< -10\%$)

Ha: group difference in expected % of subjects with anti-HAV concentrations $\geq \delta$ (P1-dose – P2-dose $\geq -10\%$)

**References used for the sample size calculation: [Racznik, 2013 and Hammitt, 2008]

8.3. Cohorts for Analyses at Years 8 and 10

The following cohorts will be defined separately for each epoch.

8.3.1. Total cohort at Year 8 or Year 10

The total cohort at Year 8 or at Year 10 will include all subjects enrolled in the study at Year 8 or Year 10 serosurveys, respectively. For the analysis of persistence, this will include subjects for whom data concerning persistence endpoint measures are available.

8.3.2. Total effective cohort at Year 8 or Year 10

The total effective cohort at Year 8 or Year 10 will include all enrolled subjects who have a valid ICF at Year 8 or Year 10 serosurveys, respectively.

8.3.3. According-to-Protocol (ATP) cohort for persistence at Years 8 or 10 (Amended: 24 July 2017)

The ATP cohort for persistence at Year 8 or at Year 10 will include all enrolled subjects:

- who have a valid ICF
- who have available assay results at Year 8 or Year 10 serosurveys, respectively.
- who have not received a hepatitis A vaccine other than Havrix.
- who do not have a history hepatitis A infection prior to study.
- who have available HAV vaccination records.
- who received either 1 or two doses of Havrix at selected health centres of Panama.
- who have ≥ 7 years and < 10 years between last dose and Persistence Visit 1 (Year 8) and children ≥ 10 years and < 13 years between last dose and Persistence Visit 1' (Year 10).

8.4. Derived and transformed data

- Age at time of enrolment in the study will be computed as the difference between the date of enrolment [date when the ICF was signed by the parent(s)/ LAR(s)] and the date of birth. The age will be expressed in years.
- For a given subject and given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, the analysis will exclude subjects with missing or non-evaluable measurements.
- The GMC calculations are performed by taking the anti-log of the mean of the log concentrations transformations. 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. The cut-off value is defined by the laboratory before the analysis and is described in Section 6.6.3.
- A seronegative subject is a subject whose antibody concentration is below the cut-off value of the assay. A seropositive subject is a subject whose antibody concentration is greater than or equal to the cut-off value of the assay. The cut-off value is defined in Section 6.6.3.

8.5. Analysis of demographics/baseline characteristics

At each cross-sectional survey (Year 8 and 10 visits):

- Demographic characteristics such as age at the study visit, gender, geographic ancestry, rural and urban residency of the subjects enrolled will be tabulated per dose group and overall.
- The mean age (with the range and standard deviation) as well as the proportion of males and females will be calculated and presented for subjects enrolled at Year 8 visit and Year 10 visit per dose group and overall.

8.6. Analysis of primary and secondary objectives

All statistical analyses will be performed using statistical analysis system version 9.2 or later. All the analyses will be described in detail in the statistical analysis plan (SAP).

The analysis for persistence at Year 8 and at Year 10 serosurveys will be based on the ATP cohort. If the percentage of enrolled subjects with available serological results excluded from this ATP cohort is more than 5%, a second analysis based on the Total effective cohort will be performed to support the ATP analysis.

Analysis of persistence

For the subjects who had received either one dose or two doses of *Havrix*, at each serosurvey (approximately 8 years and 10 years after the last vaccine dose):

- Percentage of subjects with concentrations above the proposed cut-off with exact 95% CI will be calculated.
- GMCs with 95% CI will be tabulated.
- The distribution of antibody concentrations will be tabulated and also presented using reverse cumulative curves.

Exploratory between groups analysis:

An exploratory evaluation of the differences in the immune response approximately 8 years and 10 years after the last vaccination dose with *Havrix* between the subjects who received only one dose of *Havrix* and subjects who received two doses of *Havrix* at each serosurvey (approximately 8 years and 10 years after the last received vaccine dose) will be performed in terms of:

- Differences in anti-HAV antibody concentrations between the subjects who received only one dose of *Havrix* and subjects who received two doses of *Havrix* at each serosurvey.

Computation of the asymptotic standardised 2-sided 95% CIs for the difference in the percentage of subjects with anti-HAV antibody concentrations ≥ 15 mIU/mL at Year 8 or at Year 10 after the last received *Havrix* vaccination dose (1-dose group minus the 2-dose group): if the lower limit of the 2-sided 95% CI for the difference in the percentage of subjects with anti-HAV antibody concentrations ≥ 15 mIU/mL at Year 8 or at Year 10 serosurveys, (1-dose group minus the 2-dose group) is greater than or equal to the pre-defined clinical limit of -10%, the pre-defined criteria for the exploratory objective is considered met and the 1-dose group non-inferior to the 2-dose group in terms of seropositivity rate).

- Ratio of the anti-HAV GMCs between the subjects who received only one dose of *Havrix* and subjects who received two doses of *Havrix* with their standardised asymptotic 95% CI at each serosurvey.

Computation of the GMC ratio and its 95% CI:

For all analyses (exploration evaluation of differences between groups), the GMC ratio and its 95% CI will be computed using an analysis of variance (ANOVA) model on the log10-transformed concentration with the group and area of residence as fixed effects in the model.

8.7. Interpretation of analyses

All the analyses will be descriptive with the aim to characterise the immunogenicity within and between study groups. Because of the exploratory nature of the comparisons no conclusions can be made.

There will be no comparison between the two serosurveys (Year 8 and Year 10). It will be descriptive in nature.

8.8. 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.

8.8.1. Sequence of analyses

All statistical analysis will be performed at the end of each cross-sectional survey (at Year 8 (Epoch 001) and Year 10 (Epoch 002)) on clean data and carried out on the final dataset, following recruitment of the last subject of each serosurvey.

The statistical analysis will be described in detail in the SAP.

8.8.2. Statistical considerations for interim analyses

No interim analysis is planned. However, the analysis will be done separately for the two epochs. As all analyses are descriptive, no adjustment of type I error is needed.

9. ADMINISTRATIVE MATTERS

To comply with ICH GCP or other applicable guidelines such as ISPE guidelines for GPP, administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality, ownership and publications must be met.

9.1. 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.

Once the database is archived and the clinical study report is complete and approved by all parties, each participating investigator will be provided with a CD-ROM of the final version of the data generated at his/her investigational site.

9.2. 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 a 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 will mark 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.

9.3. 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 or other applicable guidelines such as ISPE guidelines for GPP any institutional requirements or applicable laws or regulations, or GSK standards/procedures; otherwise, the minimum retention period will default to 15 years.

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

9.4. Quality assurance

To ensure compliance with GCP or other applicable guidelines such as ISPE guidelines for GPP 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.

9.5. Posting of information on publicly available registers and publication policy

Study information from this protocol will be posted on public registers before enrolment of subjects begins.

Observational studies that do not evaluate vaccines/products are progressed for publication in the scientific literature when the results provide important scientific or medical knowledge.

9.6. 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 Biologicals site or other mutually-agreed 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.

10. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

11. REFERENCES

Cervio G, Trentadue J, D'Agostino D et al. Decline in HAV-associated fulminant hepatic failure and liver transplant in children in Argentina after the introduction of a universal hepatitis A vaccination program. *Hepatic Medicine: Evidence and Research* 2011;3:99-106.

Ciocca ME, Ramonet MD, Cuarterolo ML et al. Acute liver failure in children: Experience with 210 patients. *J Pediatr Gastroenterol & Nutr.* 2004; 39 (suppl 1):S31-O0062. URL: http://journals.lww.com/jpgn/Fulltext/2004/06001/O0062_Acute_Liver_Failure_in_Children_Experience.64.aspx. Last accessed: 05 April 2016.

Ciocca M, Moreira-Silva SF, Alegria S et al. Hepatitis A as an etiologic agent of acute liver failure in Latin America. *Pediatric Infectious Disease Journal* 2007;26(8):711-715.

Ciocca M, Ramonet M, Cuarterolo M et al. Prognostic factors in paediatric acute liver failure. *Arch Dis Child.* 2008;93:48-51.

GlaxoSmithKline Biologicals Clinical Study Report 112263. Time trend analysis of the incidence of hepatitis-related outcomes (viral hepatitis A and unspecified viral hepatitis) by monitoring the reports received by the surveillance system of Panama, 2000-2010.

Hammitt LL, Bulkow L, Hennessy TW et.al. Persistence of Antibody to Hepatitis A Virus 10 Years after Vaccination among Children and Adults. *Int J Infect Dis.* 2008; 198 (12): 1776-82.

Hendrickx G, Van Herck K, Vorsters A et al. Has the time come to control hepatitis A globally? Matching prevention to the changing epidemiology. *Journal of Viral Hepatitis* 2008;15(Suppl 2):1-15.

International Society for Pharmacoepidemiology (ISPE). *Guidelines for Good Pharmacoepidemiology Practices. GPP*, http://www.pharmacoepi.org/resources/guidelines_08027.cfm; 2007. Accessed 17 April 2014.

Ott JJ and Wiersma ST. Single-dose administration of inactivated hepatitis A vaccination in the context of hepatitis A vaccine recommendation. *Int J Infect Dis.* 2013; 17(11): e939-44.

Raczniak GA, Bulkow LR, Bruce MG et.al. Long-Term Immunogenicity of Hepatitis A Virus Vaccine in Alaska 17 Years After Initial Childhood Series. *J Infect Dis.* 2013; 207(3):493-6.

Vizzotti C, González J, Gentile A et al. Impact of the single-dose immunisation strategy against hepatitis A in Argentina. *Pediatr Infect Dis J*. 2014;33(1): 84-8.

World Health Organisation (WHO). Meeting of the Strategic Advisory Group of Experts on Immunization, November 2011. URL: <http://www.who.int/wer/2012/wer8701.pdf>. Last accessed: 05 April 2016.

WHO guidelines on drawing blood. Best practices in phlebotomy. 2010. URL: http://apps.who.int/iris/bitstream/10665/44294/1/9789241599221_eng.pdf. Last accessed: 05 April 2016.

APPENDIX A LABORATORY ASSAYS

The Enzygost ELISA kit from Siemens Healthcare is a commercial labelled kit for the qualitative detection of anti-HAV antibodies in serum.

Performance characteristics of the assay are described in the GSK Vaccines internal validation document.

APPENDIX B CLINICAL LABORATORIES**Table 11 GSK Biologicals' laboratories**

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

APPENDIX C AMENDMENTS AND ADMINISTRATIVE CHANGES TO THE PROTOCOL

GlaxoSmithKline Biologicals

Vaccine Value & Health Science (VVHS)

Protocol Amendment 1

eTrack study number and Abbreviated Title	201630 (EPI-HAV-007 BOD PA)
Amendment number:	Amendment 1
Amendment date:	05 April 2016
Co-ordinating author:	PPD [REDACTED], Scientific Writer for GSK Biologicals

Rationale/background for changes:

- The mitigation strategy for risks associated with blood sample collection was revised based on the WHO guidelines on drawing blood.
- Due to unexpected low number of eligible subjects identified with 8 years post vaccination of the last dose of *Havrix* in the first survey (and subsequently 10 years post vaccination for the second survey), the post last dose vaccination period criteria will be expanded in order to increase the pool of eligible children and reach the adequate sample size for the study:
 - For the Year 8 survey: The new post last dose time interval may include children with ≥ 7 years and < 9 years between last dose.
 - For the Year 10 survey: The new post last dose time interval may include children with ≥ 9 years and < 11 years between last dose.

This post last dose time criteria have been added to inclusion criteria section for clarification.

- The date of birth requirement has also been expanded to include the 2005 birth cohort in order to increase the pool of eligible children and reach the adequate sample size for the study. The new date of birth inclusion criteria will include children born January 1, 2005 to December 31, 2007
- The age requirement has been eliminated from the study as it is neither necessary for the design nor the objectives of the study.

- The typographical error identified by the local ethics committee in section 6.6.3 “Laboratory assays” of the protocol was updated accordingly:
 - “Please refer to APPENDIX A for a detailed description of the assays performed in the study. Please refer to APPENDIX B for the address of the clinical laboratories used for sample analysis. Serological assays for the determination of antibodies against HAV will be performed by ELISA using standardised and validated procedures at a local laboratory”. The correct sentence should be “...at *GSK Biologicals' laboratory or in a laboratory designated by GSK Biologicals*.”

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

Title page:

Contributing authors

- PPD [REDACTED], Senior Manager, Epidemiology
Travellers/PPD [REDACTED], ***Senior Manager, Epidemiology***
- PPD [REDACTED]/PPD [REDACTED], GVCL ***CLS***
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- PPD [REDACTED], ***Project Data Manager***
- PPD [REDACTED]/PPD [REDACTED], GVCL ***CLS***
Study Manager
- PPD [REDACTED], ***Cluster Area***
Medical Lead, Caricam-Andina
- PPD [REDACTED], ***Study Delivery Manager***
- PPD [REDACTED]/PPD [REDACTED], Vaccine Supply
Coordinator, Aprova for GSK Biologicals
- PPD [REDACTED], ***Local Delivery Lead***

Synopsis and Section 1.2 Rationale for the study

Panama is an ideal setting to conduct the present study since it consists of an adequate size cohort of children who had received both doses of *Havrix* as well as children who had received one dose of *Havrix*. In addition, this population provides an adequate time interval to measure long term antibody persistence since the children were vaccinated ***approximately*** 8 years ago.

The present study will be conducted to evaluate the persistence of hepatitis A antibodies, ***approximately*** 8 years and 10 years post vaccination with the complete series of *Havrix* (2 doses) and the partial series completion (1 dose).

Section 2.1 Risk Assessment

Risks associated with blood collection (such as pain at blood sampling site, haematoma or thrombus, vasovagal reaction, syncope or fainting) can be reduced by following best practices listed in the [WHO guidelines] for drawing blood (2010). Some examples are provided in the table below.

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Blood sample collection- While giving blood the subject may feel faint, or locally experience mild pain, bruising, irritation or redness.	Spontaneous data	Subjects will be observed for at least 30 minutes after blood sample collection.
Study procedure: Blood sample collection.	Pain at blood sampling site	<ul style="list-style-type: none"> • Well-trained person should take the blood sample • Use needle of smaller gauge than the vein
	Haematoma or thrombus	<ul style="list-style-type: none"> • Enter vessel at an angle of 30 degrees or less • Use gauge of needle smaller than the vein • Apply pressure to a straight arm for 3–5 minutes after drawing blood
	Vasovagal reaction, Syncope, fainting	<ul style="list-style-type: none"> • Hydrate patient, take postural blood pressure if dehydrated • Reduce anxiety • Have patient lie down if the person expresses concern • Provide audio-visual distraction

Please refer to WHO guidelines for drawing blood (2010): Best practices in phlebotomy, in particular section 8.5.3 on risk assessment and risk reduction strategies.

Synopsis and Section 3 Objectives

Primary

To assess the persistence of anti- HAV antibodies, *approximately* 8 years and 10 years post vaccination with the last received vaccine dose of the complete series of *Havrix* (2 doses) and the partial series completion (1 dose) in Panama.

Secondary

- To assess geometric mean concentration (GMC) of anti-HAV antibodies, **approximately** 8 years and 10 years post vaccination with the last received dose of the complete series of *Havrix* (2 doses) and the partial series completion (1 dose) in Panama.
- To explore the non-inferiority of the 1-dose schedule of *Havrix* when compared to the 2-dose schedule in terms of the percentage of subjects with anti-HAV antibody concentrations ≥ 15 mIU/mL, **approximately** 8 years and 10 years after the last received vaccination dose.

Synopsis and Section 4 Study design overview

- Study population comprises of children who had received *Havrix* at selected health centres of Panama in **2007-2009**.
- Definition of the cohorts foreseen in the study:
 - Year 8 cohort: All subjects participating in the Year 8 cross-sectional survey (Visit 1 Epoch 001) - **May include children with ≥ 7 years and < 9 years between last *Havrix* dose and Persistence Visit 1 (Year 8)**
 - Year 10 cohort: All subjects participating in the Year 10 cross-sectional survey (Visit 1' Epoch 002) - **May include children with ≥ 9 years and < 11 years between last *Havrix* dose and Persistence Visit 1' (Year 10)**

Synopsis and Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)	Epochs	
			Epoch 001	Epoch 002
Yr 8 Havrix_1 dose	~300	8 years-9 years	x	
Yr 8 Havrix_2 dose	~300	8 years-9 years	x	
Yr 10 Havrix_1 dose	~300	10 years-11 years		x
Yr 10 Havrix_2 dose	~300	10 years-11 years		x

Synopsis and Section 4.1 Discussion of study design

A serial, cross-sectional, serological study will allow evaluating the long term persistence of anti-HAV antibodies and GMCs in children who had received 1 dose of *Havrix* vaccine (partial series) and children who had received 2 doses of *Havrix* vaccine (complete series) during the same time point.

The first cross-sectional serosurvey will evaluate the long term persistence of immunity **approximately** 8 years post vaccine administration and provide preliminary data on the non-inferiority of 1 dose versus 2 doses, in terms of long term persistence. The second cross-sectional study will evaluate long term persistence, **approximately** 10 years post vaccine administration, since the duration of protection beyond 10 years remains unknown and provide preliminary data on the non-inferiority of 1 dose versus 2 doses, in terms of long term persistence. However, the sampling and testing for Year 8 and Year 10 cross-sectional surveys will be independent of each other.

Synopsis and Section 5.1 Number of subjects/centres

Approximately 600 subjects born between 1st January 2006-2005 and 31st December 2007 (i.e., 8 to 9 years of age at Year 8 visit and 10 to 11 years of age at Year 10 visit) (about 300 subjects vaccinated with one dose of *Havrix* and about 300 subjects vaccinated with two doses of *Havrix*) are planned to be enrolled at each cross-sectional survey time points (Year 8 or 2015 and Year 10 or 2017). Two different cohorts of subjects will be enrolled at each epoch, therefore about 600 subjects will be enrolled at Epoch 001 (Year 8 cohort) and another cohort of about 600 subjects will be enrolled at Epoch 002 (Year 10 cohort). In total, approximately 1200 subjects will be enrolled in the study. Subjects in the Year 8 cross-sectional survey may be randomly enrolled for the Year 10 cross-sectional survey. In this case, the subject will not be associated to his / her previous participation and a new subject number will be re-assigned.

Synopsis and Section 5.2 Inclusion criteria for enrolment

- Children born between 1st January 2006-2005 and 31st December 2007 (i.e., 8 to 9 years of age at Year 8 visit and 10 to 11 years of age at Year 10 visit) that received either 1 or two doses of *Havrix* at selected health centres of Panama.
- Available HAV vaccination records.
- *Children who have received either 1 or two doses of Havrix at selected health centres of Panama*
- *Children with ≥ 7 years and < 9 years between last dose and Persistence Visit 1 (Year 8) and children ≥ 9 years and < 11 years between last dose and Persistence Visit 1' (Year 10)*

Section 6.4 Outline of study procedures

Table 2 List of study procedures

Age	8-9 years and 10-11 years
Epochs	001 and 002
Visits	Visit 1 – Year 8 and Visit 1' – Year 10
Time points	Day 0 and Year 2
Reporting of subjects in the individual screening log sheet	○
Informed consent/assent	●
Check inclusion/exclusion criteria	●
Subject number attribution	○
Collect socio-demographic data	●
Study group allocation	○
Record medical history including hepatitis A disease/ vaccination history	●
Blood sampling for antibody determination (~5mL)	●
Recording of SAEs related to study participation	●
Study conclusion* 3	●

Table 3 Intervals between study visits/contacts/observations

Interval	Optimal length of interval ¹	Allowed interval ²
Last Havrix dose vaccination date → Visit 1 Epoch 001 (Year 8 Survey)	≥ 2920 days to < 3285 days (8.0-8.9 years)	≥ 2555 days to < 3285 days (7.0-8.9 years)
Last Havrix dose vaccination date → Visit 1' Epoch 002 (Year 10 Survey)	≥ 3650 days to < 4015 days (10.0-10.9 years)	≥ 3285 days to < 4015 days (9.0-10.9 years)

¹. Whenever possible the investigator should arrange study visits/contacts within this interval². Subjects will not be eligible for inclusion in the ATP cohort for analysis of each survey if they make the study visit outside this interval

Section 6.5.7 Record medical history including hepatitis A disease or vaccination 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 participation in the study in the eCRF. Record any previous history of hepatitis A disease or vaccination. The investigator will also record if the subject received 1 dose or 2 doses of *Havrix* previously **along with the vaccination dates**, in order to assign the subject to the appropriate study group.

Section 6.6.3 Laboratory assays

Serological assays for the determination of antibodies against HAV will be performed by ELISA using standardised and validated procedures at **GSK Biologicals' laboratory or in a laboratory designated by GSK Biologicals** local laboratory. Subjects will be defined as being seropositive if their anti-HAV antibody concentration is ≥ 15 mIU/mL.

Table 5 Humoral immunity (antibody determination)

System	Component	Method	Kit / Manufacturer	Cut-off	Unit	Laboratory*
SER	Hepatitis A Virus Antibodies	ELI	Enzygnost (Siemens Healthcare)	15	mIU/mL	GSK Biologicals**

SER = Serum

ELI = Enzyme-linked immunosorbent assay

*Refer to APPENDIX B for the laboratory addresses

GSK Biologicals laboratory refers to the **Clinical Laboratories Sciences (CLS) in Rixensart, Belgium; Wavre, Belgium. Global Vaccines Clinical Laboratories (GVCL) in Rixensart, Belgium; Wavre, Belgium.

Section 6.6.4.1 Immunological read-outs

If subjects have anti-HAV levels below the seropositivity cut-off of 15 mIU/ml If subjects have a suboptimal response to anti-HAV (anti-HAV antibody concentration is < 15 mIU/mL), the investigator will refer them to the appropriate point of care to assess if an extra dose of the *Havrix* vaccine needs be administered.

Section 7 Safety

All SAEs related to the study procedure (blood sample collection) will be collected from the time of sample collection up to study conclusion. ~~Each subject will be monitored for a period of 30 minutes after blood sample collection.~~

Synopsis and Section 8.1 Endpoints

Primary

- Anti-HAV seropositivity status at **approximately** 8 years and 10 years after administration with the last received dose of *Havrix*.

Secondary

- Anti-HAV concentrations (GMC) at **approximately** 8 years and 10 years after administration with the last received dose of *Havrix*.
- Anti-HAV seropositivity status at **approximately** 8 years and 10 years after administration with the last received dose of *Havrix*.

Section 8.2 Determination of sample size

The primary objective of this study is to evaluate the persistence of anti-HAV antibodies, **approximately** 8 years and 10 years post vaccination with the last vaccination of the complete series of *Havrix* (2 doses) and the partial series (1 dose). Around 300 subjects will be enrolled in each group at Year 8 and Year 10 visits. Considering that 10-15% of the subjects will be non-evaluable or will not have any available immunogenicity result, it will be assumed that around 260 subjects per group will be included in the ATP cohort of persistence at Year 8 and at Year 10 for the analysis of the primary endpoint.

In terms of seropositivity rates: To explore the non-inferiority of the 1-dose of *Havrix* when compared to 2-doses in terms of the percentage of subjects with anti-HAV antibody concentrations ≥ 15 mIU/mL, **approximately** 8 years and 10 years after the last vaccination dose.

Section 8.3.3 According-to-Protocol (ATP) cohort for persistence at Years 8 or 10

The ATP cohort for persistence at Year 8 or at Year 10 will include all enrolled subjects:

- ***who have a valid ICF***
- who were born between 1st January ~~2005 2006~~ and 31st December ~~2009 2007~~ (i.e., ~~6 to 9 years of age at Year 8 visit and 8 to 11 years of age at Year 10 visit~~)
- who received either 1 or two doses of *Havrix* at selected health centres of Panama in ~~2007 2008~~
- ***who have ≥ 7 years and < 9 years between last dose and Persistence Visit 1 (Year 8) and children ≥ 9 years and < 11 years between last dose and Persistence Visit 1 (Year 10)***

Section 8.6 Analysis of primary and secondary objectives

The analysis for persistence at Year 8 and at Year 10 **serosurveys** will be based on the ATP cohort. If the percentage of enrolled subjects with available serological results excluded from this ATP cohort is more than 5%, a second analysis based on the Total effective cohort will be performed to support the ATP analysis.

Analysis of persistence

For the subjects who had received either one dose or two doses of *Havrix*, at each serosurvey (*approximately* 8 years and 10 years after the last vaccine dose):

Exploratory between groups analysis:

An exploratory evaluation of the differences in the immune response *approximately* 8 years and 10 years after the last vaccination dose with *Havrix* between the subjects who received only one dose of *Havrix* and subjects who received two doses of *Havrix* at each serosurvey (*approximately* 8 years and 10 years after the last received vaccine dose) will be performed in terms of:

- Differences in anti-HAV antibody concentrations between the subjects who received only one dose of *Havrix* and subjects who received two doses of *Havrix* at each serosurvey.

Computation of the asymptotic standardised 2-sided 95% CIs for the difference in the percentage of subjects with anti-HAV antibody concentrations ≥ 15 mIU/mL at Year 8 or at Year 10 after the last received *Havrix* vaccination dose (1-dose group minus the 2-dose group): if the lower limit of the 2-sided 95% CI for the difference in the percentage of subjects with anti-HAV antibody concentrations ≥ 15 mIU/mL at Year 8 or at Year 10 serosurveys after the last received *Havrix* vaccination dose, (1-dose group minus the 2-dose group) is greater than or equal to the pre-defined clinical limit of -10%, the pre-defined criteria for the exploratory objective is considered met and the 1-dose group non-inferior to the 2-dose group in terms of seropositivity rate)

Section 8.7 Interpretation of analyses

There will be no comparison between the two serosurveys (Year 8 and Year 10) ~~post vaccination with the last vaccine dose~~. It will be descriptive in nature.

Section 8.8.1 Sequence of analyses

All statistical analysis will be performed at the end of each cross-sectional survey (at Year 8 (Epoch 001) and Year 10 (Epoch 002)) ~~after the last received *Havrix* vaccination dose~~ on clean data and carried out on the final dataset, following recruitment of the last subject of each serosurvey.

Section 11 References

WHO guidelines on drawing blood. Best practices in phlebotomy. 2010. URL: http://apps.who.int/iris/bitstream/10665/44294/1/9789241599221_eng.pdf. Last accessed: 05 April 2016.

Appendix B Clinical Laboratories**Table 10 GSK Biologicals' laboratories**

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

GlaxoSmithKline Biologicals**Vaccine Value & Health Science (VVHS)****Protocol Amendment 2**

eTrack study number and Abbreviated Title	201630 ((EPI-HAV-007 BOD PA)
Amendment number:	Amendment 2
Amendment date:	24 July 2017
Co-ordinating author:	PPD [REDACTED], Freelance Scientific Writer for GSK Biologicals
Rationale/background for changes:	
<ul style="list-style-type: none">• The date of birth requirement has been eliminated from the study as it is not necessary for the design or the objectives of the study and will ease the recruitment constraint observed in the study.• The post last dose interval will be updated due to low number of eligible subjects identified within the existing intervals which puts at risk achieving adequate sample size.<ul style="list-style-type: none">– For the Year 8 survey: The new post last dose time interval may include children with ≥ 7 years and < 10 years between last dose.– For the Year 10 survey: The new post last dose time interval may include children with ≥ 10 years and < 13 years between last dose.– This post last dose time criteria have been added to inclusion criteria section for clarification.	

Amended text has been included in ***bold italics*** and deleted text in ~~strikethrough~~ throughout the protocol.

Title page- List of contributing authors has been updated.

- PPD ██████████, PPD ██████████, Study Delivery Leads
- PPD ██████████, PPD ██████████, Project Statisticians
- PPD ██████████, PPD ██████████, Lead Statisticians

Synopsis and Section 4 Study Design Overview

- Study population comprises of children who had received *Havrix* at selected health centres of Panama ~~in 2007-2009~~.
- Definition of the cohorts foreseen in the study:
 - Year 8 cohort: All subjects participating in the Year 8 cross-sectional survey (Visit 1 Epoch 001) - May include children with ≥ 7 years and $< 9\text{ }10$ years between last *Havrix* dose and Persistence Visit 1 (Year 8)
 - Year 10 cohort: All subjects participating in the Year 10 cross-sectional survey (Visit 1' Epoch 002) - May include children with $\geq 9\text{ }10$ years and $< 11\text{ }13$ years between last *Havrix* dose and Persistence Visit 1' (Year 10)

Synopsis and Section 5.1 Number of subjects

Approximately 600 subjects ~~born between 1st January 2005 and 31st December 2007~~ (about 300 subjects vaccinated with one dose of *Havrix* and about 300 subjects vaccinated with two doses of *Havrix*) are planned to be enrolled at each cross-sectional survey time points (Year 8 and Year 10)

Section 5.2 Inclusion criteria for enrolment

- ~~Children born between 1st January 2005 and 31st December 2007.~~
- Children with ≥ 7 years and $< 9\text{ }10$ years between last dose and Persistence Visit 1 (Year 8) and children $\geq 9\text{ }10$ years and $< 11\text{ }13$ years between last dose and Persistence Visit 1' (Year 10)

Section 6.4 Outline of study procedures

Table 3 Intervals between study visits/contacts/observations

Interval	Allowed interval ¹
Last <i>Havrix</i> dose vaccination date → Visit 1 Epoch 001 (Year 8 Survey)	≥ 2555 days to <3285-3650 days (7.0-8.99.9 years)
Last <i>Havrix</i> dose vaccination date → Visit 1' Epoch 002 (Year 10 Survey)	$\geq 3285-3650$ days to < 4045 days (9.010.0-10.912.9 years)

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

Section 8.3.3 According-to-Protocol (ATP) cohort for persistence at Years 8 or 10

The ATP cohort for persistence at Year 8 or at Year 10 will include all enrolled subjects:

- ~~who were born between 1st January 2005 and 31st December 2007.~~
- who have \geq 7 years and < 910 years between last dose and Persistence Visit 1 (Year 8) and children ≥ 910 years and < 1113 years between last dose and Persistence Visit 1' (Year 10).