Study Protocol
Sponsor:
GlaxoSmithKline Biologicals
14200 Shady Grove Road
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eTrack study number and Abbreviated Title	200318 (EPI-DENGUE-007 BOD)
Date of protocol	Final Version 2: 17 April 2015
Date of protocol amendment	Amendment 1 Final: 28 January 2016 Amendment 2 Final: 11 May 2017 Amendment 3 Final: 24 July 2017
Title	A cohort study to assess the burden of dengue illness in household members from selected communities in Southeast Asia and Latin America.
Detailed Title	A prospective, multi-centre, cohort study to assess the burden of dengue illness in household members (aged between 6 months and 50 years) from selected communities in Southeast Asia and Latin America.
Co-ordinating author	PPD, Scientific Writer
Contributing authors (Amended: 24 July 2017)	• PPD, Senior Manager, Epidemiology • PPD, Director, Epidemiology • PPD, PPD, PPD, Study Delivery Leads • PPD, Clinical Development and Research Lead • PPD, Specialist, Biostatistics • PPD, Statistician • PPD, Lead Statistician • PPD, Study Data Manager, for GSK Biologicals, from Tata Consultancy Services • PPD, Study Data Manger, for GSK Biologicals for Tata Consultancy Services • PPD, PPD, Clinical Safety representatives • PPD, Clinical read-out Lead (CRTL), CLS • PPD, Lab study manager • PPD, Director, Regional Epidemiology and Health Outcomes, Asia-Pacific • PPD, Senior Manager, Epidemiologist, LATAM

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 14.1.1

Protocol Amendment 3 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	200318 (EPI-DENGUE-007 BOD)
Date of protocol Amendment	Amendment 3 Final: 24 July 2017
Detailed Title	A prospective, multi-centre, cohort study to assess the burden of dengue illness in household members (aged between 6 months and 50 years) from selected communities in Southeast Asia and Latin America.
Sponsor signatory	Robert Paris US RDC Clinical & Epidemiology Project Lead GlaxoSmithKline Biologicals
Signature	_____
Date	_____

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Protocol Amendment 3 Rationale

Amendment number: Amendment 3
Rationale/background for changes: This amendment was developed to clarify that a blood sample will be collected for testing related to tertiary endpoints even when dengue is ruled out by the study physician during clinical examination.

Protocol Amendment 3 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 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.

eTrack study number and Abbreviated Title	200318 (EPI-DENGUE-007 BOD)
Date of protocol amendment	Amendment 3 Final: 24 July 2017
Detailed Title	A prospective, multi-centre, cohort study to assess the burden of dengue illness in household members (aged between 6 months and 50 years) from selected communities in Southeast Asia and Latin America.
Investigator name	_____
Signature	_____
Date	_____

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Sponsor Information

1. Sponsor

GlaxoSmithKline Biologicals

14200 Shady Grove Road

Rockville, MD 20850 USA

2. Sponsor Medical Expert for the Study

Refer to the local study contact information document.

3. Sponsor Study Monitor

Refer to the local study contact information document.

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

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to protocol Section 8.3.2.

SYNOPSIS

Detailed Title

A prospective, multi-centre, cohort study to assess the burden of dengue illness in household members (aged between 6 months and 50 years) from selected communities in Southeast Asia and Latin America.

Rationale for the study

The purpose of this study is to describe the burden of DENV illness among household members aged 6 months to 50 years of selected communities in Latin America and Southeast Asia. The study will describe the immune profile of study participants against DENV and possibly other flaviviruses at enrolment.

This study is also seeking to maintain/set up febrile illness surveillance and operational capacity in sites in countries of Latin America and Southeast Asia for the targeted study population (6 months to 50 years) with the perspective of preparing these sites for future dengue vaccine efficacy studies.

Objectives

Primary

- To estimate the overall incidence rate of reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR)-confirmed symptomatic DENV infection in a multi-centre cohort of household members aged 6 months to 50 years.

Secondary

- To estimate the incidence rate of virologically confirmed (through RT-qPCR or NS1) and probable (based on serological evidence) symptomatic DENV cases, separately and combined - by age, gender, site, serotype (if applicable) and anti-DENV IgG serological status at enrolment.
- To estimate the prevalence of anti-DENV immunoglobulin G (IgG) antibodies against dengue in the study population, overall and by age and site (at enrolment), for all participants and among dengue cases (confirmed and probable).
- To describe clinical presentations of dengue cases (confirmed and probable).

Tertiary

- To describe the spatial and temporal distribution of dengue cases (confirmed and probable) among cohort participants in the study areas and analyse determinants of spatio-temporal transmission (e.g. environmental, entomological, socio-demographic or ecological factors).
- To describe the DENV, and other flaviviruses (i.e. Japanese Encephalitis Virus [JEV; mostly relevant in Southeast Asia]) or West Nile Virus [WNV; mostly relevant in Latin America]) neutralising antibody profile in a subset of subjects.
- To explore other infectious aetiologies than dengue in subjects with episodes of febrile illness referred to as "suspected dengue case" (chikungunya, Zika, influenza, leptospirosis).
- To explore determinants of past dengue infection and dengue illness.
- To further characterise the immune status at enrolment (e.g. antibody avidity, etc.).

Study design

- Type of design: Prospective, multicentre, household-based, cohort study/surveillance study.
- Study population: Subjects aged between 6 months and 50 years at the time of enrolment living in geographically-defined communities in Latin America and Southeast Asia. The study population will comprise household members. The study population should include between 30% and 50% of adults (aged 18 years or above) per site.
- Recruitment: The appropriate recruitment strategy will be selected by each participating site. Two approaches may be considered: a school-based approach and a community-based approach without school involvement.

  A school-based approach: Recruitment will be initiated in primary and/or secondary schools. The parent(s)/legally acceptable representatives [LAR(s)] of the student attending participating schools will be asked to allow voluntary participation of their child/ward. The parent(s)/LAR(s) of the student or those living in the same household will also be invited to enrol himself/herself/themselves (if ≤ 50 years) and their younger/older child who may not be attending the study school(s). Other household members (if ≤ 50 years) will also be invited to participate in the study.

A community-based approach without school involvement: Recruitment will be organised by study staff at the household level and/or other participating sites. Study staff could also conduct home visits for recruitment but collection of blood sample will be done preferably at a designated facility.

- Duration of longitudinal follow-up and number of visits: Each subject will have 3 scheduled visits at enrolment, at Month 12 and a conclusion visit at Month 24. The Month 12 and Month 24 visits may occur via a telephone contact for the Mexican site. The study duration will be 24 months for each participating site. In countries with marked seasonality, the recruitment period would preferably occur outside the period of peak incidence of dengue, based on the local epidemiology of dengue in the past years and preferentially outside the holiday period (if the investigator believes that families are more likely to leave the study area during this period). These periods will be described for each site and may be modified upon mutual agreement between the investigator and the central study team based on available epidemiological information.
- Type of study: self-contained.
- Data collection: Electronic Case Report Form (eCRF).
- Duration of the study: Because of differences in local regulations, dengue seasonality and holiday period between countries, the recruitment period will be staggered. However, to facilitate site activities, sites will target to have all the subjects recruited in a 3-month period. For each subject, the study duration will be 24 months.
- Epoch 001: Prospective data collection starting at Visit 1 (Day 0) and ending at the conclusion visit (Month 24).

Synopsis Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)	Epochs
			Epoch 001
Prospective	1750	6 months to 50 years	x

Discussion of study design

A prospective cohort study design has been selected as it allows estimation of the incidence of infection in a defined population (with a known denominator), describing the spectrum of clinical outcomes and year-to-year variation.

The study population will be composed of individuals aged 6

months to 50 years. Households represent a platform of research to understand the transmission dynamics and spectrum of disease among closely-related individuals.

The study will include multiple sites, mainly because it will contribute to developing or maintaining the field infrastructure required for future vaccine phase III efficacy studies. In this perspective, multiple sites in different countries in Asia and Latin America will need to be involved to capture significant number of dengue cases caused by all four dengue serotypes. The study may allow for the detection of differences across sites in the transmission pattern.

The age range of the study population is large (6 months-50 years), including infants, young children, adolescents and adults. DENV infections affect human population of all age groups worldwide [WHO, 2012b]. Due to potential reduction of vaccine efficacy by maternal antibodies in infants and a reduced memory response [van Panhuis, 2011], infants aged < 6 months are currently not targeted for vaccination and thus, will not be included in this epidemiological study.

Number of subjects

Approximately, 1750 subjects are expected to be enrolled into this study with about 300-500 subjects expected per study site. Subjects aged between 6 months and 50 years at the time of enrolment living in geographically-defined communities in Latin America and Southeast Asia will be part of this study.

Endpoints

Primary

- RT-qPCR confirmed symptomatic DENV infection (all DENV types) during the study period.

Secondary

- DENV-type specific confirmed symptomatic DENV infection.
- Virologically confirmed (by RT-qPCR or NS1) and probable (based on serological evidence) symptomatic DENV infection.
- Status for anti-DENV IgG at enrolment (indicative of past DENV infection).
- Symptoms and severity of symptomatic dengue.

Tertiary

Exploratory objectives may be assessed based on, but not limited to the following endpoints:

- Spatial coordinates of the houses of participating households and of dengue cases and date of symptoms onset (applicable only for symptomatic cases).
- Neutralising antibodies titres against DENV 1-4.
- Neutralising antibody titres against other flaviviruses (such as JEV and WNV).
- Other infectious aetiology than dengue in subjects with episodes of febrile illness referred to as "suspected dengue case" (chikungunya, Zika, influenza, leptospirosis)
- Determinants of past dengue infection and dengue illness.
- Characteristics of immune status against DENV infection (e.g.: antibody avidity, etc.).

TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

LIST OF APPENDICES

PAGE

LIST OF ABBREVIATIONS

AE	Adverse Event
ATP	According-To-Protocol
CBC	Complete Blood Count
DENV	Dengue virus
DHF	Dengue Hemorrhagic Fever
DSS	Dengue Shock Syndrome
eCRF	electronic Case Report Form
ELISA	Enzyme-linked Immunosorbant Assay
GCP	Good Clinical Practice
GIS	Geographic Information System
GPP	Good Pharmacoepidemiology Practices
GPS	Global Positioning System
GSK	GlaxoSmithKline
HCT	Haematocrit
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IRB	Institutional Review Board
JE	Japanese Encephalitis
JEV	Japanese Encephalitis Virus
LAR	Legally Acceptable Representative
NA	Not applicable

NS1	Non-structural protein 1
RNA	Ribonucleic acid
RT-qPCR	Reverse-transcriptase quantitative polymerase chain reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SDC	Suspected Dengue Cases
SPM	Study Procedures Manual
WHO	World Health Organisation
WNV	West Nile Virus
YF	Yellow Fever

GLOSSARY OF TERMS

Adverse event:	Any untoward medical occurrence in a subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product, or temporally associated with a study procedure. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e., lack of efficacy), abuse or misuse.
Anonymised data:	Information about an individual that GSK or a third party cannot reasonably attribute to the individual, or could only attribute to the individual by expending a disproportionate amount of time, effort or expense (e.g. de-identified or aggregated information). For the purpose of this policy, Key-Coded personally identifiable information shall not be considered Anonymised Information
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.
Cohort study:	A form of epidemiological study where subjects in a study population are classified according to their exposure status/disease and followed over time (prospective/ retrospective) to ascertain the outcome(s).
Dengue naïve subject:	A subject who has no detectable anti-DENV antibodies (tested negative for anti-DENV IgG antibodies)

Designate:	A designate is defined as a person who helps the subject/subject's parent(s)/LAR(s) with performing some of the study procedures if the subject/subject's parent(s)/LAR(s) has difficulties to perform them alone (such as completion of diary log, receiving phone calls and planning of the study visits), e.g. a relative of the subject, a field worker who is linked to this study. Designates are appointed by the subject or his/ her LAR for help with the study procedures solely and cannot make decisions on behalf of the subject.
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
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 10.3 for details on criteria for evaluability).
Holiday period:	Public holidays or school holidays e.g. long school holidays or traditional holiday time for family vacations.
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.
Protocol amendment:	The International Conference on Harmonisation (ICH) defines a protocol amendment as: 'A written description of a change(s) to or formal clarification of a protocol.' GSK Biologicals further details this to include a change to an approved protocol that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study.

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.
Site Monitor:	An individual assigned by the sponsor who is responsible for assuring the proper conduct of epidemiological studies at one or more investigational sites.
Source Documents	Original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the epidemiological trial).
Study population:	Sample of population of interest.
Sub-cohort:	A subgroup of the total cohort of study subjects for whom the planned study procedures are different from those planned for the other study subjects.
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.
Surveillance:	The ongoing systematic collection, collation, analysis, and interpretation of descriptive epidemiological health data on a specific disease. Surveillance can monitor incidence and/or prevalence, and/or inform about when and where health problems are occurring and who is affected.

1. INTRODUCTION

1.1. Background

Dengue is a mosquito-borne disease endemic in large parts of Southeast Asia, Central and South America and in the Caribbean [Letson, 2010; Thomas, 2011]. Autochthonous transmission is also found in areas of Africa and Middle East. Dengue is caused by 4 flaviviruses, dengue virus (DENV) types 1-4, and results in acute febrile illness [Edelman, 2008; Thomas 2011].

Dengue fever, commonly of duration 2-7 days is accompanied by severe headache, muscle pain, joint pain, bone pain, and pain behind the eyes as well as gastrointestinal symptoms [Edelman 2008; WHO, 2012a]. In infants with dengue, high fever usually lasts 2-7 days as seen in older children. Upper respiratory tract symptoms like cough, nasal congestion, runny nose and dyspnoea, gastrointestinal symptoms and febrile convulsion are more common in infants than in older children [Capeding, 2010; Hung, 2004; Kalayanarooj, 2003]. Severe dengue is potentially fatal as plasma leakage may lead to shock and/or fluid accumulation with respiratory distress. Severe haemorrhage and severe organ impairment may develop. The critical period for complications occur 3–7 days after the first symptoms, typically coinciding with fever abatement (below 38°C/ 100°F) and can include warning signs such as severe abdominal pain, persistent vomiting, mucosal bleeding, clinical fluid accumulation, lethargy or restlessness [WHO, 2012b].

Transmission of dengue virus is variable: the intensity of transmission and distribution of serotypes typically vary significantly from one year to another. The four viruses act independently and immunity to one type only provides short-lived immunity to the other 3 viruses estimated to range from 4-6 months [Letson, 2010] to 1.6 years, as suggested by recent analysis of cohort studies [Endy, 2014]. Individuals who have recovered from a primary DENV infection are then fully susceptible to infection and disease by subsequent infection with heterologous dengue virus types [Endy, 2004]. Re-infection with the same serotype has been documented but rarely leads to disease. Neutralising antibodies elicited by a primary infection are predominantly serotype specific whereas cross-reactive responses are seen following secondary infection. Secondary DENV infection has been identified as an epidemiological risk factor for severe dengue. Third or even fourth infections are thought to be clinically mild, although the risk of developing severe dengue remains [Whitehorn, 2011].

Dengue transmission has been shown to be very focal in several settings [Mammen, 2008; Yoon, 2012; Honório, 2009]. In cluster sampling studies investigating infections in the peridomestic area of index cases, different levels of transmission intensity have been observed [Martínez-Vega, 2012]. Analysis of spatial dengue transmission is a key area of research to understand the disease and plan for control measures [Salje, 2012; Vazquez-Prokopec, 2010].

1.2. Rationale for the study

The purpose of this study is to describe the burden of DENV illness among household members aged 6 months to 50 years of selected communities in Latin America and Southeast Asia. The results will inform the design of future clinical trials, supporting decision making on target age groups and stratification, sample size estimation, recruitment method. The study will describe the population pre-existing immune status to dengue and other relevant flaviviruses reflecting past exposure. It will explore spatial and temporal characteristics of dengue transmission in the selected communities. The data generated may support modelling work to assess the impact of heterogeneity (heterogeneity of exposure, heterogeneity of individual response influenced by the pre-existing immune status) on the outcomes of an efficacy trial.

In addition, the study may shed light of other infectious aetiologies than dengue in patients with febrile illness episodes.

This study is also seeking to maintain/set up febrile illness surveillance and operational capacity in sites in countries of Latin America and Southeast Asia for the targeted study population (6 months to 50 years) with the perspective of preparing these sites for future dengue vaccine efficacy and effectiveness studies.

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 procedure: Blood sample collection.	Pain at blood sampling site	• Well-trained person should take the blood sample • Use needle of smaller gauge than the vein
	Haematoma or thrombus	• 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	• 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

The subjects may not receive any other direct benefit than facilitated access to healthcare in case of febrile illness during study conduct. Appropriate care will be provided to the subjects diagnosed with dengue or other diseases.

2.3. Overall Benefit: Risk Conclusion

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

3. OBJECTIVES

3.1. Primary objective

- To estimate the overall incidence rate of reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR)-confirmed symptomatic DENV infection in a multi-centre cohort of household members aged 6 months to 50 years.

Refer to Section 10.1.1 for the definition of the primary endpoint.

3.2. Secondary objectives

- To estimate the incidence rate of virologically confirmed (through RT-qPCR or NS1) and probable (based on serological evidence) symptomatic DENV cases, separately and combined - by age, gender, site, serotype (if applicable) and anti-DENV IgG serological status at enrolment.
- To estimate the prevalence of anti-DENV immunoglobulin G (IgG) antibodies against dengue in the study population, overall and by age and site (at enrolment), for all participants and among dengue cases (confirmed and probable).
- To describe clinical presentations of dengue cases (confirmed and probable).

Refer to Section 10.1.2 for the definition of the secondary endpoints.

3.3. Tertiary objectives

- To describe the spatial and temporal distribution of dengue cases (confirmed and probable) among cohort participants in the study areas and analyse determinants of spatio-temporal transmission (e.g. environmental, entomological, socio-demographic or ecological factors).
- To describe the DENV, and other flaviviruses (i.e. Japanese Encephalitis Virus [JEV; mostly relevant in Southeast Asia]) or West Nile Virus [WNV; mostly relevant in Latin America]) neutralising antibody profile in a subset of subjects.
- To explore other infectious aetiologies than dengue in subjects with episodes of febrile illness referred to as “suspected dengue case” (chikungunya, Zika, influenza, leptospirosis).
- To explore determinants of past dengue infection and dengue illness.
- To further characterise the immune status at enrolment (e.g. antibody avidity, etc.).

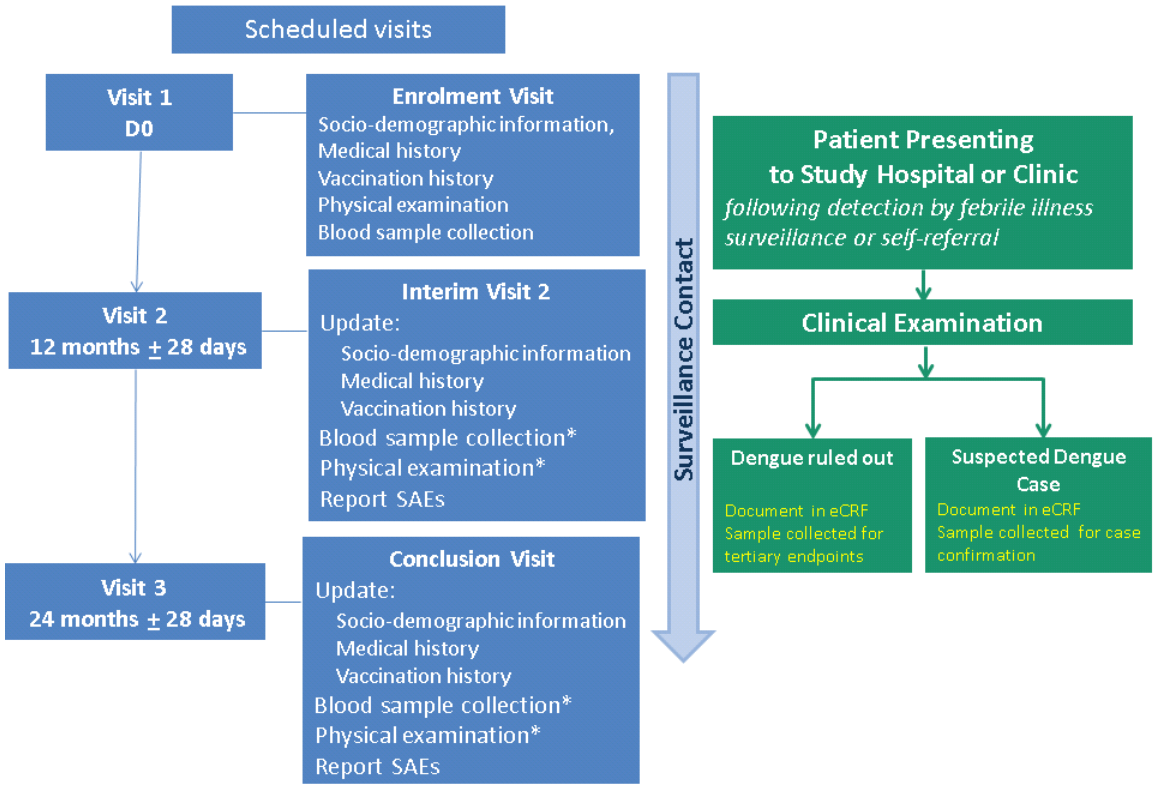
Refer to Section 10.1.3 for the definition of the tertiary endpoints.

4. STUDY DESIGN OVERVIEW

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 7.4), are essential and required for study conduct.

Figure 1 presents a schematic representation of the study design.

Figure 1 Schematic representation of the study design



***The Month 12 and Month 24 visits may occur via a telephone contact for the Mexican site and there will be no blood sample collection and physical examination at these visits for subjects in Mexico.**

- Type of design: Prospective, multi-country, multi-centre, household-based, cohort study/surveillance study.
- Study population: Subjects aged between 6 months and 50 years at the time of enrolment living in geographically-defined communities in Latin America and Southeast Asia. The study population will comprise household members. The study population should include between 30% and 50% of adults (aged 18 years or above) per site.
- Recruitment: The appropriate recruitment strategy will be selected by each participating site. Two approaches may be considered: a school-based approach and a community-based approach without school involvement (refer to Section 6.2).
- Duration of longitudinal follow-up and number of visits: The study duration will be 24 months for each participating site. Subjects will have a scheduled visit at

enrolment, one visit at Month 12 and a conclusion visit at Month 24. The Month 12 and Month 24 visits may occur via a telephone contact for the Mexican site. The number of visits in case of febrile illness, referred as "suspected symptomatic dengue case", will vary by subject.

- Type of study: self-contained.
- Data collection: Electronic Case Report Form (eCRF).
- Duration of the study: Because of differences in local regulations, dengue seasonality and holiday period between countries, the recruitment period will be staggered. However, to facilitate site activities, sites will target to have all the subjects recruited in a 3-month period. For each subject, the study duration will be approximately 24 months.
- Epoch 001: Prospective data collection starting at Visit 1 (Day 0) and ending at the conclusion visit (Month 24).

Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)	Epochs
			Epoch 001
Prospective	1750	6 months to 50 years	x

4.1. Discussion of study design

4.1.1. Rationale for study design

A prospective cohort study design has been selected as it allows estimation of the incidence of infection in a defined population (with a known denominator), describing the spectrum of clinical outcomes and year-to-year variation. This design is suited to study the natural infection history and identify marker(s) associated with protection against future symptomatic dengue infection.

The study population will be composed of individuals aged 6 months to 50 years. Households represent a platform of research to understand the transmission dynamics and spectrum of disease among closely-related individuals. Dengue cases tend to cluster within households, which could be the result of a single or very few infected mosquitoes biting different household members during a short period of time [Dussart, 2012].

The study will include multiple sites, mainly because it will contribute to developing or maintaining the field infrastructure required for future vaccine phase III efficacy studies. In this perspective, several sites in different countries in Asia and Latin America will need to be involved to capture significant number of dengue cases caused by all four dengue serotypes. The study may allow for the detection of differences across sites in the transmission pattern.

The age range of the study population is large (6 months-50 years), including infants, young children, adolescents and adults. Dengue virus infections affect human population of all age groups worldwide [WHO, 2012b]. The clinical burden of dengue (in particular severe dengue) in much of Southeast Asia primarily falls on children/adolescents aged less than 15 years. However, dengue also affects adults throughout the world including several Southeast Asian countries that include Malaysia, Sri Lanka, and Singapore [Cuong, 2011]. In a recent study in Hanoi, Vietnam, 85% of notified dengue cases were aged over 15 years [Cuong, 2011]. In several countries in Latin America, incidence of dengue was highest in teenagers and young adults [San Martín, 2010]. In Mexico, the incidence of dengue peaks in the age range of 10 to 19 years [Epidemiological Panorama Dengue Fever and Hemorrhagic Fever, 2014]. Due to potential reduction of vaccine efficacy by maternal antibodies in infants and a reduced memory response [Van Panhuis, 2011], infants aged < 6 months are currently not targeted for vaccination and thus, will not be included in this epidemiological study. Because dengue is endemic in the countries where study sites are located, the study population should have a mixed anti-DENV immunological background, providing an opportunity to describe different response patterns to new infections.

5. CASE DEFINITIONS

The following case definitions will be used in this study.

5.1. Suspected symptomatic dengue case (SDC)

Acute febrile illness measured as ≥ 38.0°C with a thermometer by any route or recent history of febrile illness (onset in the past 8 days) reported by the subject/the parent(s)/LAR(s)/ designate (if applicable) of the subject for at least two consecutive days (a duration of approximately 36-48 hours) and of less than 7 days duration, which may be accompanied by other dengue symptoms or signs and does not have a defined focus or an obvious reason unrelated to dengue (based on physician judgement).

Note: Besides fever, other dengue associated signs and symptoms include but are not limited to: upper respiratory tract symptoms like cough, nasal congestion, runny nose and dyspnoea, gastrointestinal symptoms and febrile convulsion in infants/older children and fatigue, headache, pain behind the eyes, abdominal pain, nausea, vomiting, muscle ache, joint ache, diffuse rash on the trunk, itching of skin (pruritis) in adults/adolescents.

A number of infectious and non-infectious diseases mimic dengue and severe dengue. Physicians should be careful not to rule out dengue diagnosis too quickly, when the differential diagnosis cannot be accurately made. Early symptoms of dengue may include gastrointestinal and upper respiratory symptoms and may be misdiagnosed as diarrhoeal diseases or other diseases with flu-like symptoms. The WHO handbook for clinical management of dengue provides a list of conditions that mimic the febrile phase and the critical phase of dengue infection [WHO, 2012b].

A SDC presenting at the health care facility within 5 days following the onset of symptoms will be defined as an 'early presenter'. A SDC presenting at the health care facility 6 days or more after the onset of symptoms will be defined as 'late presenter'. Both early and late presenters will have a scheduled visit at the study healthcare centre including blood sampling for laboratory diagnosis.

Seven consecutive calendar days without fever (body temperature ≥ 38.0°C), in the absence of antipyretic medication, are required to separate two episodes of SDC.

5.2. Laboratory confirmation

- **RT-qPCR confirmed symptomatic DENV infection**

A SDC confirmed by RT-qPCR

- **A virologically confirmed symptomatic DENV infection**

A SDC confirmed by RT-qPCR or NS1.

- **Probable dengue case**

A SDC with:

  – DENV RT-qPCR negative or not performed (late presenter)

and

  – DENV Non-structural protein 1 (NS1) negative or undetermined (early or late presenter)

and

  – Anti-DENV Immunoglobulin M (IgM) positive with a rapid immunochromatographic (ICT) assay or an ELISA assay)

Or

  – Anti-DENV Ig G positive (rapid ICT assay or 'capture ELISA' assay)

5.3. Classification of severity of dengue

The clinical and biological signs at presentation of SDC, potential return visits and during hospitalisation will be recorded. The physician will record in the eCRF the occurrence of warning signs (2009 WHO definition) during the course of illness.

When the episode is considered terminated (which may be at the follow-up contact for subjects with ambulatory management or at hospital discharged for hospitalized subjects), the physician will record in the eCRF whether the patient met criteria for dengue haemorrhagic fever or dengue shock syndrome according to the 1997 WHO definition and for severe dengue according to the 2009 WHO definition.

The classification of severity for research purpose within the study should not interfere with patient management, which will be conducted as per local guidelines.

5.3.1. The 1997 WHO classification [WHO, 1997]

Criteria for dengue hemorrhagic fever (DHF): The following criteria must all be present:

- Fever, or history of acute fever, lasting 2–7 days, occasionally biphasic,
- Haemorrhagic- tendencies, evidenced by at least one of the following
  - A positive tourniquet test,
  - Petechiae, ecchymoses or purpura,
  - Bleeding from the mucosa, gastrointestinal tract, injection sites or other locations,
  - Haematemesis or melaena,
- Thrombocytopenia (100,000 cells per mm^3 or less),
- Evidence of plasma leakage due to increased vascular permeability, manifested by at least one of the following:
  - A rise in the haematocrit (HCT) equal to or greater than 20% above average for age, sex and population,
  - A drop in the HCT following volume-replacement treatment equal to or greater than 20% of baseline,
  - Signs of plasma leakage such as pleural effusion, ascites and hypoproteinaemia,

Criteria for dengue shock syndrome (DSS): The criteria mentioned for DHF must all be present, plus evidence of circulatory failure manifested by:

- Rapid and weak pulse, and
- Narrow pulse pressure [<20mmHg (2.7 kPa)]

OR

- Hypotension for age (this is defined as systolic pressure < 80 mmHg for those aged less than 5 years, or < 90 mmHg for those ages 5 years and older), and
- Cold, clammy skin and restlessness.

5.3.2. The 2009 WHO classification [WHO, 2009, Gutiérrez, 2013; Macedo, 2014]

Criteria for dengue with warning signs: At least one of the following should be present:

- Abdominal pain or tenderness,
- Persistent vomiting (three or more emesis in a period of one hour, or five or more in a period of six hours),

- Clinical fluid accumulation (peri-orbital, facial or lower limb oedema as reported by the study physician, or pleural effusion, ascites or gall-bladder wall thickening ≥3 mm as observed via X-ray radiography or ultrasonography),
- Mucosal bleed (any of the following: hemoptysis, epistaxis, gingival bleeding, melena, hematemesis, hematuria, menorrhagia, vaginal bleeding, or subconjunctival hemorrhage as observed by a study physician or reported by the patient),
- Liver enlargement (liver enlarged >2 cm below the edge of the ribs as palpated by a study physician),
- Increase in HCT concurrent with rapid decrease in platelet count (interpreted as any HCT >20% over baseline with platelet 50,000/mm^3),
- Lethargy (Glasgow coma scale score <15 for children aged 5 years or more or Blantyre coma scale <5 for children under 5, as evaluated by a study physician) and restlessness.

Criteria for severe dengue: dengue with at least one of the following criteria:

- Severe Plasma Leakage leading to Shock (DSS),[1]

  Fluid accumulation with respiratory distress[2],
- Severe Bleeding[3],
- Severe organ involvement;

  Liver: AST or ALT ≥ 1000 IU/L,

  Central nervous system: impaired consciousness[4] ,

  Failure of heart and other organs[5].

[1] A clinical state of reduced perfusion to end-organs (such as the skin), defined as presence of a weak pulse, and/or narrowing of pulse pressure, and/or hypotension for age and one of the following: 1) Cold, clammy skin, 2) Increased capillary refill time, 3) Peripheral cyanosis, 4) Skin mottling

[2] defined as respiratory discomfort, dyspnea, respiratory failure, or increased respiratory rate of >60 breaths/min for ages <2 months; >50 breaths/min for ages 2 months to 1 year; >40 breaths/min for ages 1 to 5 years; >30 breaths/min for ages 5 to 8 years; and >20 breaths/min for those older than 8 years);

[3] As evaluated by clinician WHO Grade 2 or above: haematemesis, melena, menorrhagia or clinical drop in haemoglobin requiring whole blood or packed red cell transfusion;

[4] Any reduction in coma score that may be accompanied by convulsions and/or meningism and/or abnormal neurological signs

[5] For renal impairment: Stage 2 Acute Kidney Injury defined as serum creatinine increase of 100% over baseline or calculated norm for age/gender/race)

6. STUDY POPULATION

The study will be conducted in countries of Latin America and Southeast Asia.

6.1. Number of subjects/ centres

Approximately, 1750 subjects are expected to be enrolled into this study with about 300 to 500 subjects expected per study site. Subjects aged between 6 months and 50 years at the time of enrolment living in geographically-defined communities in Latin America and Southeast Asia will be part of this study. The number of households will depend on the number of subjects enrolled per household. For a site enrolling 500 subjects, the number of households would likely range between 125 (assuming 4 subjects on average per household) and 250 (assuming 2 subjects on average per household).

6.2. Overview of recruitment plan

The recruitment strategy will be selected by each participating site. One of the two approaches may be considered:

- **A school-based approach**

Recruitment will be initiated in primary and/or secondary schools. The parent(s)/ LAR(s) of the student attending participating schools will be asked to allow voluntarily participation of their child/ward. The parent(s)/LAR(s) of these students will also be invited to enrol himself/herself/themselves (if ≤ 50 years) and their younger/older child who may not be attending the study school(s). Other household members will also be invited to participate in the study (if ≤ 50 years).

Recruitment will be organised by study staff, preferably at participating schools (primary and/or secondary) or designated facility (e.g. healthcare centre, local community centre, etc.). If any member of a given household refuses to participate in the study, it will not preclude inclusion of other household members. Study staff could also conduct home visits for recruitment but collection of blood sample will be done preferably at a designated facility.

- **A community-based approach (without school involvement)**

Recruitment will be organised by study staff at the household level and/or at other designated facilities (e.g. healthcare centre, local community centre, etc.), using various modes of communication (e.g.: public announcements or community meetings to inform about the study). Study staff could also conduct home visits for recruitment but collection of blood sample will be done preferably at a designated facility.

Whichever approach is chosen, the various modes of communication used for recruitment will be reviewed and approved by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) according to the local legislation (for e.g.: public announcements or community meetings to inform about the study).

Note: For the first 3 months of the study after overall study start (in each centre), the recruitment target will be fixed for every centre. At month 3 of enrolment at each centre, the recruitment per country will be evaluated. If at the time of evaluation, recruitment at one or more countries is below the expected target, the number of subjects to be recruited will be re-evaluated.

Recruitment period

In countries with marked seasonality, the recruitment period should preferably occur outside the period of peak incidence of dengue, based on the local epidemiology of dengue in the past years. It should also preferably occur outside the holiday period if the investigator believes that families are more likely to leave the study area during this period.

These periods will be described for each site and may be modified upon mutual agreement between the investigator and the central study team based on available epidemiological information.

6.2.1. Recruitment of study centres

Selection of communities and schools

The following criteria will be considered as guidance for selecting the communities.

- Areas which are known to be endemic for dengue with high rates of transmission in most recent years.
- Easy access for the study population to a healthcare centre that can evaluate SDC (fast track) and manage the enrolled subjects.
- Safe access by study personnel.

In addition, the following criteria will be considered as guidance for selecting schools, in case the school based approach is selected for recruitment:

- Of sufficient size to allow the enrolment target of 300-500 subjects (children and adults) per site in one geographically-defined community.

6.3. Inclusion criteria for subject enrolment

Deviations from inclusion criteria are not allowed because they can potentially jeopardise the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

All subjects must satisfy ALL the following criteria at study entry:

- Written and signed informed consent (and assent if the subject is below the legal age of consent) obtained from the subject/from subject's parent(s)/LAR(s). For a subject below the legal age of consent is, his/her signature will be obtained on the informed assent form, if applicable.

- A male or female between, and including 6 months and 50 years of age at the time of enrolment (Subjects become ineligible on their 51st birthday).
- Subject and/or the subject's parent(s)/LAR(s) who the investigator believes can comply with the requirements of the protocol (e.g., willingness to go to the hospital/healthcare centre for visit(s) in case of acute febrile illness, able to observe the signs of dengue and to understand how to take and report body temperature, etc).
- Subject who plans, at the time of enrolment, to remain at same residence/study area during the one or two year study period (as applicable).
- Household should be reachable by phone (residence phone or mobile phone).

Note: Pregnant or lactating female or female planning to become pregnant can be recruited into the study.

6.4. Exclusion criteria for subject 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.*
- Participation (current or planned) in another epidemiological study or in a clinical trial that would conflict with the current study, based on investigator's judgement.
- Terminal illness or severe mental incapacity.

7. CONDUCT OF THE STUDY

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

The study will be conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP), 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:

- IRB/ IEC review and favourable opinion/approval of study protocol and any subsequent amendments.
- Subject/ 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 and/or 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, 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 GCP, a subject who can only be enrolled in the study with the consent of his/her parent(s) or LAR(s) (e.g., minors/ subjects below the legal age for consent), should be informed about the study to the extent compatible with the subject's understanding and, if capable, the subject should sign and personally date a written informed assent form (IAF). It is required that the assent be signed by each subject, if capable, in addition to the informed consent that is to be signed by his/her 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.

7.2. Subject and household number attribution

Subject numbers will be assigned sequentially to subjects consenting to participate/to be included in the study, according to the range of subject numbers allocated to each study centre. Household numbers will also be assigned sequentially at each site to eligible household.

7.3. General study aspects

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying study procedures manual (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.

7.4. Outline of study procedures

Table 2 presents the list of study procedures.

Table 2 List of study procedures

Age		6 months to 50 Years					
Epoch		Epoch 001					
Data Collection		Prospective data Collection					
Visit		Scheduled visits			Unscheduled visits (for acute febrile illness and suspected dengue at hospital/healthcare centre)		
Time points	Visit 1 Day 0	Regular Surveillance contact between visits[a]	Visit 2 Month 12 [g]	Conclusion visit 3 Month 24[g]	First visit	Return visit[*]	Follow-up contact[**]
Informed consent and assent as applicable	●						
Check inclusion/exclusion criteria	●						
Check evidence of subject being member of the household (family book or other administrative document)	O						
Attribute subject number and household number attribution	●						
Collect spatial coordinates of households[e]	●[e]						
Record/ update household and socio-demographic information	●		●	●			
Medical history and Japanese encephalitis (JE)/Yellow fever (YF)/ Dengue (if applicable) vaccination history or updates	●		●	●			
Distribute subject ID card and instruction kit[d]	O						
Collect blood sample	●		●[f]	●[f]			
Instruct/ remind subjects/subjects' parent(s)/LAR(s) or designate (if applicable) on assessment procedures in case of acute febrile illness	●	O					

Age		6 months to 50 Years					
Epoch		Epoch 001					
Data Collection		Prospective data Collection					
Visit		Scheduled visits			Unscheduled visits (for acute febrile illness and suspected dengue at hospital/healthcare centre)		
Time points	Visit 1 Day 0	Regular Surveillance contact between visits[a]	Visit 2 Month 12 [g]	Conclusion visit 3 Month 24[g]	First visit	Return visit[*]	Follow-up contact[**]
Issue diary logs to subject/subject's parent(s)/LAR(s)/ designate and train them on how it is to be filled out in the event of an acute febrile illness[†]	O	O			O		
Contact the subject/subject's parent(s)/LAR(s)/ designate regarding any acute febrile illness and remind him/her/them of the procedures		O					
Physical examination and medical history (see Section 7.5.4)	●		●[f]	●[f]	●	●	
Collect or verify diary logs if applicable[b]					O	O	
Record if subject meets the definition of a SDC; if dengue is discarded, record the alternative diagnosis					●		
Record laboratory results prescribed as per local guidelines by the treating physician (may include CBC, liver enzymes, bilirubin)					●	●	
Record patient management [c]					●	●	
Collect blood sample for diagnosis[#]					●		
Outcome of dengue episode							●
Report Serious Adverse Events (SAEs) related to study procedures[‡]	●		●	●	●	●	●
Study conclusion				●			

● is used to indicate a study procedure that requires documentation in the individual eCRF.
O is used to indicate a study procedure that does not require documentation in the individual eCRF.
* A return visit is a visit linked to the first visit for acute febrile illness, occurring if the subject's physical condition necessitates medical evaluation, according to local medical practice. The investigator will determine whether this return visit is necessary and whether it is considered as part of the same episode. See Section 7.5.2 for details.
** A follow-up contact will be done to record the outcome of the dengue episode
[a]Refer to section 7.5.3 for the more details on regular contact between study visits.

[b]A diary log given at visit 1 and potentially subsequently (via surveillance contacts or mail) are only to be filled out in the event that the subject has acute febrile illness or suspected dengue symptoms. See Section 7.5.2for details.

[c] Depending on the clinical manifestations and other circumstances, patients may be either be sent home, be referred for in-hospital management for close observation or require emergency treatment and urgent referral. In case of in hospital/emergency room management, data on clinical evolution and results of biological and paraclinical examinations will be collected in the eCRF.

[d]The instruction kit includes a thermometer, study contact information (phone numbers) and an instruction card with information about dengue symptoms and steps to take if acute febrile illness occurs.

[e]Collection of spatial coordinates can be done at any time during the recruitment period and not necessarily at Visit 1.

[f]The blood sample collection, physical examination and collection of medical history procedures at Month 12 and Month 24 were included as part of the protocol amendment 2 and will not be applicable for Mexico.

[g]The Month 12 and Month 24 visits may occur via a telephone contact for the Mexican site.

[#]Testing includes RT-qPCR and NS1/IgG capture/ IgM rapid ICT or ELISA. One purpose is to provide rapid results to the physician and the subject.

[‡]SAE = Serious adverse event- see Section 7.1.1for definition.

Table 3 presents the procedures involved in case of hospitalisation or urgent care visit at any non-study health care facility for acute febrile illness and SDC detected through active surveillance.

Table 3 Procedures in case of urgent care visit or hospitalisation or at any non-study health care facility for acute febrile illness and SDC detected through active surveillance

Procedures involved	Time point/Data to be collected	Documentation
Timeframe to record the event	As soon as possible after detection of event	○
Contact with hospital/health care facility visited by the patient	Phone call/visit, as appropriate	○
Data to be collected	Date of visit/entry, main symptoms and severity, diagnosis, date of discharge (if applicable)	●
Verification of diagnoses	By phone contact/visit with treating physician/written report by treating physician/hospital discharge report	○

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

Table 4 presents the intervals between study visits/contacts.

Table 4 Intervals between study visits/contacts

Interval	Optimal length of interval*	Allowed interval
Visit 1 (Day 28) → Visit 2 (Month12)	12 months	±28 days
Visit 2 (Month 12) → Conclusion visit (Month 24)	12 months	± 28 days
Interval between First visit for a SDC and the final follow-up contact for a case	21 days	± 7 days

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

7.5. Detailed description of study procedures

There will be one scheduled visit (Visit 1) at enrolment, a second visit at Month 12 (Visit 2) and a final conclusion visit at Month 24 (Visit 3). The Month 12 and Month 24 visits may occur via a telephone contact for the Mexican site. During the study, detection of febrile illness in the cohort will be performed using active surveillance and enhanced passive surveillance. Subjects with febrile illness will have a medical visit; if the subject meets the case definition of SDC, a blood sample will be collected for laboratory diagnosis.

7.5.1. Procedures at scheduled visit 1 (enrolment)

The visit 1 (enrolment) will take place at a designated facility, as determined by each participating site. Home visits may also be organised, at the convenience of the study team.

7.5.1.1. Informed consent and assent

The informed consent/assent will be obtained from all eligible subjects of the household, participating in the study.

The signed/witnessed/thumb printed informed consent of the subject/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 7.1 for the requirements on how to obtain informed consent and assent, as appropriate.

7.5.1.2. Check inclusion and exclusion criteria

Check all applicable inclusion and exclusion criteria as described in Sections 6.3 and 6.4 before enrolment.

7.5.1.3. Check evidence of subject being member of the household

The evidence of the subjects being members of the same household (e.g. family book or other administrative documents) will be checked by the site and then documented in the source notes.

7.5.1.4. Attribute subject number and household number

Refer to Section 7.2.

7.5.1.5. Collect spatial coordinates of the households

The participating households may be geo-referenced with GPS (Global Positioning System) devices. This method has been used in other studies characterising spatial transmission and using households or individual dengue cases as the primary unit of analysis [Martínez-Vega, 2012; Rabaa, 2013; Siqueira-Junior, 2008; Paz-Soldan, 2014].

A geographic masking technique may be applied (see Section 11.7) to remove the possibility of re-identification of participants.

Collection of spatial coordinates may be done at any time during the recruitment period and not necessarily at Visit 1.

7.5.1.6. Record socio-demographic and household information

Socio-demographic and household information such as age, gender, type of dwelling and parental relationship with other participants in the house will be collected and recorded in the eCRF at each scheduled visit.

7.5.1.7. Physical examination, medical history and JE/YF/dengue vaccination history

A detailed clinical examination will be carried out to assess the subject's general condition, cardiac and respiratory rates, blood pressure, dengue associated clinical signs/symptoms.

Information on subject's medical history and JE, YF and dengue vaccination history (if applicable, should a vaccine be licensed and available) will be collected and recorded in the eCRF.

7.5.1.8. Distribute subject ID card and instruction kit

Each household will be given a instruction kit which includes a thermometer, study contact information (phone numbers) and an instruction card with information about dengue symptoms and steps to take if acute febrile illness occurs.

7.5.1.9. Collect blood sample (Amended: 24 July 2017)

At Visit 1, a blood sample of 3.5 mL will be collected from subjects aged between 6 months and 2 years (all sites), and a blood sample of 10 mL will be collected from subjects aged between 2 and 50 years (all sites).

7.5.1.10. Instruct/ remind subjects/subject's parent(s)/LAR(s) or designate on assessment procedures in case of acute febrile illness

Study personnel will train subjects/subject's parent(s)/LAR(s) or designate (if applicable) to recognise acute febrile illness, and will instruct them to contact the study personnel or come to a designated study healthcare centre/ hospital for medical evaluation as soon as possible, preferably within 5 days of the occurrence of acute febrile illness.

7.5.1.11. Issue diary logs to subject/subject's parent(s)/LAR(s) or designate and train them on how it is to be filled out in the event of an acute febrile illness

The subjects'/subjects' parent(s)/LAR(s) or designate (if applicable) will be instructed to record temperature and main symptoms in a diary log in case of suspected acute febrile illness until the medical appointment. The diary log will complement the patient interview and will facilitate the recording of symptoms in the eCRF. Please refer to the SPM for more details on the dengue kit and diary log. If a subject/subject's parent(s)/LAR(s) is illiterate and is unable to complete the diary log on his/her own, he/she may be helped by a designate (refer to the GLOSSARY OF TERMS for the definition of designate).

7.5.1.12. Report Serious Adverse Events (SAEs) related to study procedures

All SAEs related to study procedures (blood collection) will be recorded in the eCRF.

7.5.2. Procedures at Visit 2 and conclusion visit (Visit 3) (Amended: 24 July 2017)

The Visit 2 and Visit 3 will take place at a designated facility, as determined by each participating site. Home visits may also be organised, at the convenience of the study team. The socio-demographic information will be updated in the eCRF (e.g. change of household during the conduct of the study). The Japanese encephalitis (JE) /Yellow fever (YF)/Dengue (if applicable) vaccination history will be updated.

A detailed clinical examination will be carried out to assess the subject's general condition, cardiac and respiratory rates, blood pressure, dengue associated clinical signs/symptoms.

A blood sample of 3.5 mL will be collected from subjects aged between 6 months and 2 years (all sites), and a blood sample of 10mL will be collected from subjects aged between 2 and 50 years (all sites). The Month 12 and Month 24 visits may occur via a telephone contact for the Mexican site and there will be no physical examination and blood sample collection at these visits for subjects in Mexico.

At study conclusion (Visit 3) for each subject, the investigator will:

- Review all the data collected to ensure accuracy and completeness
- Complete the study conclusion screen in the eCRF.

7.5.3. Dengue surveillance

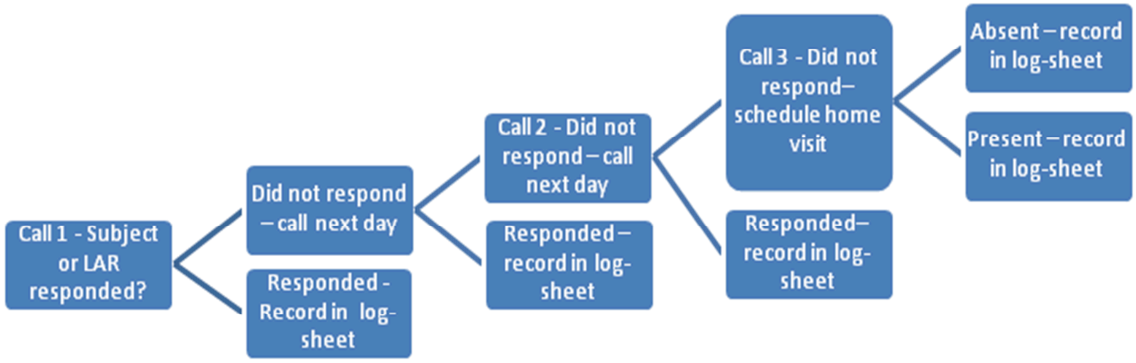
Between scheduled visits, detection of SDC in the cohort will be performed using active surveillance of acute febrile illness and enhanced passive surveillance.

- **Active surveillance**: Households will be contacted regularly to enquire about the occurrence of febrile illness among the study subjects. Preferably, the frequency of contacts will be once per week. In sites with marked dengue seasonality, the frequency of contact may be twice per month during periods of low transmission of dengue. These periods may be modified upon decision of the central study team based on available epidemiological information. The mode of contact may include phone calls and/or house visits. In addition to the regular phone contact, some sites may opt to check the school absenteeism log and contact the household of the children who are absent.
- If febrile illness is identified during active surveillance, an appointment will be arranged at the designated study healthcare centre/ hospital as soon as possible (preferably within 5 days following the onset of symptoms).

A household visit may be planned if the subject cannot come to the designated healthcare centre/hospital. This situation should, however, remain exceptional.

- The surveillance operations will be tracked through an active tracking algorithm. In sites performing surveillance through phone contacts in first intention, there should be at least three documented attempts to contact the household before a home visit is planned (refer Figure 2). In sites where surveillance is performed routinely by other means (e.g. home visits), there should also be three documented attempts to contact the household.

Figure 2 Active tracking algorithm



- **Enhanced passive surveillance (spontaneous referral):** This form of surveillance will be key in maximising the likelihood to detect SDC early, during the course of illness. Study personnel will instruct subjects/subject's parent(s)/LAR(s)/ designate (if applicable) to contact the study personnel or come to a designated study healthcare centre/hospital for medical evaluation in case of febrile illness (preferably within 5 days of onset of febrile illness).

7.5.4. Procedures at unscheduled visits and management of SDC (Amended: 24 July 2017)

- Suspected cases in the cohort may arise from three sources: spontaneous referral (enhanced passive surveillance), referral by study personnel during scheduled study visits, or as a result of active surveillance.
- All subjects with acute febrile illness should be seen at a designated study healthcare centre/hospital by a study physician. If dengue is ruled out by the study physician during clinical examination because of another obvious alternative diagnosis (identified focus of fever), the physician will document his alternative diagnosis, and ***collect a blood sample for testing related to tertiary endpoints***.

  Examples of obvious alternative diagnosis are abscess with cellulitis, dental/ gingival problems with cellulitis, skin infections with cellulitis, eye infections with periorbital cellulitis, otitis media with pain, pneumonia, meningitis, bacillary dysentery, purulent tonsillitis, urinary tract infections/pyelonephritis, cholecystitis, peritonitis, appendicitis, moderate to severe trauma from accidents, post operation fever.

- Subjects who, for any reason, seek medical assistance at any non-study health care facility may be identified, for instance by active surveillance, and if feasible, clinical data will be retrospectively collected on the eCRF from those non-study healthcare facilities. A study physician will be responsible for collecting the retrospective data and informing the local study coordinator for appropriate follow-up. Refer to Table 3 for further details.
- The first visit to the study healthcare centre/hospital will include a detailed clinical examination to assess the subject's general condition, cardiac and respiratory rates, blood pressure, dengue associated clinical signs/symptoms and other relevant clinical signs/symptoms or paraclinical examinations (for example detection of clinical fluid accumulation with ultrasonography). A blood sample should be collected by the healthcare centre/hospital for confirmation of dengue by RT-qPCR technique for early presenters. Rapid dengue diagnostic tests (NS1, IgM, and IgG) will be performed as well because a result can be provided rapidly to the physician and subject and support the diagnosis. These tests will also provide indications on the diagnosis for late presenters, in which RT-qPCR will not be performed. If other tests (such as CBC, liver enzymes, bilirubin) are prescribed by the treating physician as part of routine assessment, the results will be recorded in the eCRF.
- Record patient management:
  Depending on the clinical manifestations and other circumstances (co-morbidities, etc.), patients may either be sent home, be referred for in-hospital management for close observation or require emergency treatment and urgent referral. In case of hospital/emergency room management, data on clinical evolution and main results of biological (platelet counts, evidence of hemoconcentration, etc.) and paraclinical examinations (e.g. chest X-ray, ultrasonography, etc.) will be collected in the eCRF. The duration of hospitalisation, potential management in the intensive care unit and the treatment administered (fluid therapy/ blood product transfusion) will also be collected in the eCRF.
- Subsequent visits ('return visits') may be needed and will be conducted as directed by national guidelines by the study physician. Medical data related to any SDC will be collected for hospitalised subjects and for subjects in outpatient settings at return visits. Treatment will be given according to local standard routines, following national guidelines.
- The physician will classify the severity of the episode in the eCRF at the time of follow-up contact or hospital discharge, if applicable.

7.6. Biological sample handling and analysis

Please refer to the SPM and any document provided e.g.: Investigator manual, 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/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 on the samples collected for the study except those described in the protocol or its amendment(s). Other biological assays prescribed as part of routine care or local guidelines should be performed on separate biological samples.

Collected samples will be stored for a maximum of 20 years (counting from when the last subject performed the last study contact), 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.

7.6.1. 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 10.3 for the definition of study cohorts/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.

7.6.2. Biological samples (Amended: 24 July 2017)

Table 5 presents the volume of blood that will be collected from subjects for study-related testing during each visit.

Table 5 Volume of whole blood for study related testing (Amended: 24 July 2017)

Age group	Type of Visit			
	Visits 1, 2 and 3	SDC – first visit		Cases where dengue is ruled out
		Early presenter	Late Presenter	
6 months to <2 years	3.5 ml	3.5 ml	3.5 ml	***3.5 mL***
2-50 years	10 ml	10 ml	10 ml	***9 mL***

If blood collection is not feasible, a finger prick test may alternatively be performed for testing with a IgM/IgG/NS1 ICT.

7.6.3. Laboratory assays *for primary and secondary endpoints*

Please refer to APPENDIX A for a detailed description of the assays performed in the study. Please refer to APPENDIX C for the address of the clinical laboratories used for sample analysis.

Table 6 presents the possible laboratory assays for primary and secondary endpoints on the blood sample that will be drawn from the subject.

Table 6 Possible laboratory assays on study blood samples for primary and secondary endpoints

Time point	Possible parameters/assays measured
Scheduled visit 1, 2 and 3*	- Anti-DENV indirect IgG Enzyme Linked Immunosorbant Assay (ELISA)
Unscheduled Visit: SDC, early presenter (≤ 5 days following onset of symptoms)	- DENV RT-qPCR - DENV isolation for sequencing purpose - DENV sequence - DENV NS1 rapid test (ICT) or ELISA - IgM/Ig G rapid test (ICT) or SD BIOLINE Dengue Duo (Dengue NS1 Ag + IgG/IgM)A

*Blood sample will not be collected at Visit 2 and Visit 3 for subjects in Mexico

Assays for the determination of DENV types will be performed using standardised and qualified or validated procedures (refer to Table 7 and Table 8).

Diagnostic tests (NS1, IgG and IgM) will be available at each site. The purpose of these diagnostic tests is to provide prompt laboratory results to the physician and the subject. Rapid ICT (point of care) will be used. Providing rapid results to the subject may be perceived as a benefit of study participation and may help in retaining subjects.

Table 7 presents humoral immunity to DENV.

Table 7 Humoral immunity (DENV antibody determination) for primary and secondary endpoints (Amended: 24 July 2017)

System	Component	Method	Kit / Manufacturer	Laboratory
Serum	IgM/IgG to DENV	Rapid ICT assay or Dengue NS1 Ag + IgM/IgG	Standard Diagnostics (ICT) or equivalent/ SD BIOLINE Dengue Duo(Dengue NS1 Ag +IgG/IgM) or equivalent	Local
	IgG to DENV (for scheduled visit 1, ***2 and 3****)	ELISA	Indirect IgG Panbio or equivalent	Central or local GSK designated laboratory
	DENV types 1-4 neutralising antibodies	Dengue neutralisation assay	In-house	GSK designated laboratory

Blood sample will not be collected at Visit 2 and Visit 3 for subjects in Mexico.

Additional potential exploratory tests including neutralising antibodies against JEV and other flaviviruses may be performed. Table 8 presents virology.

Table 8 Virology for primary and secondary endpoints

System	Component	Method	Laboratory
Serum	DENV RNA	RT-qPCR	Central or local GSK designated laboratory

Note: Viral isolation may be conducted if needed.

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.

7.6.4. Laboratory assays to be performed for tertiary endpoints (Amended: 24 July 2017)

Table 9 presents the possible laboratory assays for tertiary endpoints on the blood sample that will be drawn from the subject.

Table 9 Possible laboratory assays on study blood samples for tertiary endpoints (Amended: 24 July 2017)

Time point	Possible parameters/assays measured
Scheduled visit 1, 2 and 3*	- DENV neutralising antibody assay - JEV neutralising antibody assay - WNV neutralising antibody assay - Tertiary exploratory assays to characterize DENV immune status (e.g. antibody avidity assay, etc.) - Chikungunya serology (IgM/IgG detection) and neutralizing antibodies - Zika serology (IgM/IgG detection) and neutralizing antibodies
Unscheduled Visit: SDC, early presenter (≤ 5 days following onset of symptoms)	- PCR/serology for other infectious agents causing febrile illness (chikungunya, Zika, influenza, leptospirosis)
Unscheduled Visit: SDC, late presenter (> 6 days following onset of symptoms)	- DENV NS1 rapid test - IgM/IgG rapid test or IgM/IgG capture ELISA - PCR/serology for other infectious agents causing febrile illness (chikungunya, Zika, influenza, leptospirosis)
Unscheduled Visit: Cases where dengue is ruled out	***-PCR/serology for other infectious agents causing febrile illness (chikungunya, Zika, influenza, leptospirosis)***

*Blood sample will not be collected at Visit 2 and Visit 3 for subjects in Mexico.

Laboratory assays related to tertiary endpoints will only be performed after the primary and secondary endpoints have been ensured, and the local epidemiology of dengue virus disease is better understood.

The results of the primary and secondary endpoints, public health needs, and availability of biological samples will guide:

- The subset of subjects selected for which assays related to tertiary endpoints will be conducted
- Which pathogens should be tested (JEV, WNV, chikungunya, Zika, influenza, leptospirosis)
- The assays and tests to be performed.

If testing for tertiary endpoints will be conducted, these parameters and rationale will be provided to the local ethical committees and relevant health authorities for approval. Testing may include, but are not limited to the following tests for humoral immunity and virology.

Table 10 Humoral immunity for tertiary objectives

System	Component	Method	Kit / Manufacturer	Laboratory
Serum	Antibodies against Zika virus	To be determined	To be determined	GSK designated lab
Serum	Zika virus neutralizing antibody	Zika neutralization assay	To be determined	GSK designated laboratory
Serum	Antibodies against chikungunya virus	To be determined	To be determined	To be determined
Serum	YF virus neutralizing antibody	YF neutralization assay	To be determined	GSK designated laboratory
Serum	JEV neutralizing antibody	JEV neutralization assay	To be determined	GSK designated laboratory
Serum	WNV neutralizing antibody	WNV neutralization assay	To be determined	GSK designated laboratory

Table 11 Virology for tertiary endpoints

System	Component	Method	Kit/Manufacturer	Laboratory
Serum	Zika RNA	RT-PCR	To be determined	GSK designated laboratory
Serum	Chikungunya RNA	RT-PCR	To be determined	GSK designated laboratory
Serum	JEV RNA	RT-PCR	To be determined	GSK designated laboratory
Serum	WNV RNA	RT-PCR	To be determined	GSK designated laboratory

7.6.5. Biological samples evaluation

Table 12 presents the aliquoting of blood samples collected at scheduled visits.

Table 12 Possible assays for primary and secondary endpoints for blood samples collected at scheduled visits

Blood sampling time point		Possible assays	Estimated number of subjects	Serum aliquot	Components priority rank
Type of contact/	Sampling time point				
Scheduled Visit 1, Visit 2 and Visit 3	Day 0 Month 12 Month 24	Anti-DENV IgG indirect ELISA	All	200 µl	1
		DENV virus 1-4 neutralising antibody assay	Subset	1x 1 ml or remaining volume	2

Blood sample will not be collected at Visit 2 and Visit 3 for subjects in Mexico

Table 13 presents the possible assays and aliquot preparation for blood samples collected at first unscheduled visit for suspected dengue (for early and late presenters).

Table 13 Aliquot preparation for blood samples collected at visits for suspected dengue and follow-up contact

Blood sampling time point	Serum aliquot volume	Possible Assays	Estimated number of subjects	Components priority rank
First visit – presentation within 5 days following onset of symptoms (early presenter)	750 µl	DENV RT-qPCR	Unknown	1
	Approximately 500 µl	DENV NS1/IgM/IgG rapid test (ICT)	Unknown	2
	1 ml or remaining volume	PCR/serology of other infectious aetiologies (chikungunya, Zika, influenza, leptospirosis)	Unknown	3
		Viral isolation and sequencing		
First visit – presentation 6 days or more following onset of symptoms (late presenter)	Approximately 500 µl	DENV NS1/IgM/IgG rapid test (ICT)	Unknown	1
	1 ml or remaining volume	PCR/serology of other infectious aetiologies (chikungunya, Zika, influenza, leptospirosis)	Unknown	2

Collected samples may be used for purposes related to the quality assurance of data generated within the scope of this protocol, such as for maintenance of assays described in this protocol and comparison between analytical methods and/or laboratories.

8. SAFETY (AMENDED: 24 JULY 2017)

In this prospective cohort study no test product/vaccine will be given. However, blood samples will be collected at the enrolment visit ***(Visit 1), Visit 2, conclusion visit (Visit 3),*** for suspected dengue ***and for subjects where dengue is ruled out***. SAEs related to any study procedure will be recorded.

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

8.1. Safety definitions

8.1.1. 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.

or

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

   Medical or scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation.

8.1.1.1. 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 8.1.1). 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. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the study and do not worsen, will not be reported as SAEs.

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

8.2. Detecting and recording SAEs

8.2.1. Time periods for detecting and recording SAEs

SAEs related to study participation (blood sample collection) will be collected and recorded from the time of the first study visit until the subject is discharged from the study, by the investigator or his/ her designate on the SAE form, within 24 hours of being aware of the SAE, irrespective of intensity.

All reported SAEs related to the study procedure will be followed-up by the investigator until they have resolved, subsided, stabilised, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up.

See Section 8.3 for instructions on reporting of SAEs.

An overview of the protocol-required reporting periods for SAEs is given in Table 14.

Table 14 Reporting periods for SAEs

Study activity	Visit 1	Visit 2	Visit 3	Conclusion visit/ Follow-up contact for unscheduled visits (whichever occurs last)
Report SAEs related to study procedures				

8.2.2. Evaluation of SAEs

8.2.2.1. Active questioning to detect SAEs

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

All SAEs either observed by the investigator or his/ her staff or reported by the subject/ subject's parents/ LAR(s) / designate (if applicable) 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.

8.2.2.2. Assessment of the intensity of SAEs

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 (in a young child, such an SAE would, for example, prevent attendance at school/ kindergarten/ a day-care centre and would cause the parent(s)/ LAR(s) to seek medical advice. In adults/ adolescents, such an SAE would, for example, prevent attendance at work/ school and would necessitate the administration of corrective therapy.)

An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 8.1.1**.**

8.2.2.3. 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.

Is there a reasonable possibility that the SAE may have been caused by the study procedure (blood sample collection)?

YES	**:**	There is a reasonable possibility that the study procedure contributed to the SAE.
NO	**:**	There is no reasonable possibility that the SAE is causally related to the study procedure.

If an event meets the criteria to be considered as 'serious' (see section 8.1.1), 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.

8.2.2.4. 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).

8.3. Reporting of SAEs

8.3.1. Prompt reporting of SAEs related to study participation to GSK

SAEs that occur in the time period defined in Section 8.2.1 will be reported promptly to GSK within the timeframes described in Table 15 once the investigator determines that the event meets the protocol definition of an SAE.

Table 15 Timeframes for submitting SAEs related to study participation to GSK

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*	SAE screen	24 hours*	SAE screen

* Timeframe allowed after receipt or awareness of the information.
‡ The investigator will be required to confirm review of the SAE causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE.

8.3.2. Contact information for reporting SAEs to GSK

Back-up Study Contact for Reporting SAEs
24/24 hour and 7/7 day availability: **GSK Biologicals Clinical Safety & Pharmacovigilance** Outside US & Canada sites: Fax: PPD or PPD Email address: PPD

8.3.3. Completion and transmission of SAEs reports related to study participation to GSK

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.

8.3.3.1. 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.

8.3.4. 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 15.

8.3.5. Regulatory reporting requirements for SAEs

The investigator will promptly report all SAEs to GSK Biologicals in accordance with the procedures detailed in Section 8.3.1. 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.

8.4. Follow-up of SAEs

8.4.1. 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 15).

All SAEs documented at a previous visit/ contact and recorded as not recovered/ not resolved or recovering/ resolving will be reviewed at subsequent visits/ contacts until the end of the study.

8.4.2. 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.

9. SUBJECT COMPLETION AND WITHDRAWAL

9.1. Subject completion

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

9.2. Subject withdrawal

Subjects who are withdrawn because of SAEs related to study participation must be clearly distinguished from subjects who are withdrawn for other reasons. The investigator will follow subjects who are withdrawn as the result of an SAE until resolution of the event (see Section 8.4.2).

Withdrawals will not be replaced.

From an analysis perspective, a 'withdrawal' from the study refers to any subject who was not available for the concluding visit foreseen in the protocol.

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

A subject is considered a 'withdrawal' from the study when no study procedure has occurred, no follow-up has been performed and no further information has been collected for this subject from the date of withdrawal/last contact. The withdrawal of one subject does not have impact on the participation of other subjects from the same household.

Investigators will make an attempt to contact those subjects who do not return for scheduled visits or follow-up.

Information relative to the withdrawal will be documented in the eCRF. The investigator will document whether the decision to withdraw a subject from the study was made by the subject himself/herself, by the subject's parent(s) or LAR(s), or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- SAE.
- Protocol violation.
- Consent withdrawal, not due to an SAE*
- Moved from the study area.
- Lost to follow-up.
- Others

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

Subjects who are withdrawn from the study because of SAEs must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn from the study as result of an SAE until resolution of the event (see Section 8.4.2).

10. STATISTICAL METHODS

10.1. Endpoints

10.1.1. Primary endpoint

- RT-qPCR confirmed symptomatic DENV infection (all DENV types) during the study period.

10.1.2. Secondary endpoints

- DENV-type specific confirmed symptomatic DENV infection.
- Virologically confirmed (by RT-qPCR or NS1) and probable (based on serological evidence) symptomatic DENV infection.
- Status for anti-DENV IgG at enrolment (indicative of past DENV infection).
- Symptoms and severity of symptomatic dengue.

10.1.3. Tertiary endpoints

Exploratory objectives may be assessed based on, but not limited to the following endpoints:

- Spatial coordinates of the houses of participating households and of dengue cases and date of symptoms onset (applicable only for symptomatic cases).
- Neutralising antibodies titres against DENV 1-4.
- Neutralising antibody titres against other flaviviruses (such as JEV and WNV).
- Other infectious aetiology than dengue in subjects with episodes of febrile illness referred to as “suspected dengue cases” (chikungunya, Zika, influenza, leptospirosis).
- Determinants of past dengue infection and dengue illness.
- Characteristics of immune status against DENV infection.

10.2. Determination of sample size

The target population to be enrolled is estimated at 1750 subjects (approximately 300 to 500 per site). A dropout rate of 5% per year is estimated, which would lead to approximately 1662 subjects completing 1 year of follow-up and approximately 1579 completing 2 years of follow-up, accumulating 3327 patient-years.

The incidence of dengue is likely to vary by site and by age group. The study population should include between 30% and 50% of adults, but the distribution is not further specified as it would make operational feasibility more complex.

Supposing that the study population is composed of 70% of children with an expected incidence of 8 RT-qPCR-confirmed dengue case per 1000 person-years and 30% of adults with an expected incidence of 5 RT-qPCR confirmed dengue cases per 1000 person-years, the study would detect about 19 cases in children and 5 cases in adults (12 cases in year one with a cohort of 1750 subjects and 12 cases in year 2 with a cohort of about 1662 subjects).

The enrolment will be done by household. Each household can be considered as a cluster and this induces a design effect to account for the between-cluster variability when estimating the confidence intervals (CI) of the incidence rates. The design effect measures the increase in the standard error of the incidence rate estimate due to the sampling design used and is given by: $D = 1 + (b - 1)$ rho, where rho is the intra-cluster correlation (a measure of rate of homogeneity within clusters) and b is the average number of subjects sampled per household. Here, we assume b is assumed to be 3. Although in theory 'rho' can have a value up to 1, in practice values higher than 0.4 are uncommon. We have used a conservative estimate of 0.4 for this study [Bennett, 1991]. The design effect is then estimated to 1.8.

Table 16 shows the 95% CI for a range of scenarios in term of cohort size, dropout rates and incidence rates. With an overall sample size of a minimum of about 1750 evaluable subjects, a follow-up period of two years and the expected incidences of 8 cases per 1000 person-years in children and 5 cases per 1000 person-years in adults, the overall incidence rate would be 7.1 with an exact Poisson 95% CI of [4.5; 10.6], and with a CI based on the normal approximation and accounting for the design effect of [3.3; 10.9].

Table 16 Expected 95% confidence intervals for the incidence rate of virologically confirmed dengue cases

Cohort size	Proportion of adults	Dropout rate (per year)	Incidence rates		Person-years	Number of cases	Overall incidence (1000 p-y)	Confidence intervals (1000 p-y)			
			Children (p-y)*	Adults (p-y)				Exact (Poisson)		Normal approx + DE**	
								Lower	Upper	Lower	Upper
1400	30%	5%	0.008	0.003	2662	17	6.5	3.8	10.4	2.4	10.6
1575	30%	5%	0.008	0.003	2994	19	6.5	3.9	10.1	2.6	10.4
1750	30%	5%	0.008	0.003	3327	22	6.5	4.1	9.9	2.8	10.2
1400	30%	5%	0.008	0.005	2662	19	7.1	4.2	11.1	2.8	11.4
1575	30%	5%	0.008	0.005	2994	21	7.1	4.4	10.8	3.1	11.1
1750	30%	5%	0.008	0.005	3327	24	7.1	4.5	10.6	3.3	10.9
1400	30%	5%	0.01	0.003	2662	21	7.9	4.9	12.1	3.4	12.4
1575	30%	5%	0.01	0.003	2994	24	7.9	5.0	11.8	3.6	12.2
1750	30%	5%	0.01	0.003	3327	26	7.9	5.2	11.5	3.8	12.0
1400	30%	5%	0.01	0.005	2662	23	8.5	5.4	12.8	3.8	13.2
1575	30%	5%	0.01	0.005	2994	25	8.5	5.5	12.5	4.1	12.9
1750	30%	5%	0.01	0.005	3327	28	8.5	5.7	12.3	4.3	12.7

*p-y: Person-years.

**: Confidence intervals based on the normal approximation and accounting for a design effect of 1.8.

10.3. Cohorts for Analyses

10.3.1. Total cohort

The Total cohort will include all subjects enrolled in the study.

10.3.2. According-To-Protocol (ATP) cohort

The ATP cohort will include all evaluable subjects, that is, subjects who meet all eligibility criteria, complying with the procedures defined in the protocol and for whom data of at least one follow-up contact are available. The surveillance period for one subject will be defined as the duration from the date of enrolment until the date of the last follow-up contact for this subject. Reasons for elimination from ATP analyses will be established at the time of data cleaning and documented.

10.4. Derived and transformed data

RT-qPCR-confirmed symptomatic DENV cases and probable symptomatic DENV cases will be detected on the basis of the definitions presented in Section 5.

The severity of the dengue symptomatic infections will be derived as described in Section 5.3.

10.5. Analysis of demographics

Demographic characteristics (age at study entry and gender), medical history and vaccination history for JE and YF will be summarised by group using descriptive statistics:

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

10.6. Descriptive analyses of laboratory results

The primary analysis will be based on the ATP cohort. If the percentage of enrolled subjects with serological results excluded from the ATP cohort is 5% or more, a second analysis based on the Total cohort will be performed to complement the ATP analysis.

Descriptive analyses will be performed at enrolment (Visit 1) for each laboratory test available, by site and overall:

- The seroprevalence of each DENV serotype, and other flaviviruses (i.e. JEV; (Southeast Asia only) or WNV; (Latin America only) will be calculated with 95% CI.
- If applicable, GMT with 95% CI will be tabulated for antibodies against each virus.

- If applicable, the range and distribution of antibody concentrations for each virus will be tabulated.

Descriptive analyses will be performed at visits for suspected dengue for each laboratory test available, by site and overall:

- The seroprevalence of each DENV serotype, and other flaviviruses (i.e. JEV; (Southeast Asia only) or WNV; (Latin America only) will be calculated with 95%CI. The denominator will be the number of visits.
- If applicable, GMT with 95% CI will be calculated for antibodies against each virus.
- If applicable, the range and distribution of neutralizing antibody titres for each virus will be tabulated.

95% CI will account for clustering of observations within households for the analyses by centre and also the stratification by centres for the overall analyses. Details of the methods will be described in the statistical analysis plan (SAP).

10.7. Analysis of primary objective

The primary analysis will be based on the ATP cohort. If the percentage of enrolled subjects with serological results excluded from the ATP cohort is 5% or more, a second analysis based on the Total cohort will be performed to complement the ATP analysis.

The following analyses will be performed:

Incidence rate of RT-qPCR confirmed symptomatic dengue infection with 95% CI: the numerator will be the number of subjects with RT-qPCR confirmed symptomatic dengue infection during the study period. The denominator will be the total person-years at risk, i.e., from enrolment until the first RT-qPCR confirmed symptomatic dengue infection during the entire study period.

CIs for incidence rates and prevalence will account for clustering of observations within households for the analyses by centre and also the stratification by centres for the overall analyses. Details of the methods will be described in the statistical analysis plan (SAP).

10.8. Analysis of secondary objectives

The following analyses will be performed within each centre and overall, and for each year/season and overall:

- The analyses planned for the primary objective will be repeated by age class, gender, serotype, and dengue serological status at the beginning of the analysis period (visit 1 for the first season and overall, or the last visit for suspected dengue cases prior to season for the second season). The analysis period for the second season will be determined according to the peak of the season.

- Incidence rates of probable symptomatic DENV infection with 95% CI by age class, gender, dengue serological status at the beginning of the analysis period, and overall: the numerator will be the number of subjects with probable dengue infection during the analysis period in each subset. The denominator will be the total person-years at risk in each subset, i.e. from the beginning of the analysis period until the first probable symptomatic dengue infection.
- Incidence rate of RT-qPCR confirmed and probable symptomatic DENV infection combined with 95% CI by age class, gender, dengue serological status at the beginning of the analysis period, and overall: the numerator will be the number of subjects with RT-qPCR confirmed symptomatic and probable dengue infection during the analysis period in each subset. The denominator will be the total person-years at risk in each subset, i.e. from the beginning of the analysis period until the first RT-qPCR confirmed symptomatic or probable symptomatic dengue infection.

The following analyses will be performed within each centre and overall:

- The (crude) seroprevalence of DENV infection with 95% CI at enrolment (visit 1), by age class and overall: the proportion of dengue IgG positive subjects among all subjects and among dengue cases (confirmed and probable) with available results.
- The clinical characteristics of symptomatic DENV infection (symptoms, hospitalisations, severity) will be presented as overall and per sub-group (type (DENV 1-4), gender and age class).

CIs for incidence rates and proportions will account for clustering of observations within households for the analyses by centre and also the stratification by centres for the overall analyses. Details of the methods will be described in the SAP.

10.9. Other analyses

All analyses will be performed on the ATP cohort.

The following analyses will be performed within each centre and overall, and for each year/season and overall:

- The attack rate within households with an original RT-qPCR-confirmed symptomatic DENV infection. All types of DENV infections will be considered. The attack rate will be the proportion of subjects with symptomatic DENV infection among subjects with an original RT-qPCR-confirmed symptomatic DENV infection within their household.
- Analysis of spatio-temporal transmission

  The proportion of dengue infections among study participants living in circles of various radius (less than 20 metres, 20-40 metres, 60-80 metres, 80-100 metres and up to 200 metres) in the neighbourhood of laboratory confirmed dengue cases (positive index case) and participants without evidence of dengue infection (negative index case) will be estimated.

The time distribution of laboratory-confirmed symptomatic dengue infections will be described. The cumulative distribution of the cases every month will be presented in graphs for each season.

The spatial distribution of participants will support in making a model to simulate the conduct of an efficacy trial and assess potential sources of biases (heterogeneity of spatial transmission, baseline immune status, etc.)

The analyses for the other exploratory objectives will be described in detail in the SAP.

10.10. Interpretation of analyses

There is no hypothesis testing in this study. 95% CI will be computed for incidence rates and proportions. CIs for incidence rates and proportions will account for clustering of observations within households for the analyses by centre and also the stratification by centres for the overall analyses. Details of the methods will be described in the SAP.

10.11. Conduct of analyses

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the final study report.

10.11.1. Sequence of analyses

The final analysis will be performed when all data have been collected and cleaned.

10.11.2. Statistical considerations for interim analyses

No interim analysis is planned for this study.

11. ADMINISTRATIVE MATTERS

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

11.1. Electronic Case Report Form instructions

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

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

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

11.2. Study monitoring by GSK Biologicals

GSK will monitor the study to verify that, amongst others, the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol, any other study agreements, GCP and all applicable regulatory requirements.

The subject information will be anonymised (see GLOSSARY OF TERMS) using a code number to assure confidentiality of the subjects' data. No GSK Biologicals personnel will have the ability to link data to an identifiable individual. 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.

11.3. Record retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (e.g., audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. However, the investigator/institution should seek the written approval of the sponsor before proceeding with the disposal of these records. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP or other applicable guidelines, 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.

11.4. Quality assurance

To ensure compliance with GCP or other applicable guidelines and all applicable regulatory requirements, GSK may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

11.5. Posting of information on publicly available 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.

11.6. Provision of study results to investigators

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

11.7. Collection, analysis, storage of spatial data

Access to spatial data will be restricted to the site having collected the data and to study team members performing the spatial and temporal analysis described in Section 10.9.

Spatial coordinates will be modified using an algorithm respecting the relative position of all subjects, but removing the link with the original location of the household. Only modified coordinates will be archived.

12. COUNTRY SPECIFIC REQUIREMENTS

12.1. Thailand specific information and requirements

12.1.1. List name, affiliation and contact details of all investigators

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12.1.2. Definition of burden on the study protocol (Amended: 24 July 2017)

Burden ***in*** the study protocol means incidence and severity of Dengue infection and ***hospital*** admission of volunteer/subjects.

12.1.3. Duration of the study, site address and number of participating subjects in Thailand (Amended: 24 July 2017)

Trial Site in Thailand

There is 1 trial site participating in Thailand at Sam Kwai Phuek Health Promoting Hospital, Muang District, Nakhon Pathom Province. The site is planning to enrol 500 subjects in this study. Study team members are from Department of Pediatric Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, and Deputy Nakhonpathom Provincial Chief Medical Officer, Nakornpathom provincial Health Office, consultant of the study.

This study is ***an international*** multicentre (International) ***cohort study*** that will be conducted in 4 countries. ***Sample sizes are*** list***ed*** below:

- Thailand is planning to enrol 500 subjects
- Malaysia is planning to enrol 400 subjects
- Philippines is planning to enrol 500 subjects
- Mexico is planning to enrol 350 subjects

Duration of the study in Thailand

The study duration for Thailand is 2.0 years (24 months).

Planning of recruitment period is ***October 2017-June 2018***.

Follow up visit period of the study is ***October 2017- October 2020***.

Planning of the study closure is ***October*** 2020.

For each subject, the study duration is approximately 2 years (24 months)

12.1.4. Study population groups and Calculation of study population groups in Thailand (Amended: 24 July 2017)

The size of Thailand study population is derived from the average yearly incidence of dengue fever in Sam Khwai Phuak Sub-district from 2009 to 2013 of 283 per 100,000 people. If ***a lower than actual incidence is assumed,*** according to population data of Sam Khwai Phuak Sub-District of approximately 10,900 people, a sample size of 500 people will be able to detect the incidence of dengue fever in Sam Khwai Phuak Sub-district of close to 600 per 100,000 people, with a confidence level of 80 percent and an estimated data deficiency of no more than 15 percent.

In Thailand, a total of approximately 500 subjects will be recruited and there will be a total of 1 study site participating in this study, which is Sam Khwai Phuek Health Promotion Hospital, Muang District, Nakhon Pathom Province.

Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University, is the main site for administration with no recruitment. Investigator and study team are affiliated with Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University.

Deputy Nakhonpathom Provincial Chief Medical Officer [Expert Public Health Physician (Preventive Medicine)] from Nakhon Pathom Province, a local person, is the project consultant.

The roles of study team at the Sam Kwai Phuek Health Promotion Hospital is:

To be the local person coordinating public utility payments at the study site for which the research project is the party responsible for, and coordinating with community leaders and the community in the area.

12.1.5. Overview of the Subject Recruitment Plan for Thailand

The method of finding/recruiting appropriate subjects will be selected by each research site participating in the study. One of the following 2 methods may be selected:

- School recruitment method
- Community recruitment method (without schools being involved)

For Thailand, however, subjects will be recruited using the community recruitment method (without schools being involved).

12.1.6. Adding Secondary Objective Explanations

To describe the clinical symptoms of patients infected with the dengue virus (which have been confirmed and which are within the scope of suspected infection)*

*This includes studying clinical symptoms and the severity of the dengue virus infection. The severity of the dengue virus infection in this study will be monitored with the subject by taking their physical examination history, and tracking the progress of the disease and hospitalization during the follow-up contact after 21 days of the first visit.

12.1.7. Informed Consent and Consent for Thailand

Obtaining consent from subjects in this study means requesting consent from individual subjects.

Subjects/guardians of subjects will be given an explanation of various details, including both the natures and risks involved with the study. The above process will be carried out by the head of protocol or protocol participants prior to obtaining signatures of consent from the subjects/imprinting the fingerprints of the subjects and/or the father/mother/legally acceptable representative of the subjects prior to their participation

in the study, including the signature of the person providing protocol details (investigator) and relevant witnesses. The person providing protocol details must allow sufficient time for the subjects to decide whether or not to participate in the study, with the signing of the Subject Information Sheet and Informed Consent Form for adults, and Subject Information Sheet and Informed Assent Form for children, or Subject Information Sheet and Informed Consent Form for the father/mother/legally acceptable representative of the subjects to the amount of 2 copies of each. The first copy will be kept at the study site and the second copy will be kept by the subject. In addition, if the subjects or the father/mother/legally acceptable representative of the subjects would like to appoint a designee* to help with certain parts of the study procedures, including to help in monitoring and checking the body temperature of the subjects in the event they have a fever, completing daily records, answering calls from study staff who will ask whether or not the participant has a fever, and reminding the subjects of the study procedure, and help planning study visits, this designee is not able to make any decision on behalf of the subjects, or the father/mother/legally acceptable representative of the subjects. Moreover, the above designee must sign their name to receive consent from the subjects or the father/mother/legally acceptable representative of the subjects, or the father/mother/legally acceptable representative of the subjects together with the signature of the person providing protocol details (investigator) and the witnesses in the designee information document and letter of consent to participate in the study, to the amount of 2 copies. The first copy will be kept at the research site and the second copy will be kept by the subject.

The Subject Information Sheet and Informed Consent Form for this study are as follows:

- Subject Information Sheet for research subject that are between 20 – 50 years old
- Informed Consent Form for subjects that are between 20 – 50 years old
- Subject Information Sheet for the father/mother/legally acceptable representative of subject from 6 months old to less than 20 years old
- Informed Consent Form for the father/mother/legally acceptable representative of subject from 6 months old to less than 20 years old
- Subject Information Sheet for subject from 7 years to less than 13 years old
- Assent Form for subject from 7 years to less than 13 years old.
- Subject Information Sheet for subject from 13 years to less than 20 years old.
- Assent Form for subject from 13 years to less than 20 years old.
- Subject Information Sheet for designees.
- Informed Consent Form for designees.

12.1.8. Compiling Area Coordinates of Households in Thailand

This study involves the compilation of the geographical area coordinates of households. The compilation of the area coordinates of subjects participating in the protocol with a Global Positioning System (GPS) device will deliver only subject number data and their area coordinates. It will not specify the name, surname, home address, or any data that could affect the privacy of the subjects.

12.1.9. Managing Biological Samples in Thailand

For Thailand, samples of subjects will be stored for a period of 5 years after the study is completed (counting from the date of the last subject's last contact in the study). In the event that investigator needs to store blood samples for longer than 5 years, the investigator and the study team will request approval from the Ethical Review Committee for Research in Human Subjects for a further period of 5 years, and the samples remaining after the period approved will be destroyed.

Subject samples will only be used for tests in this study.

12.1.10. Potential Risks and Inconveniences, and Prevention Measures

Blood drawing may cause slight pain in the area blood is drawn from. There may be swelling or bruising, or dizziness. After blood is drawn some people may feel faint, or nauseous, and in very rare cases infection may arise in the area blood is drawn. Investigator will use sterile blood drawing techniques to prevent infection from blood drawing. We will use hospitals with experience in blood drawing, especially for small children, and will use a scalp vein of appropriate size. For any side effects that may be caused by blood drawing, investigator will offer treatment until full recovery free of charge.

12.1.11. Benefits, Compensation, Reimbursement, Treatment and Solution in the Event of Complications for the Investigator

Benefits, Compensation, Reimbursement, Treatment and Solution in the Event of Complications for the Investigator

Subjects may not receive direct benefits from this study. However, knowledge regarding dengue fever derived from this study will be of great benefit in the prevention and management of dengue fever and administration of vaccines to prevent dengue fever in the future.

Subjects will receive treatment for any fever symptoms from a doctor or healthcare professional at the Sam Khwai Phuak Sub-District Health Promotion Hospital in accordance with standard medical practice guidelines.

Subjects will be tested for dengue virus diagnoses (NS1, IgG and IgM) as specified in the protocol, and will know the results within 15 minutes. This will benefit subject treatment at no expense.

12.1.12. Compensation and Reimbursement (Amended: 24 July 2017)

Compensation

None

Reimbursement

Subjects will receive the amount of 1,000 baht per person per visit as specified in the protocol as a reimbursement for time and travel costs, except the case where you have a fever when attending a scheduled visit, but the person providing treatment suspects that ***he/she*** may have dengue fever, in which case a return visit appointment will be made on the following day.

12.1.13. Other Ethical Issues

Confidentiality

Investigator will keep personal information, study documents, and study results related to this study confidential, without disclosing them publicly. The investigator certifies that the personnel working in this project and the study sponsor will act in the same way. This study will keep subject information confidential and will disclose it in the form of overall study results that does not include any names or other data which may be able to identify the subjects, by using a numerical code instead of a name. This information, including medical records, may be examined by authorized personnel from Ministry of Public Health of Thailand or the Ethical Review Committee for Research in Human Subjects, only for the benefit of oversight, monitoring, and research evaluation. Regardless, the study sponsor and representatives, including those involved in all parts, must act in accordance with good clinical practices, and must, to the best of their ability, endeavour to keep personal information of subjects confidential.

Budget Details and Funding Sources

Study sponsor GlaxoSmithKline Biologicals

Budget Details: As per documents showing research budget details.

12.1.14. Signature of all investigators

Principal Investigator

(Assoc. Prof. Dr. Pornthep Chanthavanich)	**Day / Month/ Year**

Consultant

(Dr. PPD)	**Day / Month/ Year**

Sub-Investigator

(Assoc. Prof. Dr. Chukiat Sirivichayakul)	**Day / Month/ Year**
(Assist. Dr. Kriengsak Limkittikul)	**Day / Month/ Year**
(Assist. Prof. Dr. Watcharee Chokejindachai)	**Day / Month/ Year**
(Dr. Weerawan Hattasingh)	**Day / Month/ Year**
(Assoc. Prof. Dr. Wijitr Fungladda)	**Day / Month/ Year**
(Dr. Supawat Chatchen)	**Day / *Month/ Year***

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APPENDIX A LABORATORY ASSAYS

SD Bioline Dengue Duo (NS1 Ag + Ab Combo)

The SD Bioline Duo rapid test is commercial CE labelled individual test from Standard Diagnostics, Inc. It is an in-vitro immunochromatographic, one step assay designed to detect both dengue virus NS1 antigen and differential IgG/IgM antibodies to dengue virus in human serum, plasma or whole blood.
Performance characteristics of the assay are described in the manufacturer package insert.

NS1-antigen ELISA

Platelia™ Dengue NS1 Ag is a one step sandwich format microplate enzyme immunoassay for the qualitative or semi-quantitative detection of Dengue virus NS1 antigen in human serum or plasma.
Performance characteristics of the assay are described in the manufacturer package insert.

Dengue IgG Indirect ELISA

The Panbio Dengue IgG Indirect ELISA is a commercial CE labelled kit for the qualitative detection of IgG antibodies to dengue antigen serotypes (1, 2, 3 and 4) in serum.
Performance characteristics of the assay are described in the manufacturer package insert.

Dengue IgG Capture ELISA

The Panbio Dengue IgG Capture ELISA is a commercial CE labelled kit for the qualitative presumptive detection of elevated IgG antibodies to dengue virus (serotypes 1-4) in patients with secondary infection.
Performance characteristics of the assay are described in the manufacturer package insert.

Dengue IgM Capture ELISA

The Panbio Dengue IgM Capture ELISA is a commercial CE labelled kit for the qualitative detection of IgM antibodies to dengue antigen in serum, as an aid in the clinical laboratory diagnosis of patients with clinical symptoms consistent with dengue fever.
Performance characteristics of the assay are described in the manufacturer package insert.

Dengue serotype-specific RT-qPCR

The dengue serotype-specific RT-qPCR assay has been developed for the detection of DENV serotype RNA in serum samples. Viral RNA is extracted from serum samples and reverse-transcribed into cDNA. The resulting cDNA is quantified by real time PCR using specific DNA primers targeting the DENV capsid gene. PCR is performed as 2 duplex amplification (DENV-1/-3 and DENV-2/-4).

Simplexa TM Dengue REF MOL3100 Rev. C

A real-time RT-PCR assay for the in vitro detection and typing of dengue virus serotypes 1, 2, 3 and 4.

Principles of the procedure: The assay is a real-time RT-PCR that discriminates serotypes 1 and 4 in one reaction (well), and serotypes 2 and 3 in another reaction (well). The assay is composed of two principal steps: (1) extraction of RNA from specimens, and (2) amplification of the extracted RNA using bi-functional fluorescent probe-primers and reverse primers. The assay amplifies four serotype specific regions: dengue 1 (NS5 gene), dengue 2 (NS3 gene), dengue 3 (NS5 gene) and dengue 4 (capsid gene). An RNA internal control is used to monitor the extraction process and to detect RT-PCR inhibition.

Viral micro-plaque-reduction neutralisation (µPRN) assay

The anti-DENV 1-4 µPRN assay is performed in 96-well plates on Vero cells Serial serum dilutions are incubated with a fixed target amount of 75 PFU DENV. The serum-virus mixture is then added to Vero cells and incubated for 2 days in parallel to control wells with virus only. The replication of the infectious (non-neutralised) virus is monitored by detecting the expression of DENV envelope (gE) protein by infected cells with pan-flaviviruses anti-gE mouse monoclonal antibody (4G2, from ATCC, Rockville, MD, USA) conjugated to peroxidase. Using a precipitating chromogenic substrate for peroxidase, plaques of infected cells are counted. The number of plaques in a given serum-virus well is compared to the number of plaques in virus control wells. The Neutralisation titre corresponds to the inverse of the serum dilution giving 50 % reduction in the number of plaques as compared to the virus control wells.

APPENDIX B CLINICAL LABORATORIES

Table 17 Outsourced laboratories

Laboratory	Address
Laboratório de Tecnologia Virológica, Bio-Manguinhos, Fiocruz	Rio de Janeiro, Brazil
Q^2 Solutions	26081 Avenue Hall, Suite 150, Valencia Ca, 91355-1241, United States

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	200318 (EPI-DENGUE-007 BOD)
Amendment number:	Amendment 1
Amendment date:	28 January 2016
Co-ordinating author:	PPD, freelance writer for GSK Biologicals

Rationale/background for changes: This amendment was primarily developed to incorporate comments from the ethics committees of the countries in which the study will be conducted.

- The mitigation strategy for risks associated with blood sample collection was revised based on the WHO guidelines on drawing blood.
- The amount of blood collected in subjects 2-50 years enrolled in Vietnam was reduced from 7 ml to 5 ml at the enrolment visit and at visit for dengue suspicion, for early presenters, based on country-specific acceptability requirements.
- The list of other infectious aetiologies than dengue for which the samples collected in subjects with episodes of febrile illness (referred to as "suspected dengue cases") may be tested has been fully defined to include chikungunya, influenza, Zika and leptospirosis.
- Details on anonymisation of subject data were added based on comments from the Ethics Committee in Thailand.
- The study duration for each country was specified under the 'Country specific requirements' section of the protocol.
- The name of one of the laboratories performing testing was updated from 'Quest Diagnostics' to 'Q^2 solutions'.

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

The name of the Global Vaccines Clinical Laboratories (GVCL) department was changed to "Clinical Laboratory Sciences (CLS). The name of this function has been updated in the title page.

- PPD, ***Clinical read-out Lead (CRTL)***~~Project Manager~~, ~~GVCL~~ ***CLS***

Synopsis and Section 3.3 Tertiary Objectives

- To explore other infectious aetiologies than dengue in subjects with episodes of febrile illness referred to as "suspected dengue case" (~~e.g.:~~ chikungunya, ***Zika, influenza, leptospirosis,*** ~~etc.~~).

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**~~	
Study procedure: Blood sample collection ~~While giving blood the subject may feel faint, or locally experience mild pain, bruising, irritation or redness~~.	~~Spontaneous data~~ ***Pain at blood sampling site***	• ~~Subjects will be observed for at least 30 minutes after blood sample collection.~~ • ***Well-trained person should take the blood sample*** • ***Use needle of smaller gauge than the vein***
	Haematoma or thrombus	• ***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	• ***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.

Section 2.2 Benefit Assessment

The subjects ***may not receive any other direct benefit than facilitated access to healthcare in case of febrile illness during study conduct.*** ~~will be followed up for febrile illness due to dengue or other infectious aetiologies throughout the study.~~ Appropriate care will be provided to the subjects diagnosed with dengue or other diseases.

Section 5.1 Suspected symptomatic dengue case (SDC)

Acute febrile illness measured as ≥ 38.0°C with a thermometer by any route or recent history of febrile illness (onset in the past 8 days) reported by the subject/the parent(s)/LAR(s)/ designate (if applicable) of the subject for at least two consecutive days (a duration of ***approximately*** 36-48 hours) and of less than 7 days duration, which may be accompanied by other dengue symptoms or signs and does not have a defined focus or an obvious reason unrelated to dengue (based on physician judgement).

Section 7.5.1.9 Collect blood sample

At Visit 1, a blood sample of 3.5 mL will be collected from subjects aged between 6 months and 2 years ***(all sites)***, and a blood sample of 7 mL will be collected from subjects aged between 2 and 50 years (in all sites except Indonesia ***and Vietnam***).

At Visit 1, a blood sample of 3.5 mL will be collected from subjects enrolled in Indonesia aged between 6 months and 50 years. The volume ***in Indonesia*** is lower than in other countries as testing for DENV neutralising antibodies ***and other flaviviruses*** will not be performed for these subjects. ***The lower volume collected for subjects enrolled in Indonesia and Vietnam may result occasionally in the impossibility to repeat certain assays if needed (for instance in case of invalid results after the first assay run).***

At visit 1, a blood sample of 5 ml will be collected for subjects aged between 2 and 50 years enrolled in Vietnam to comply with local acceptability requirement. However, this lower volume may result occasionally in the impossibility to repeat certain assays if needed (for instance in case of invalid results after the first assay run).

Sectin 7.6 Biological sample handling and analysis

Please refer to the SPM ***and any document provided e.g.: Investigator manual,*** for details of biospecimen management (handling, storage and shipment).

Section 7.6.2 Biological samples

Table 5 presents the volume of blood that will be collected from subjects for study-related testing during each visit (all sites except Indonesia ***and Vietnam***), ***Table 6 presents the volume of blood that will be collected from subjects in Vietnam*** and ***Table 7*** ~~Table 6~~ presents the volume of blood that will be collected from subjects enrolled in Indonesia for study-related testing during each visit. The volume of blood collected for subjects enrolled in Indonesia at the enrolment visit will be lower than in other countries because neutralising antibodies against DENV and other flaviviruses will not be

measured. ***The volume of blood collected for subjects enrolled in Vietnam at the enrolment visit and at the initial visit for dengue suspicion will be lower than in other countries to comply with local acceptability criteria; however, this may result occasionally in the impossibility, due to limited serum volume, to repeat certain assays if needed (for instance in case of invalid results after the first assay run).***

Table 6 Volume of blood for study related testing in subjects enrolled in Vietnam

Age group	***Type of Visit***		
	Visit 1 (enrolment) Day 0	***SDC – first visit***	
		Early presenter	***Late Presenter***
6 months to <2 years	***3.5 ml***	***3.5 ml***	***3.5 ml***
2-50 years	***5 ml***	***5 ml***	***3.5 ml***

Section 7.6.3 Laboratory assays
Table 8 Possible laboratory assays on study blood samples

Time point	Possible parameters/assays measured
Scheduled visit 1	- Anti-DENV indirect IgG Enzyme Linked Immunosorbant Assay (ELISA) - DENV neutralising antibody assay - JEV neutralising antibody assay* - WNV neutralising antibody assay* - Tertiary exploratory assays to characterize DENV immune status (e.g. antibody avidity assay, etc.)
Unscheduled Visit: SDC, early presenter (≤ 5 days following onset of symptoms)	- DENV RT-qPCR - DENV isolation for sequencing purpose - DENV sequence - DENV NS1 rapid test (ICT) or ELISA - IgM/Ig G rapid test (ICT) or IgM/IgG capture ELISA - PCR/serology for other infectious agents causing febrile illness ***(chikungunya, Zika, influenza, leptospirosis)*** ~~(e.g. Chikungunya, etc.)~~
Unscheduled Visit: SDC, late presenter (> 6 days following onset of symptoms)	- DENV NS1 rapid test or ELISA - IgM/IgG rapid test or IgM/IgG capture ELISA - PCR/serology for other infectious agents causing febrile illness ~~(e.g. Chikungunya, etc.)~~ ***(chikungunya, Zika, influenza, leptospirosis)***

*DENV, JEV and WNV neutralising antibody assays will not be done for Indonesia.

Section 7.6.4 Biological samples evaluation

Table 11 Possible assays for blood samples collected at scheduled visits

Blood sampling time point		Possible assays	Estimated number of subjects	Serum aliquot	Components priority rank
Type of contact/	Sampling time point				
Scheduled Visit 1	(Day 0)	Anti-DENV IgG indirect ELISA	All	200 µl	1
		DENV virus 1-4 neutralising antibody assay, JEV and WNV neutralising antibody assay, ***serum bank for*** other tertiary exploratory assays	Subset**	2 x 1 ml or remaining volume	2

Table 12 Aliquot preparation for blood samples collected at visits for suspected dengue and follow-up contact

Blood sampling time point	Serum aliquot volume	Possible Assays	Estimated number of subjects	Components priority rank
First visit – presentation within 5 days following onset of symptoms (early presenter)	750 µl	DENV RT-qPCR	Unknown	1
	Approximately 500 µl	DENV NS1/IgM/IgG rapid test (ICT) or ELISA	Unknown	2
	750 µl or remaining volume	DENV RT-qPCR PCR/serology of other infectious aetiologies (~~e.g.:~~chikungunya, ***Zika, influenza, leptospirosis***~~, etc.~~)	Unknown	3
		Viral isolation and sequencing		
First visit – presentation 6 days or more following onset of symptoms (late presenter)	Approximately 500 µl	DENV NS1/IgM/IgG rapid test (ICT) or ELISA	Unknown	1
	750 µl or remaining volume	PCR/serology of other infectious aetiologies (~~e.g.:~~ chikungunya, ***Zika, influenza, leptospirosis***~~, etc.~~)	Unknown	2

Synopsis and Section 10.1.2

- Other infectious aetiology than dengue in subjects with episodes of febrile illness referred to as "suspected dengue cases" (~~e.g.:~~ chikungunya, ***zika, influenza, leptospirosis*** ~~etc.~~).

Section 11.2 Study monitoring by GSK Biologicals

The subject information will be anonymised (see GLOSSARY OF TERMS) using a code number to assure confidentiality of the subjects' data. No GSK Biologicals personnel will have the ability to link data to an identifiable individual. The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

Section 12 Country Specific Requirements

The duration of individual follow-up is 12 month for 2 sites (one site out of 2 in Mexico, one site in Indonesia) and 24 months for all the other sites.

Section 13 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: 23 November 2015.

Appendix A Laboratory Assays

Dengue serotype-specific RT-qPCR

The dengue serotype-specific RT-qPCR assay has been developed for the detection of DENV serotype RNA in serum samples. Viral RNA is extracted from serum samples and reverse-transcribed into cDNA. The resulting cDNA is quantified by real time PCR using specific DNA primers targeting the DENV capsid gene. PCR is performed as 2 duplex amplification (DENV-1/-3 and DENV-2/-4).

Simplexa TM Dengue REF MOL3100 Rev. C

A real-time RT-PCR assay for the in vitro detection and typing of dengue virus serotypes 1, 2, 3 and 4.

Principles of the procedure: The assay is a real-time RT-PCR that discriminates serotypes 1 and 4 in one reaction (well), and serotypes 2 and 3 in another reaction (well). The assay is composed of two principal steps: (1) extraction of RNA from specimens, and (2) amplification of the extracted RNA using bi-functional fluorescent probe-primers and reverse primers. The assay amplifies four serotype specific regions: dengue 1 (NS5 gene), dengue 2 (NS3 gene), dengue 3 (NS5 gene) and dengue 4 (capsid gene). An RNA internal control is used to monitor the extraction process and to detect RT-PCR inhibition.

Appendix B Clinical Laboratories

The name of one of the laboratories has been changed from Quest Diagnostics to Q^2 Solutions.

Table 16 Outsourced laboratories

Laboratory	Address
~~Quest Diagnostics~~ ***Q^2 Solutions***	26081 Avenue Hall, Suite 150, Valencia Ca, 91355-1241, United States
~~Local labs in Indonesia~~ ***Eijkman Institute for Molecular Biology***	~~To be determined~~ ***Jalan Diponegoro 69, Jakarta 10430, Indonesia, (within the Cipto Mangunkusumo Hospital / RSCM)***

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

eTrack study number and Abbreviated Title	200318 (EPI-DENGUE-007 BOD)
Amendment number:	Amendment 2
Amendment date:	11 May 2017
Co-ordinating author:	PPD, freelance writer for GSK Biologicals

Rationale/background for changes:

This amendment was primarily developed:

- To incorporate comments from the Thai Ethics Committee
  - Provide more detail on the testing for the tertiary endpoints.
- To facilitate meeting the secondary endpoint (To describe clinical presentations of dengue cases (confirmed and probable) and the tertiary endpoint (To explore other infectious aetiologies than dengue in subjects with episodes of febrile illness referred to as "suspected dengue case" (chikungunya, Zika, influenza, leptospirosis)).
  - Add blood draws at 12 months and 24 months in study sites located in the Asia Pacific (Incidence in Guadalajara Mexico, where study enrolment is complete at the time of the protocol amendment, is too low to justify to add these blood draws for Mexican subjects)
- To facilitate meeting the tertiary endpoint (To describe the spatial and temporal distribution of dengue cases (confirmed and probable) among cohort participants in the study areas and analyse determinants of spatio-temporal transmission (e.g. environmental, entomological, socio-demographic or ecological factors)).
  - Update spatial coordinates, household information, etc., during SDC visits, where applicable.
- To account for study sites that will no longer participate in the study (Indonesia, Vietnam and Monterrey Mexico)
  - Adjust sample size
  - Delete country specific wording that is no longer relevant
- To clarify
  - The cut-off time for Late SDC value
- To remove ELISA kits that are no longer applicable.
- To decrease the response time to address events of public health significance (such as an epidemic of arbovirus aetiology), and to address unforeseen principle investigator opinion on the needs of the study (based on GSK experience with investigators participating in the EPI-DEN-006 study in Brazil):
  - Increase the blood draw values in study sites located in the Asia Pacific (Incidence in Guadalajara Mexico, where study enrolment is complete at the time of the protocol amendment, is too low to justify to increase blood draw values for Mexican subjects)
- To eliminate the option for 12 month study duration (subjects at all sites will have a study duration of 24 months)

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

The list of contributing authors was updated:

- PPD ***, Director, Epidemiology***
- PPD , PPD , Study Delivery Lead***s***
- PPD ***, Statistician***
- PPD ***, Lead Statistician***
- PPD ***, Study Data Manger, for GSK Biologicals for Tata Consultancy Services***
- PPD , PPD **,** Clinical Safety representative***s***
- PPD ***, Lab study manager***

The address of the sponsor was updated in the protocol:

GlaxoSmithKline Biologicals
~~Rue de l'Institut, 89~~ ***14200 Shady Grove Road***
Rockville, MD 20850 USA~~B-1330 Rixensart - Belgium~~

Synopsis and Section 4 Study Design Overview

- Duration of longitudinal follow-up and number of visits: The study duration will be ~~determined~~ ***24 months*** for each participating site ~~(12 or 24 months)~~. Each subject will have ~~one~~ ***3*** scheduled visit at enrolment, ***at Month 12*** and a conclusion ***visit*** at ***Month 24. The Month 12 and Month 24 visits may occur via a telephone contact for the Mexican site.*** ~~Overall, asbout 75% of the subjects are expected to enrol in a two-year longitudinal follow-up and 25% in a one-year follow-up.~~

  ~~These periods will be described for each site and may be modified upon mutual agreement between the investigator and the central study team based on available epidemiological information.~~

  In countries with marked seasonality, the recruitment period ***would preferably*** occur outside the period of peak incidence of dengue, based on the local epidemiology of dengue in the past years and preferentially outside the holiday period (if the investigator believes that families are more likely to leave the study area during this period).

- Duration of the study: Because of differences in local regulations, dengue seasonality and holiday period between countries, the recruitment period will be staggered. However, to facilitate site activities, sites will target to have all the subjects recruited in a 3-month period. For each subject, the study duration will be ~~either~~ approximately ~~12 or~~ 24 months ~~(determined by site)~~.

- Epoch 001: Prospective data collection starting at Visit 1 (Day 0) and ending at the conclusion ***visit***~~contact~~ (Month ~~12 or~~ 24, ~~as applicable~~).

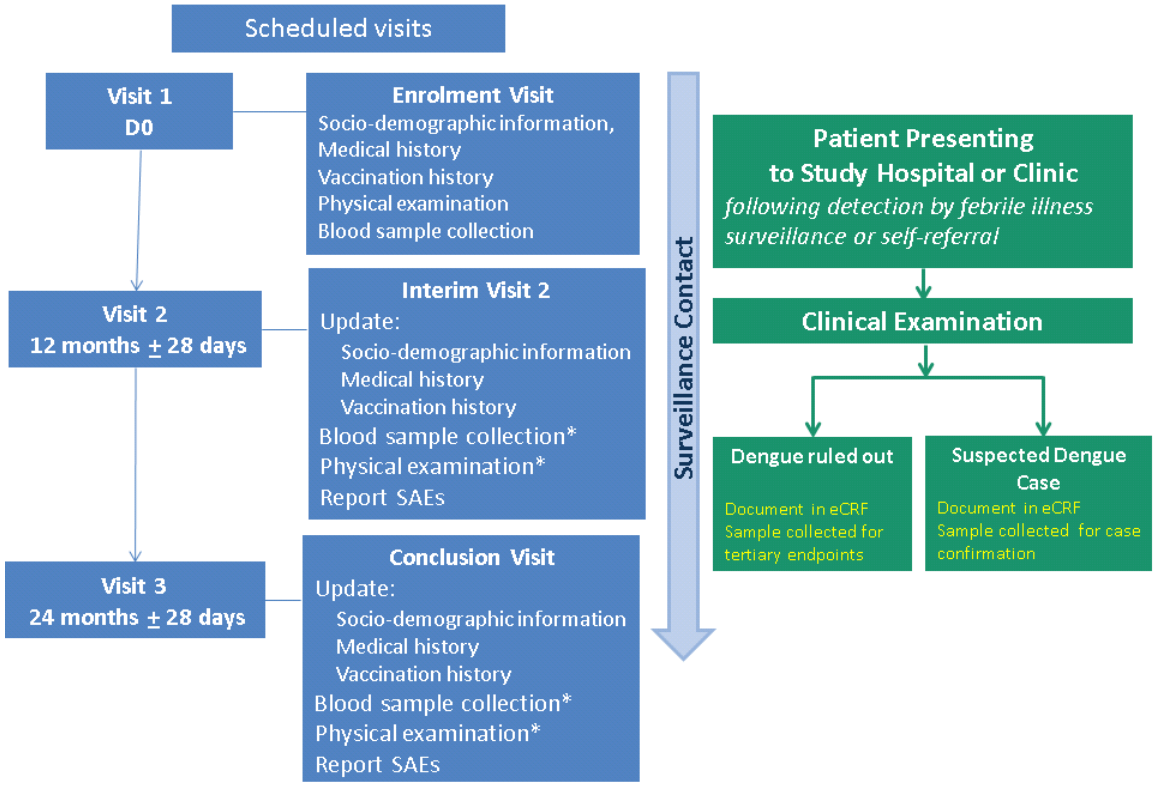
Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)	Epochs
			Epoch 001
Prospective	***1750*** ~~3000~~	6 months to 50 years	x

Section 4 Study design overview

Figure 1 Schematic representation of the study design

The Month 12 and 24 time points and the procedures to be carried out at these visits were added in the figure and the footnote was modified in the figure.



**** The Month 12 and Month 24 visits may occur via a telephone contact for the Mexican site and there will be no blood sample collection and physical examination at these visits for subjects in Mexico.***

Synopsis and Section 6.1 Number of subjects

Approximately, ***1750*** ~~3000~~ subjects are expected to be enrolled into this study with about 300-500 subjects expected per study site. Subjects aged between 6 months and 50 years at the time of enrolment living in geographically-defined communities in Latin America and Southeast Asia will be part of this study.

Section 4.1.1 Rationale for study design

Because dengue is endemic in the countries where study sites are located, the study population ~~will~~ ***should*** have a mixed anti-DENV immunological background, providing an opportunity to describe different response patterns to new infections.

Section 6.2 Overview of recruitment plan

Recruitment period

In countries with marked seasonality, the recruitment period should ***preferably*** occur outside the period of peak incidence of dengue, based on the local epidemiology of dengue in the past years.

Section 7.4 Outline of study procedures

Table 2 List of study procedures

Age		**6 months to 50 Years**					
Epoch		**Epoch 001**					
Data Collection		**Prospective data Collection**					
Visit		**Scheduled visits~~/contacts~~**			**Unscheduled visits (for acute febrile illness and suspected dengue at hospital/healthcare centre)**		
Time points	**Visit 1 Day 0**	**Regular Surveillance contact between visits[a]**	***Visit 2 Month 12***	**Conclusion ~~contact~~ *Visit 3* Month~~12 or~~ 24 §**	**First visit**	**Return visit[*]**	**Follow-up contact[**]**
Informed consent and assent as applicable	●						
Check inclusion/exclusion criteria	●						
Check evidence of subject being member of the household (family book or other administrative document)	O						
Attribute subject number and household number attribution	●						
Collect spatial coordinates of households[e]	●[e]						
Record/ update household and socio-demographic information	●		●	●			
Medical history and Japanese encephalitis (JE)/Yellow fever (YF)/ Dengue (if applicable) vaccination history or updates	●		●	●			
Distribute subject ID card and instruction kit[d]	O						
Collect blood sample	●		●[f]	●[f]			
Instruct/ remind subjects/subjects' parent(s)/LAR(s) or designate (if applicable) on assessment procedures in case of acute febrile illness	●	O					

Age		**6 months to 50 Years**					
Epoch		**Epoch 001**					
Data Collection		**Prospective data Collection**					
Visit		**Scheduled visits~~/contacts~~**			**Unscheduled visits (for acute febrile illness and suspected dengue at hospital/healthcare centre)**		
Time points	**Visit 1 Day 0**	**Regular Surveillance contact between visits[a]**	***Visit 2 Month 12***	**Conclusion ~~contact~~ *Visit 3* Month~~12 or~~ 24 §**	**First visit**	**Return visit[*]**	**Follow-up contact[**]**
Issue diary logs to subject/subject's parent(s)/LAR(s)/ designate and train them on how it is to be filled out in the event of an acute febrile illness[†]	O	O			O		
Contact the subject/subject's parent(s)/LAR(s)/ designate regarding any acute febrile illness and remind him/her/them of the procedures		O					
Physical examination and medical history (see Section 7.5.4)	●		●[f]	●[f]	●	●	
Collect or verify diary logs if applicable[b]					O	O	
Record if subject meets the definition of a SDC; if dengue is discarded, record the alternative diagnosis					●		
Record laboratory results prescribed as per local guidelines by the treating physician (may include CBC, liver enzymes, bilirubin)					●	●	
Record patient management[c]					●	●	
Collect blood sample for diagnosis[#]					●		
Outcome of dengue episode							●
Report Serious Adverse Events (SAEs) related to study procedures[‡]	●		●	●	●	●	●
Study conclusion			~~●~~	●			

~~§The conclusion contact may be done by phone or by a home visit (at the site's discretion).~~

[f]These procedures were included as part of the protocol amendment 2 and will not be applicable for Mexico.

Table 4 Intervals between study visits

Interval	Optimal length of interval[*]	Allowed interval
Visit 1 (Day 28) → ~~Conclusion contact~~ ***Visit 2*** (Month ~~12 or 24~~***12***~~, as applicable~~)	~~12 or 24~~ ***12*** months	±28 days
Visit 2 (Month 12) → Conclusion visit (Month 24)	***12 months***	***±28 days***
Interval between First visit for a SDC and	21 days	±7 days

the final follow-up contact for a case		

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

Section 7.5 Detailed description of study procedures

There will be one scheduled visit (Visit 1) at enrolment, ***a second visit at Month 12 (Visit 2) and*** a ***final*** conclusion ***visit at Month 24 (Visit 3)***~~as well as a conclusion contact which may be done by phone or via a home visit, at the discretion of the site. The conclusion contact will take place 12 or 24 months (+/- 28 days) after visit 1, depending on the duration of follow-up determined for the study site~~. ***The Month 12 and Month 24 visits may occur via a telephone contact for the Mexican site.***

Section 7.5.1.5 Collect spatial coordinates of the households

The participating households ~~will~~ ***may*** be geo-referenced with GPS (Global Positioning System) devices.

A geographic masking technique ~~will~~ ***may*** be applied (see Section 15.7 ~~11.7~~) to remove the possibility of re-identification of participants.

Collection of spatial coordinates ~~can~~ ***may*** be done at any time during the recruitment period and not necessarily at Visit 1.

Section 7.5.1.7 *Physical examination*, medical history and JE/YF/dengue vaccination history

A detailed clinical examination will be carried out to assess the subject's general condition, cardiac and respiratory rates, blood pressure, dengue associated clinical signs/symptoms.

Section 7.5.1.9 Collect blood sample

At Visit 1, a blood sample of 3.5 ***- 5*** mL will be collected from subjects aged between 6 months and 2 years (all sites), and a blood sample of ~~7~~ ***10 – 15*** mL will be collected from subjects aged between 2 and 50 years (~~in~~ all sites ~~except Indonesia and Vietnam~~).

~~At Visit 1, a blood sample of 3.5 mL will be collected from subjects enrolled in Indonesia aged between 6 months and 50 years. The volume in Indonesia is lower than in other countries as testing for DENV neutralising antibodies and other flaviviruses will not be performed for these subjects. The lower volume collected for subjects enrolled in Indonesia and Vietnam may result occasionally in the impossibility to repeat certain assays if needed (for instance in case of invalid results after the first assay run).~~

~~At visit 1, a blood sample of 5 ml will be collected for subjects aged between 2 and 50 years enrolled in Vietnam to comply with local acceptability requirement. However, this lower volume may result occasionally in the impossibility to repeat certain assays if needed (for instance in case of invalid results after the first assay run)~~.

Section 7.5.2 Procedures at *Visit 2 and* conclusion visit (Visit 3) ~~contact~~

The Visit 2 and Visit 3 will take place at a designated facility, as determined by each participating site. Home visits may also be organised, at the convenience of the study team. ~~The conclusion contact will be done by phone contact or by home visit, at the convenience of the site.~~

A detailed clinical examination will be carried out to assess the subject's general condition, cardiac and respiratory rates, blood pressure, dengue associated clinical signs/symptoms.

A blood sample of 3.5 - 5mL will be collected from subjects aged between 6 months and 2 years (all sites), and a blood sample of 10 mL will be collected from subjects aged between 2 and 50 years (all sites). The Month 12 and Month 24 visits may occur via a telephone contact for the Mexican site and there will be no blood sample collection at these visits for subjects in Mexico.

At study conclusion ***(Visit 3)*** for each subject, the investigator will:

- Review all the data collected to ensure accuracy and completeness
- Complete the study conclusion screen in the eCRF.

Section 7.5.4 Procedures at unscheduled visits and management of SDC

Rapid dengue diagnostic tests (NS1, IgM, and IgG) ~~or ELISA tests (depending on the country local standards)~~ will be performed as well because a result can be provided rapidly to the physician and subject and support the diagnosis.

Section 7.6.2 Biological samples

Table 5 presents the volume of blood that will be collected from subjects for study-related testing during each visit ~~(all sites except Indonesia and Vietnam), Table 6 presents the volume of blood that will be collected from subjects in Vietnam and Table 7 presents the volume of blood that will be collected from subjects enrolled in Indonesia for study-related testing during each visit. The volume of blood collected for subjects enrolled in Indonesia at the enrolment visit will be lower than in other countries because neutralising antibodies against DENV and other flaviviruses will not be measured. The volume of blood collected for subjects enrolled in Vietnam at the enrolment visit and at the initial visit for dengue suspicion will be lower than in other countries to comply with local acceptability criteria; however, this may result occasionally in the impossibility, due to limited serum volume, to repeat certain assays if needed (for instance in case of invalid results after the first assay run)~~

Table 5 Volume of whole blood for study related testing ~~related to sites with the exception of Indonesia and Vietnam~~

Age group	Type of Visit		
	Visit 1, *2 and 3* ~~(enrolment)~~ ~~Day 0~~	SDC – first visit	
		Early presenter	Late Presenter
6 months to <2 years	3.5 ml ***-5ml***	3.5 ml ***-5ml***	3.5 ml***-5ml***
2-50 years	~~7~~ ***10 – 15*** ml	~~7~~ ***10*** ml	~~3.5~~ ***10*** ml

~~Table 6 Volume of blood for study related testing in subjects enrolled in Vietnam~~

~~Age group~~	~~Type of Visit~~		
	~~Visit 1 (enrolment) Day 0~~	~~SDC – first visit~~	
		~~Early presenter~~	~~Late Presenter~~
~~6 months to <2 years~~	~~3.5 ml~~	~~3.5 ml~~	~~3.5 ml~~
~~2-50 years~~	~~5 ml~~	~~5 ml~~	~~3.5 ml~~

~~Table 7 Volume of blood for study related testing in subjects enrolled in Indonesia~~

~~Age group~~	~~Type of Visit~~		
	~~Visit 1 (enrolment) Day 0~~	~~SDC – first visit~~	
		~~Early presenter~~	~~Late Presenter~~
~~6 months to <2 years~~	~~3.5 ml~~	~~3.5 ml~~	~~3.5 ml~~
~~2-50 years~~	~~3.5 ml~~	~~7 ml~~	~~3.5 ml~~

If blood collection is not feasible in a young child with SDC, a finger prick test may alternatively be performed for testing with a IgM/IgG/NS1 ICT.

Section 7.6.3 Laboratory assays *for primary and secondary endpoints*

~~Table 8~~ Table 6 presents the possible laboratory assays ***for primary and secondary endpoints*** on the blood sample that will be drawn from the subject.

~~Table 8~~ Table 6 Possible laboratory assays on study blood samples ***for primary and secondary endpoints***

Time point	Possible parameters/assays measured
Scheduled visit 1***, 2 and 3****	- Anti-DENV indirect IgG Enzyme Linked Immunosorbant Assay (ELISA) ~~- DENV neutralising antibody assay~~ ~~- JEV neutralising antibody assay~~ ~~- WNV neutralising antibody assay*~~ ~~- Tertiary exploratory assays to characterize DENV immune status (e.g. antibody avidity assay, etc.)~~
Unscheduled Visit: SDC, early presenter (≤ 5 days following onset of symptoms)	- DENV RT-qPCR - DENV isolation for sequencing purpose - DENV sequence - DENV NS1 rapid test (ICT) or ELISA - IgM/Ig G rapid test (ICT) or ***SD BIOLINE Dengue Duo (Dengue NS1 Ag + IgG/IgM)*** ~~IgM/IgG capture ELISA~~ ~~- PCR/serology for other infectious agents causing febrile illness (chikungunya, zika, influenza, leptospirosis)~~
~~Unscheduled Visit: SDC, late presenter (> 6 days following onset of symptoms)~~	~~- DENV NS1 rapid test or ELISA~~ ~~- IgM/IgG rapid test or IgM/IgG capture ELISA~~ ~~- PCR/serology for other infectious agents causing febrile illness (chikungunya, zika, influenza, leptospirosis)~~

* ***Blood sample will not be collected at Visit 2 and Visit 3 for subjects in Mexico***
~~*DENV, JEV and WNV neutralising antibody assays will not be done for Indonesia.~~

~~The subset of subjects for which assays related to tertiary endpoints (e.g. neutralising antibodies, tests to further characterise the immune status to dengue, PCR/serology to identify other infectious agents causing febrile illness) will be performed will be determined based on preliminary results of the study (identification of laboratory confirmed and probable symptomatic dengue cases during the surveillance, medical history/vaccination history for other flaviviruses, etc.).~~

The purpose of these diagnostic tests is to provide prompt laboratory results to the physician and the subject. Rapid ICT (point of care) will be used, ~~unless local guidelines require the use of ELISA tests~~.

~~Table 9~~ Table 7 presents humoral immunity to DENV.

~~Table 9~~ Table 7 Humoral immunity (DENV antibody determination) ***for primary and secondary endpoints***

System	Component	Method	Kit / Manufacturer	Laboratory
Serum	IgM/IgG to DENV	Rapid ICT assay or ***Dengue NS1 Ag + IgM/IgG*** ~~IgM/Ig G capture ELISA~~	Standard Diagnostics (ICT) or equivalent/ ~~PanBio (Capture ELISA)~~ ***SD BIOLINE Dengue Duo(Dengue NS1 Ag +IgG/IgM)*** or equivalent	Local
	IgG to DENV (for scheduled visit 1)	ELISA	Indirect IgG Panbio or equivalent	Central or local GSK designated laboratory
	DENV types 1-4 neutralising antibodies	Dengue neutralisation assay~~*~~	In-house	GSK designated laboratory

~~*Dengue Neutralisation assay will not be done for Indonesia.~~

~~Table 10~~ **Table 8 Virology** ***for primary and secondary endpoints***

System	Component	Method	Laboratory
Serum	DENV RNA	RT-qPCR	Central or local GSK designated laboratory
	~~NS1 antigen~~	~~Rapid test (ICT) or ELISA~~	~~Local~~

Note: Viral isolation may be conducted if needed.

Section 7.6.4 Laboratory assays to be performed for tertiary endpoints

Table 9 presents the possible laboratory assays for primary and secondary endpoints on the blood sample that will be drawn from the subject.

Table 9 Possible laboratory assays on study blood samples for tertiary endpoints

Time point	*Possible parameters/assays measured*
Scheduled visit 1, 2 and 3*	***- DENV neutralising antibody assay*** ***- JEV neutralising antibody assay*** ***- WNV neutralising antibody assay*** ***- Tertiary exploratory assays to characterize DENV immune status (e.g. antibody avidity assay, etc.)*** ***- Chikungunya serology (IgM/IgG detection) and neutralizing antibodies*** ***- Zika serology (IgM/IgG detection) and neutralizing antibodies***
Unscheduled Visit: SDC, early presenter (≤ 5 days following onset of symptoms)	***- - PCR/serology for other infectious agents causing febrile illness (chikungunya, Zika, influenza, leptospirosis)***
Unscheduled Visit: SDC, late presenter (> 6 days following onset of symptoms)	***- DENV NS1 rapid test*** ***- IgM/IgG rapid test or IgM/IgG capture ELISA*** ***- PCR/serology for other infectious agents causing febrile illness (chikungunya, Zika, influenza, leptospirosis)***

**** Blood sample will not be collected at Visit 2 and Visit 3 for subjects in Mexico.***

Laboratory assays related to tertiary endpoints will only be performed after the primary and secondary endpoints have been ensured, and the local epidemiology of dengue virus disease is better understood.

The results of the primary and secondary endpoints, public health needs, and availability of biological samples will guide:

- ***The subset of subjects selected for which assays related to tertiary endpoints will be conducted***
- ***Which pathogens should be tested (JEV, WNV, chikungunya, Zika, influenza, leptospirosis)***
- ***The assays and tests to be performed.***

If testing for tertiary endpoints will be conducted, these parameters and rationale will be provided to the local ethical committees and relevant health authorities for approval. Testing may include, but are not limited to the following tests for humoral immunity and virology.

Table 10 Humoral immunity for tertiary objectives

System	Component	Method	Kit / Manufacturer	Laboratory
Serum	Antibodies against Zika virus	To be determined	To be determined	GSK designates lab
Serum	Zika virus neutralizing antibody	Zika neutralization assay	To be determined	GSK designated laboratory
Serum	Antibodies against chikungunya virus	To be determined	To be determined	To be determined
Serum	YF virus neutralizing antibody	YF neutralization assay	To be determined	GSK designated laboratory
Serum	JEV neutralizing antibody	JEV neutralization assay	To be determined	GSK designated laboratory
Serum	WNV neutralizing antibody	WNV neutralization assay	To be determined	GSK designated laboratory

Table 11 Virology for tertiary endpoints

System	Component	Method	Kit/Manufacturer	Laboratory
Serum	Zika RNA	RT-PCR	To be determined	GSK designated laboratory
Serum	Chikungunya RNA	RT-PCR	To be determined	GSK designated laboratory
Serum	JEV RNA	RT-PCR	To be determined	GSK designated laboratory
Serum	WNV RNA	RT-PCR	To be determined	GSK designated laboratory

Section ~~7.6.4~~ 11.1.1 Biological samples evaluation

~~**Table 9**~~ Table 12 presents the aliquoting of blood samples collected at scheduled visits.

~~Table 9~~ Table 12 Possible assays *for primary and secondary endpoints* for blood samples collected at scheduled visits

Blood sampling time point		Possible assays	Estimated number of subjects	Serum aliquot	Components priority rank
Type of contact/	Sampling time point				
Scheduled Visit 1, ***Visit 2 and Visit 3****	Day 0 Month 12 Month 24	Anti-DENV IgG indirect ELISA	All	200 µl	1
		DENV virus 1-4 neutralising antibody assay, ~~JEV and WNV neutralising antibody assay, serum bank for other tertiary exploratory assays~~	Subset	***1***x 1 ml or remaining volume	2

~~*~~Blood sample will not be collected at Visit 2 and Visit 3 for subjects in Mexico ~~**The DENV neutralising assay and JEV, WNV and tertiary exploratory assays will not be done for Indonesia.~~

Table 13 Aliquot preparation for blood samples collected at visits for suspected dengue and follow-up contact

Blood sampling time point	Serum aliquot volume	Possible Assays	Estimated number of subjects	Components priority rank
First visit – presentation within 5 days following onset of symptoms (early presenter)	750 µl	DENV RT-qPCR	Unknown	1
	Approximately 500 µl	DENV NS1/IgM/IgG rapid test (ICT) ~~or ELISA~~	Unknown	2
	1 ml ~~750 µl~~ or remaining volume	~~DENV RT-qPCR~~ PCR/serology of other infectious aetiologies (chikungunya, Zika, influenza, leptospirosis)	Unknown	3
		Viral isolation and sequencing		
First visit – presentation 6 days or more following onset of symptoms (late presenter)	Approximately 500 µl	DENV NS1/IgM/IgG rapid test (ICT) ~~or ELISA~~	Unknown	1
	1 ml ~~750 µl~~ or remaining volume	PCR/serology of other infectious aetiologies (chikungunya, Zika, influenza, leptospirosis)	Unknown	2

Section 13 Subject completion and withdrawal

Section 13.1 Subject completion

A subject who is available for the concluding ~~contact~~ ***visit*** foreseen in the protocol is considered to have completed the study.

Subject 13.2 Subject withdrawal

From an analysis perspective, a 'withdrawal' from the study refers to any subject who was not available for the concluding ~~contact~~ ***visit*** foreseen in the protocol.

All data collected until the date of withdrawal/last ***visit*** ~~contact~~ of the subject will be used for the analysis.

Section ~~10.2~~ 14.2 Determination of Sample size

The target population to be enrolled is estimated at ~~3000~~ ***1750*** subjects (approximately 300 to 500 per site)~~, with at least 75% expected to enrol for a 2-year longitudinal follow-up and 25% expected to enrol for a 1-year follow-up~~. A dropout rate of 5% per year is estimated, which would lead to approximately ~~2850~~ ***1662*** subjects completing 1 year of follow-up and approximately ~~2031~~ ***1579*** completing 2 years of follow-up, accumulating ~~5008~~ ***3327***patient-years.

The incidence of dengue is likely to vary by site and by age group. The study population should include between 30% and 50% of adults, but the distribution is not further specified as it would make operational feasibility more complex.

Supposing that the study population is composed of 70% of children with an expected incidence of 8 RT-qPCR-confirmed dengue case per 1000 person-years and 30% of adults with an expected incidence of 5 RT-qPCR confirmed dengue cases per 1000 person-years, the study would detect about ~~28~~ ***19*** cases in children and ~~8~~ ***5*** cases in adults (~~21~~***12*** cases in year one with a cohort of ~~3000~~ ***1750*** subjects and ~~15~~ ***12*** cases in year 2 with a cohort of about ~~2250~~ ***1662v***subjects).

The enrolment will be done by household. Each household can be considered as a cluster and this induces a design effect to account for the between-cluster variability when estimating the confidence intervals (CI) of the incidence rates. The design effect measures the increase in the standard error of the incidence rate estimate due to the sampling design used and is given by: D = 1 + (b – 1) rho, where rho is the intra-cluster correlation (a measure of rate of homogeneity within clusters) and b is the average number of subjects sampled per household. Here, we assume b is assumed to be 3. Although in theory ‘rho’ can have a value up to 1, in practice values higher than 0.4 are uncommon. We have used a conservative estimate of 0.4 for this study [Bennett, 1991]. The design effect is then estimated to 1.8.

Table 15 shows the 95% CI for a range of scenarios in term of cohort size, dropout rates and incidence rates. With an overall sample size of a minimum of about ~~3000~~ ***1750*** evaluable subjects, a follow-up period of two years and the expected incidences of 8 cases per 1000 person-years in children and 5 cases per 1000 person-years in adults, the overall incidence rate would be ~~7.0~~ ***7.1*** with an exact Poisson 95% CI of [~~5.0, 9.5~~ ***4.5; 10.6***], and with a CI based on the normal approximation and accounting for the design effect of [~~4.1, 9.9~~***3.3;10.9***].

Table 16 Expected 95% confidence intervals for the incidence rate of virologically confirmed dengue cases

Cohort size	Proportion of adults	Dropout rate (per year)	Incidence rates		Person-years	Number of cases	Overall incidence (1000 p-y)	Confidence intervals (1000 p-y)			
			Children (p-y)*	Adults (p-y)				Exact (Poisson)		Normal approx + DE**	
								Lower	Upper	Lower	Upper
1400	*30%*	*5%*	*0.008*	*0.003*	*2662*	*17*	*6.5*	*3.8*	*10.4*	*2.4*	*10.6*
1575	*30%*	*5%*	*0.008*	*0.003*	*2994*	*19*	*6.5*	*3.9*	*10.1*	*2.6*	*10.4*
1750	*30%*	*5%*	*0.008*	*0.003*	*3327*	*22*	*6.5*	*4.1*	*9.9*	*2.8*	*10.2*
1400	*30%*	*5%*	*0.008*	*0.005*	*2662*	*19*	*7.1*	*4.2*	*11.1*	*2.8*	*11.4*
1575	*30%*	*5%*	*0.008*	*0.005*	*2994*	*21*	*7.1*	*4.4*	*10.8*	*3.1*	*11.1*
1750	*30%*	*5%*	*0.008*	*0.005*	*3327*	*24*	*7.1*	*4.5*	*10.6*	*3.3*	*10.9*
1400	*30%*	*5%*	*0.01*	*0.003*	*2662*	*21*	*7.9*	*4.9*	*12.1*	*3.4*	*12.4*
1575	*30%*	*5%*	*0.01*	*0.003*	*2994*	*24*	*7.9*	*5.0*	*11.8*	*3.6*	*12.2*
1750	*30%*	*5%*	*0.01*	*0.003*	*3327*	*26*	*7.9*	*5.2*	*11.5*	*3.8*	*12.0*
1400	*30%*	*5%*	*0.01*	*0.005*	*2662*	*23*	*8.5*	*5.4*	*12.8*	*3.8*	*13.2*
1575	*30%*	*5%*	*0.01*	*0.005*	*2994*	*25*	*8.5*	*5.5*	*12.5*	*4.1*	*12.9*
1750	*30%*	*5%*	*0.01*	*0.005*	*3327*	*28*	*8.5*	*5.7*	*12.3*	*4.3*	*12.7*

Cohort size	Proportion of adults	Percentage followed during two years	Dropout rate (over 2 years)	Incidence rates		Person-years	Number of cases	Overall incidence (1000 p-y)	Confidence intervals (1000 p-y)			
				Children (p-y)*	Adults (p-y)				Exact (Poisson)		Normal approx + DE**	
									Lower	Upper	Lower	Upper
2400	30%	75%	5%	0.008	0.003	4006	26	6.5	4.2	9.5	3.1	9.8
2700	30%	75%	5%	0.008	0.003	4507	29	6.4	4.3	9.2	3.3	9.6
3000	30%	75%	5%	0.008	0.003	5008	32	6.4	4.4	9.0	3.4	9.4
2400	30%	75%	5%	0.008	0.005	4006	28	7.0	4.6	10.1	3.5	10.5
2700	30%	75%	5%	0.008	0.005	4507	32	7.1	4.9	10.0	3.8	10.4
3000	30%	75%	5%	0.008	0.005	5008	35	7.0	4.9	9.7	3.9	10.1
2400	30%	75%	5%	0.01	0.003	4006	31	7.7	5.3	11.0	4.1	11.4
2700	30%	75%	5%	0.01	0.003	4507	35	7.8	5.4	10.8	4.3	11.2
3000	30%	75%	5%	0.01	0.003	5008	39	7.8	5.5	10.6	4.5	11.1
2400	30%	75%	5%	0.01	0.005	4006	34	8.5	5.9	11.8	4.7	12.3
2700	30%	75%	5%	0.01	0.005	4507	38	8.4	6.0	11.6	4.8	12.0
3000	30%	75%	5%	0.01	0.005	5008	42	8.4	6.1	11.3	5.0	11.8

Section ~~12~~ 16 Country Specific Requirements

~~The duration of individual follow-up is 12 month for 2 sites (one site out of 2 in Mexico, one site in Indonesia) and 24 months for all the other sites.~~

Section 16.1 Thailand specific information and requirements

Section 16.1.1 List name, affiliation and contact details of all investigators

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Section 16.1.2 Definition of burden on the study protocol

Burden on the study protocol is means to incidence and severity of Dengue infection and admission in hospital of volunteer/subjects.

Section 16.1.3 Duration of the study, site address and number of participating subjects in Thailand

Trial Site in Thailand

There is 1 trial site participating in Thailand at Sam Kwai Phuek Health Promoting Hospital, Muang District, Nakhon Pathom Province. The site is planning to enrol 500 subjects in this study. Study team members are from Department of Pediatric Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, and Deputy Nakhonpathom Provincial Chief Medical Officer, Nakornpathom provincial Health Office, consultant of the study.

This study is multicentre (International) that will be conducted in 4 countries. As listing below:

- ***Thailand is planning to enrol 500 subjects***
- ***Malaysia is planning to enrol 400 subjects***
- ***Philippines is planning to enrol 500 subjects***
- ***Mexico is planning to enrol 350 subjects***

<u>Duration of the study in Thailand</u>

The study duration for Thailand is 2.0 years (24 months).

Planning of recruitment period is June – December 2017.

Follow up visit period of the study is June 2017 – June 2020.

Planning of the study closure is June 2020.

For each subject, the study duration is approximately 2 years (24 months)

Section 16.1.4 Study population groups and Calculation of study population groups in Thailand

The size of Thailand study population is derived from the average yearly incidence of dengue fever in Sam Khwai Phuak Sub-district from 2009 to 2013 of 283 per 100,000 people. If it is assumed that this incidence is lower than actual incidence, according to population data of Sam Khwai Phuak Sub-District of approximately 10,900 people, a sample size of 500 people will be able to detect the incidence of dengue fever in Sam Khwai Phuak Sub-district of close to 600 per 100,000 people, with a confidence level of 80 percent and an estimated data deficiency of no more than 15 percent.

A total of approximately 500 subjects will be recruited from Thailand.

In Thailand, a total of approximately 500 subjects will be recruited and there will be a total of 1 study site participating in this study, which is Sam Khwai Phuek Health Promotion Hospital, Muang District, Nakhon Pathom Province, which will recruit approximately 500 subjects.

Department of Tropical Paediatrics, Faculty of Tropical Medicine, Mahidol University, is the main site for administration with no recruitment. Investigator and study team are affiliated with Department of Tropical Paediatrics, Faculty of Tropical Medicine, Mahidol University.

Deputy Nakhonpathom Provincial Chief Medical Officer [Expert Public Health Physician (Preventive Medicine)] from Nakhon Pathom Province, a local person, is the project consultant.

The roles of study team at the Sam Kwai Phuek Health Promotion Hospital is:

To be the local person coordinating public utility payments at the study site for which the research project is the party responsible for, and coordinating with community leaders and the community in the area.

Section 16.1.5 Overview of the Subject Recruitment Plan for Thailand

The method of finding/recruiting appropriate subjects will be selected by each research site participating in the study. One of the following 2 methods may be selected:

- ***School recruitment method***
- ***Community recruitment method (without schools being involved)***

For Thailand, however, subjects will be recruited using the community recruitment method (without schools being involved).

Section 16.1.6 Adding Secondary Objective Explanations

To describe the clinical symptoms of patients infected with the dengue virus (which have been confirmed and which are within the scope of suspected infection).*

****This includes studying clinical symptoms and the severity of the dengue virus infection. The severity of the dengue virus infection in this study will be monitored with the subject by taking their physical examination history, and tracking the progress of the disease and hospitalization during the follow-up contact after 21 days of the first visit.***

Section 16.1.7 Informed Consent and Consent for Thailand

Obtaining consent from subjects in this study means requesting consent from individual subjects.

Subjects/guardians of subjects will be given an explanation of various details, including both the natures and risks involved with the study. The above process will be carried out by the head of protocol or protocol participants prior to obtaining signatures of consent from the subjects/imprinting the fingerprints of the subjects and/or the father/mother/legally acceptable representative of the subjects prior to their participation in the study, including the signature of the person providing protocol details (investigator) and relevant witnesses. The person providing protocol details must allow sufficient time for the subjects to decide whether or not to participate in the study, with the signing of the Subject Information Sheet and Informed Consent Form for adults, and Subject Information Sheet and Informed Assent Form for children, or Subject Information Sheet and Informed Consent Form for the father/mother/legally acceptable representative of the subjects to the amount of 2 copies of each. The first copy will be kept at the study site and the second copy will be kept by the subject. In addition, if the subjects or the father/mother/legally acceptable representative of the subjects would like to appoint a designee* to help with certain parts of the study procedures, including to help in monitoring and checking the body temperature of the subjects in the event they have a fever, completing daily records, answering calls from study staff who will ask whether or not the participant has a fever, and reminding the subjects of the study procedure, and help planning study visits, this designee is not able to make any decision on behalf of the subjects, or the father/mother/legally acceptable representative of the subjects. Moreover, the above designee must sign their name to receive consent from the subjects or the father/mother/legally acceptable representative of the subjects, or the father/mother/legally acceptable representative of the subjects together with the signature of the person providing protocol details (investigator) and the witnesses in the designee information document and letter of consent to participate in the study, to the amount of 2 copies. The first copy will be kept at the research site and the second copy will be kept by the subject.

The Subject Information Sheet and Informed Consent Form for this study are as follows:

- ***Subject Information Sheet for research subject that are between 20 – 50 years old***
- ***Informed Consent Form for subjects that are between 20 – 50 years old***
- ***Subject Information Sheet for the father/mother/legally acceptable representative of subject from 6 months old to less than 20 years old***
- ***Informed Consent Form for the father/mother/legally acceptable representative of subject from 6 months old to less than 20 years old***
- ***Subject Information Sheet for subject from 7 years to less than 13 years old***
- ***Assent Form for subject from 7 years to less than 13 years old.***
- ***Subject Information Sheet for subject from 13 years to less than 20 years old.***
- ***Assent Form for subject from 13 years to less than 20 years old.***
- ***Subject Information Sheet for designees.***
- ***Informed Consent Form for designees.***

Section 16.1.8 Compiling Areal Coordinates of Households in Thailand

This study involves the compilation of the geographical areal coordinates of households. The compilation of the areal coordinates of subjects participating in the protocol with a Global Positioning System (GPS) device will deliver only subject number data and their areal coordinates. It will not specify the name, surname, home address, or any data that could affect the privacy of the subjects.

Section 16.1.9 Managing Biological Samples in Thailand

For Thailand, samples of subjects will be stored for a period of 5 years after the study is completed (counting from the date of the last subject's last contact in the study). In the event that investigator needs to store blood samples for longer than 5 years, the investigator and the study team will request approval from the Ethical Review Committee for Research in Human Subjects for a further period of 5 years, and the samples remaining after the period approved will be destroyed.

Subject samples will only be used for tests in this study.

Section 16.1.10 Potential Risks and Inconveniences, and Prevention Measures

Blood drawing may cause slight pain in the area blood is drawn from. There may be swelling or bruising, or dizziness. After blood is drawn some people may feel faint, or nauseous, and in very rare cases infection may arise in the area blood is drawn. Investigator will use sterile blood drawing techniques to prevent infection from blood drawing. We will use hospitals with experience in blood drawing, especially for small children, and will use a scalp vein of appropriate size. For any side effects that may be caused by blood drawing, investigator will offer treatment until full recovery free of charge.

Section 16.1.11 Benefits, Compensation, Reimbursement, Treatment and Solution in the Event of Complications for the Investigator

Benefits, Compensation, Reimbursement, Treatment and Solution in the Event of Complications for the Investigator

Subjects may not receive direct benefits from this study. However, knowledge regarding dengue fever derived from this study will be of great benefit in the prevention and management of dengue fever and administration of vaccines to prevent dengue fever in the future.

Subjects will receive treatment for any fever symptoms from a doctor or healthcare professional at the Sam Khwai Phuak Sub-District Health Promotion Hospital in accordance with standard medical practice guidelines.

Subjects will be tested for dengue virus diagnoses (NS1, IgG and IgM) as specified in the protocol, and will know the results within 15 minutes. This will benefit subject treatment at no expense.

Section 16.1.12 Compensation and Reimbursement

Compensation

None

Reimbursement

Subjects will receive the amount of 1,000 baht per person per visit as specified in the protocol as a reimbursement for time and travel costs, except the case where you have a fever when attending a scheduled visit, but the person providing treatment suspects that you may have dengue fever, in which case a return visit appointment will be made on the following day.

Section 16.1.13 Other Ethical Issues

Confidentiality

Investigator will keep personal information, study documents, and study results related to this study confidential, without disclosing them publicly. The investigator certifies that the personnel working in this project and the study sponsor will act in the same way. This study will keep subject information confidential and will disclose it in the form of overall study results that does not include any names or other data which may be able to identify the subjects, by using a numerical code instead of a name. This information, including medical records, may be examined by authorized personnel from Ministry of Public Health of Thailand or the Ethical Review Committee for Research in Human Subjects, only for the benefit of oversight, monitoring, and research evaluation. Regardless, the study sponsor and representatives, including those involved in all parts, must act in accordance with good clinical practices, and must, to the best of their ability, endeavour to keep personal information of subjects confidential.

Budget Details and Funding Sources

Study sponsor GlaxoSmithKline Biologicals

Budget Details: As per documents showing research budget details.

Section 16.1.14 Signature of all investigators

Principal Investigator

_____ _____

(Assoc. Prof. Dr. Pornthep Chanthavanich) ***Day / Month/ Year***

Consultant

_____ _____

(Dr. PPD) ***Day / Month/ Year***

Sub-Investigator

_____ _____

(Assoc. Prof. Dr. Chukiat Sirivichayakul) ***Day / Month/ Year***

_____ _____

(Assist. Dr. Kriengsak Limkittikul) ***Day / Month/ Year***

_____ _____

(Assist. Prof. Dr. Watcharee Chokejindachai) ***Day / Month/ Year***

_____ _____

(Dr. Weerawan Hattasingh) ***Day / Month/ Year***

_____ _____

(Assoc. Prof. Dr. Wijitr Fungladda) ***Day / Month/ Year***

_____ _____

(Dr. Supawat Chatchen) ***Day / Month/ Year***

Appendix B Clinical laboratories

Table 16 Outsourced laboratories

Laboratory	Address
Laboratório de Tecnologia Virológica, Bio-Manguinhos, Fiocruz	Rio de Janeiro, Brazil
Q^2 Solutions	26081 Avenue Hall, Suite 150, Valencia Ca, 91355-1241, United States
~~*Eijkman Institute for Molecular Biology*~~	~~*Jalan Diponegoro 69, Jakarta 10430, Indonesia, (within the Cipto Mangunkusumo Hospital / RSCM)*~~

GlaxoSmithKline Biologicals
Vaccine Value & Health Science (VVHS)
Protocol Amendment 3

eTrack study number and Abbreviated Title	200318 (EPI-DENGUE-007 BOD)
Amendment number:	Amendment 3
Amendment date:	24 July 2017
Co-ordinating author:	PPD, freelance writer for GSK Biologicals

Rationale/background for changes:

This amendment was developed to clarify that an additional blood sample will be collected for testing related to tertiary endpoints even when dengue is ruled out by the study physician during clinical examination.

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

Title page- Contributing authors

PPD, PPD, PPD, Study Delivery Leads**Section 7.5.1.9 Collect blood sample**

At Visit 1, a blood sample of 3.5 mL~~ 5 mL~~ will be collected from subjects aged between 6 months and 2 years (all sites), and a blood sample of 10~~ 15~~ mL will be collected from subjects aged between 2 and 50 years (all sites).

Section 7.5.2 Procedures at Visit 2 and conclusion visit (Visit 3)

A blood sample of 3.5 ~~ 5~~mL will be collected from subjects aged between 6 months and 2 years (all sites), and a blood sample of 10~~ *15*~~ mL will be collected from subjects aged between 2 and 50 years (all sites).

Section 7.5.4 Procedures at unscheduled visits and management of SDC

- All subjects with acute febrile illness should be seen at a designated study healthcare centre/hospital by a study physician. If dengue is ruled out by the study physician during clinical examination because of another obvious alternative diagnosis (identified focus of fever), the physician will document his alternative diagnosis, and ***collect a blood sample for testing related to tertiary endpoints*** ~~no further study procedures will be conducted~~.

Section 7.6.2 Biological samples

Table 5 Volume of whole blood for study related testing

Age group	Type of Visit			
	Visits 1, 2 and 3	SDC – first visit		Cases where dengue is ruled out
		Early presenter	Late Presenter	
6 months to <2 years	3.5 ml~~ 5ml~~	3.5 ml~~ 5ml~~	3.5 ml~~ 5ml~~	***3.5 mL***
2-50 years	10 ~~ 15~~ ml	10 ml	10 ml	***9 mL***

If blood collection is not feasible ~~in a young child with SDC~~, a finger prick test may alternatively be performed for testing with a IgM/IgG/NS1 ICT.

Section 7.6.3 Laboratory assays to be performed for primary and secondary endpoints

Table 7 Humoral immunity (DENV antibody determination) for primary and secondary endpoints

System	Component	Method	Kit / Manufacturer	Laboratory
Serum	IgM/IgG to DENV	Rapid ICT assay or Dengue NS1 Ag + IgM/IgG	Standard Diagnostics (ICT) or equivalent/ SD BIOLINE Dengue Duo(Dengue NS1 Ag +IgG/IgM) or equivalent	Local
	IgG to DENV (for scheduled visit 1, ***2 and 3****)	ELISA	Indirect IgG Panbio or equivalent	Central or local GSK designated laboratory
	DENV types 1-4 neutralising antibodies	Dengue neutralisation assay	In-house	GSK designated laboratory

Blood sample will not be collected at Visit 2 and Visit 3 for subjects in Mexico.

Section 7.6.4 Laboratory assays to be performed for tertiary endpoints

Table 9 Possible laboratory assays on study blood samples for tertiary endpoints

Time point	Possible parameters/assays measured
Scheduled visit 1, 2 and 3*	- DENV neutralising antibody assay - JEV neutralising antibody assay - WNV neutralising antibody assay - Tertiary exploratory assays to characterize DENV immune status (e.g. antibody avidity assay, etc.) - Chikungunya serology (IgM/IgG detection) and neutralizing antibodies - Zika serology (IgM/IgG detection) and neutralizing antibodies
Unscheduled Visit: SDC, early presenter (≤ 5 days following onset of symptoms)	- - PCR/serology for other infectious agents causing febrile illness (chikungunya, Zika, influenza, leptospirosis)
Unscheduled Visit: SDC, late presenter (> 6 days following onset of symptoms)	- DENV NS1 rapid test - IgM/IgG rapid test or IgM/IgG capture ELISA - PCR/serology for other infectious agents causing febrile illness (chikungunya, Zika, influenza, leptospirosis)
Unscheduled Visit: Cases where dengue is ruled out	***-PCR/serology for other infectious agents causing febrile illness (chikungunya, Zika, influenza, leptospirosis)***

Section 8 Safety

In this prospective cohort study no test product/vaccine will be given. However, blood samples will be collected at the enrolment visit ***(Visit 1), Visit 2, conclusion visit (Visit 3),*** ~~and~~ for suspected dengue ***and for subjects where dengue is ruled out***. SAEs related to any study procedure will be recorded.

Section 12.1.3 Duration of the study, site address and number of participating subjects in Thailand

The study duration for Thailand is 2.0 years (24 months).

Planning of recruitment period is ~~June – December 2017~~***October 2017-June 2018***.

Follow up visit period of the study is ~~June 2017 – June 2020~~***October 2017- October 2020***.

Planning of the study closure is ***October***~~June~~ 2020.

Section 12.1.2 Definition of burden on the study protocol

Burden ~~on~~ ***in*** the study protocol ~~is~~ means ~~to~~ incidence and severity of Dengue infection and ***hospital*** admission ~~in hospital~~ of volunteer/subjects.

Section 12.1.3 Duration of the study, site address and number of participating subjects in Thailand

This study is ***an international*** multicentre ~~(International)~~ ***cohort study*** that will be conducted in 4 countries. As ***Sample sizes*** are list***ed***ing below:

Section 12.1.4 Study population groups and calculation of study population groups in Thailand

The size of Thailand study population is derived from the average yearly incidence of dengue fever in Sam Khwai Phuak Sub-district from 2009 to 2013 of 283 per 100,000 people. If ***a lower than actual incidence is assumed,*** ~~it is assumed that this incidence is lower than actual incidence,~~ according to population data of Sam Khwai Phuak Sub-District of approximately 10,900 people, a sample size of 500 people will be able to detect the incidence of dengue fever in Sam Khwai Phuak Sub-district of close to 600 per 100,000 people, with a confidence level of 80 percent and an estimated data deficiency of no more than 15 percent.

~~A total of approximately 500 subjects will be recruited from Thailand.~~

In Thailand, a total of approximately 500 subjects will be recruited and there will be a total of 1 study site participating in this study, which is Sam Khwai Phuek Health Promotion Hospital, Muang District, Nakhon Pathom Province~~, which will recruit approximately 500 subjects~~.

Section 12.1.12 Compensation and Reimbursement

Subjects will receive the amount of 1,000 baht per person per visit as specified in the protocol as a reimbursement for time and travel costs, except the case where you have a fever when attending a scheduled visit, but the person providing treatment suspects that ~~you~~ ***he/she*** may have dengue fever, in which case a return visit appointment will be made on the following day