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

eTrack study number and Abbreviated Title	200274 (DPIV-021 EXPLO)
Date of protocol	Final Version 2: 31 July 2014
Date of protocol amendment	Amendment 1 Final: 16 December 2014 Amendment 2 Final: 02 April 2015 Amendment 3 Final: 01 March 2017
Title	A cohort study to determine the incidence of dengue fever and to build capacity for dengue vaccine trials in South Asia. (**Amended 01 March 2017**)
Detailed Title	A prospective, cohort study to determine the incidence of acute febrile dengue illness and to build capacity for dengue vaccine clinical endpoint trials in South Asian communities. (**Amended 01 March 2017**)
Co-ordinating authors	PPD (4Clinics Belgium for GSK Biologicals)/ PPD / PPD /PPD ***(XPE Belgium for GSK)*** (**Amended 01 March 2017**)
Contributing authors	• PPD, Clinical Research and Development Lead • PPD, ***Clinical and Epidemiol. Project Leader*** • PPD, ***Senior Epidemiologist*** • PPD, Epidemiologist • PPD, Project Statistician • PPD, Project Statistician • PPD, ***Project Statistician*** • PPD, Lead Statistician • PPD, PPD, Study Delivery Lead • PPD, Study Delivery Manager • PPD, Study Delivery Manager • PPD, PPD, Clinical Safety ***Physician*** • PI, PPD, Study Data Manager • PPD, ***Clinical Laboratory Science Clinical Readout Team Leader***

eTrack study number and Abbreviated Title	200274 (DPIV-021 EXPLO)
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GSK Biologicals' protocol template for observational studies and interventional studies without administration of medicinal products as described in a research protocol based on the Protocol Document Standard version 14.1

Protocol Amendment 3 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	200274 (DPIV-021 EXPLO)
Date of protocol amendment	Amendment 3 Final: 01 March 2017
Detailed Title	A prospective, cohort study to determine the incidence of acute febrile dengue illness and to build capacity for dengue vaccine clinical endpoint trials in South Asian communities. (**Amended 01 March 2017**)
Sponsor signatory	Robert Paris, Clinical and Epidemiology Project Lead

Signature _____

Date _____

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Protocol Amendment 3 Rationale

Amendment number: Amendment 3
Rationale/background for changes: After study enrolment was initiated, the 3 sites in India were still delayed due to the inability to export the blood samples for dengue testing. Following Sponsor review, the India portion of the study was cancelled due to logistical difficulties. Therefore, sites in India will no longer be included this study protocol. Among the 5000 subjects planned in the study, 3000 were to come from India. The planned number of study subjects changed from 5000 to 2000 and all subjects will be enrolled from Sri Lanka. Sample size calculations were adjusted accordingly. Added text to clarify that an unscheduled visit (either for first, return, or follow-up dengue visit) will be considered as the weekly contact for the week in which it occurs.

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) 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	200274 (DPIV-021 EXPLO)
Date of protocol amendment	Amendment 3 Final: 01 March 2017
Detailed Title	A prospective, cohort study to determine the incidence of acute febrile dengue illness and to build capacity for dengue vaccine clinical endpoint trials in South Asian communities (**Amended 01 March 2017**)
Investigator name	_____
Signature	_____
Date	_____

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Sponsor Information

1. **Sponsor**

GlaxoSmithKline Biologicals
Rue de 1'Institut, 89
B-1330 Rixensart - Belgium

2. **Sponsor Medical Expert for the Study**

Refer to the local study contact information document

3. **Sponsor Study Monitor**

Refer to the local study contact information document

4. **Study Contact for Report of a Serious Adverse Event (SAE) related to study participation**

GSK Biologicals Central Back-up Study Contact for Reporting SAEs realted to study participation: refer to protocol Section7.3.2

SYNOPSIS

Detailed Title

A prospective, cohort study to determine the incidence of acute febrile dengue illness and to build capacity for dengue vaccine clinical endpoint trials in South Asian communities. (**Amended 01 March 2017**):

Rationale for the study

This study aims to estimate the burden of dengue illness in selected sites in South Asia and to prepare sites for the conduct of future vaccine efficacy trials.

Operational goals include:

- Build long-term collaboration with sites in dengue-endemic regions of South Asia where the incidence of clinical dengue illness can be studied.
- Establish dengue surveillance cohorts that can be followed long-term.
- Establish operational feasibility of future Phase III studies with regard to recruitment, case capture and sampling procedures.
- Prepare sites for participation in Phase III clinical endpoint studies.

Scientific Objectives

Primary

- To determine the incidence of acute febrile illness (AFI) due to Laboratory Confirmed Dengue (LCD) in the study population.

Secondary

- To determine the incidence of AFI due to non-LCD in the study population.
- To describe the signs and symptoms of AFI due to LCD and of AFI due to non-LCD.
- To estimate the incidence of AFI due to LCD by Dengue Virus (DENV) type, study site, and age group.

Tertiary (Research)

- To describe DENVand Japanese Encephalitis Virus (JEV) antibody profiles in subset***s*** of subjects with AFI.
- To assess the concordance between dengue Reverse transcriptase-quantitative Polymerase Chain Reaction (RT-qPCR) and Non-Structural protein 1 (NS1)-antigen ImmunoChromaTographic (ICT) test and/or Enzyme-Linked Immunosorbent Assay (ELISA) in the assessment of dengue infection.
- To gather clinical data that will support the definition of clinical endpoints for future efficacy trials.
- To describe spatio-temporal clustering of LCD cases.
- To describe entomological characteristics at selected site(s).
- To estimate the prevalence and incidence of tuberculosis diagnosis in the study population.
- To describe the incidence of hospitalisation and discharge diagnosis in the study population.
- To isolate and characterise infectious agents from a subset of subjects with AFI.
- To evaluate the antibody response to infectious agents in a subset of subjects with AFI.To isolate and characterise infectious agents from a subset of subjects with AFI.
- To evaluate the antibody response to infectious agents in a subset of subjects with AFI.

Study design

- **Type of design** (**Amended 01 March 2017**): Prospective, community-based, cohort study, household-sampling, ***single country***
- **Study population** (**Amended 01 March 2017**)**:** approximately ***2,000*** subjects aged between 6 months and 50 years at the time of enrolment living in randomly selected households in geographically-defined communities. Households including at least one member aged less than 18 years will be considered eligible if at least one adult (aged no more than 50 years) and one child (aged less than 18 years) consent (and assent if applicable) to participate in the study.
- **Study visits** The study will consist of scheduled visits (the enrolment visit [Visit 1], weekly contacts, and the close-out visit [Visit 2]) and, in case of AFI*, unscheduled Suspected Dengue Visits:

- Visit 1 and Visit 2 will take place at home or at the hospital/clinic, in accordance with local ethics committee requirements and/or national laws or regulations
- Weekly contacts will be home visits (at least every other week) or telephone contacts (TCs)
- Unscheduled Suspected Dengue Visits will take place in case of AFI*

Note that an unscheduled visit (either for first, return, or follow-up dengue visits) will be considered as the weekly contact for the week in which it occurs. **(Amended 01 March 2017)**

*AFI is defined as fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days, measured at least twice, at least 8 hours apart.

- **Biological samples**: Blood samples will be collected at each Suspected Dengue First Visit.
- **Case definitions**

  AFI due to LCD: ALL of the following findings must be met for an AFI due to LCD:

  - Fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days**,** measured at least twice, at least 8 hours apart, and
  - Laboratory confirmation of dengue through dengue RT-qPCR on the acute serum sample taken during the 7-day period (Days 2-7) from the onset of fever (body temperature ≥ 38°C/≥ 100.4°F)

  AFI due to non-LCD: ALL of the following findings must be met for an AFI due to non-LCD:

  - Fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days, measured at least twice, at least 8 hours apart and
  - Dengue RT-qPCR result on the acute serum sample taken during the 7-day period (Days 2-7) from the onset of fever (body temperature ≥ 38°C/≥ 100.4°F) is (valid but) negative for dengue

Undetermined case: at least ONE of the following findings must be met for an undetermined case:

- Fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days**,** measured at least twice, at least 8 hours apart in a subject who has not been evaluated (at a Suspected Dengue First Visit or directly in a hospital/clinic) within the 7-day period (Days 2-7) from the onset of fever or who had an obvious alternative diagnosis other than dengue (i.e., an identified focus of fever), and therefore dengue RT-qPCR was not requested, or
- Dengue RT-qPCR result on the acute serum sample taken during the 7-day period (Days 2-7) from the onset of fever (body temperature ≥ 38°C/≥ 100.4°F) is invalid or missing

Note: Two consecutive calendar days without fever (body temperature ≥ 38°C/≥ 100.4°F), in the absence of antipyretic medication, are required to separate two episodes of fever.

Note: Subjects with fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days**,** measured at least twice, at least 8 hours apart who fail to attend a Suspected Dengue First Visit as they were directly hospitalised will be recorded as LCD, non-LCD or undetermined cases depending on the availability and result of the dengue RT-qPCR test.

- **Type of study**: self-contained.
- **Data collection**: Electronic Case Report Form (eCRF).
- **Duration of the study**: approximately 2 years per subject:

  Epoch 001: prospective data collection starting at Visit 1 (Month 0) and ending at Visit 2 (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	***≈2000***	6 months to 50 years	x

(Amended 01 March 2017)

Discussion of study design	This prospective cohort study including children aged less than 18 years and adults aged up to 50 years is designed to support the selection and definition of clinical and laboratory endpoints for future dengue vaccine efficacy trials and to provide incidence data for sample size calculation. In addition, the study aims to train investigators and their teams on protocol specific processes and procedures, in preparation for the conduct of anticipated future vaccine efficacy studies. (**Amended 01 March 2017**)
Number of subjects	Approximately ***2000*** (**Amended 01 March 2017**)
Endpoints	**Primary** • Occurrence of AFI due to LCD. **Secondary** • Occurrence of AFI due to non-LCD. • Occurrence and intensity of signs and symptoms of interest, during the 7-day period following the onset of each episode of AFI due to LCD and due to non-LCD. • Occurrence of AFI due to LCD by DENV type, study site, and age group. **Tertiary (Research) (Amended 01 March 2017)** During the Suspected Dengue First Visit, additional blood volumes will be collected for tertiary study endpoint assays. These tertiary study endpoints include, but are not limited to, determination of neutralising antibody titres against dengue virus (DENV), Japanese encephalitis virus (JEV), and chikungunya virus, tissue culture to isolate infectious agents, and sequencing to characterise infectious agents. Tertiary study endpoint assays other than dengue serology ***may*** be performed for a subset of samples (for example, JEV assays will only be conducted if additional studies indicate that JEV serology is needed to interpret the DENV antibody response), i.e., not all of these endpoint assays will be performed on all of the sera collected from adult study subjects. Research objectives may be assessed based on, but not limited to, the following endpoints: • Neutralising antibody titres against DENV 1-4. • Neutralising antibody titres against JEV. • Neutralising antibody titres against other viruses, including but not limited to, Chikungunya virus.

- Incidence of NS1-antigen (ICT test and/or ELISA)-positive AFI by DENV type.
- Concordance between dengue RT-qPCR and NS1-antigen (ICT and/or ELISA) assays.)
- Occurrence of AFI due to LCD and due to non-LCD having ≥ 3 days of fever (body temperature ≥ 38°C/≥ 100.4°F).
- Occurrence of AFI due to LCD and due to non-LCD resulting in hospitalisation.
- Occurrence of combinations of signs and symptoms during the 7-day period following the onset of each episode of AFI due to LCD and due to non-LCD.
- Occurrence of AFI due to LCD within 2 weeks from an index case, in the same household or within 50 metres of the household of the index case.
- Entomological characteristics for LCD cases (e.g., vector species, density and number of breeding sites) at selected site(s).
- Occurrence (by medical history only) of a diagnosis of tuberculosis, from birth up to study conclusion, in the study population.
- Occurrence of hospitalisation and discharge diagnosis.
- Identification and characterisation of infectious agents isolated from blood (e.g., DENV, influenza viruses, chikungunya viruses), in subjects with AFI.

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LIST OF ABBREVIATIONS

µPRN	Micro-Plaque-Reduction Neutralisation
AE	Adverse Event
AFI	Acute Febrile Illness
ATP	According-To-Protocol
CBC	Complete Blood Count
CI	Confidence Interval
DENV	Dengue Virus
DSS	Dengue Shock Syndrome
eCRF	electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
GCP	Good Clinical Practice
GIS	Geographic Information System
GMT	Geometric Mean Titre
GSK	GlaxoSmithKline
IAF	Informed Assent Form
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICT	ImmunoChromaTographic
IEC	Independent Ethics Committee
IRB	Institutional Review Board
JEV	Japanese Encephalitis Virus
LAR	Legally Acceptable Representative
LCD	Laboratory Confirmed Dengue
MedDRA	Medical Dictionary for Regulatory Activities
NS1	Non-Structural protein 1
PII	Personal Identifiable Information
PRN	Plaque-Reduction Neutralisation
PT	Preferred Term
RBC	Red Blood Cells
RT-qPCR	Reverse Transcriptase quantitative Polymerase Chain Reaction
SAE	Serious Adverse Event
SBIR	GSK Biologicals' randomisation system on internet

SDV	Source Document Verification
SPM	Study Procedures Manual
TC	Telephone Contact
WBC	White Blood Cells
WHO	World Health Organisation

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

Assent: When a subject is below the legal age of consent (i.e., a minor) and is able to give assent to decisions about his/her participation in a clinical study, the investigator is encouraged to obtain it, in addition to obtaining the consent from the subject's parent(s)/ Legally Acceptable Representative(s) (LAR[s]). The study investigator is accountable for determining a child's capacity to assent to participation in a research study, taking into consideration any standards set by the responsible Institutional Review Board (IRB) / Independent Ethics Committee (IEC) (or local requirements).

Assent refers to the child's affirmative agreement to participate in the research.

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.

Coded: Data from which personal identifier information has been removed and replaced by a key. These data are not anonymised since a decode listing exists and it is therefore possible to identify the patient under certain circumstances by an authorised or legally appointed third party data custodian, or by the original holder of the data.

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).
Community health worker:	A member of the local community who is selected, supported, and trained to work by the health system as a health aide in the community from which they come.
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
Epoch:	An epoch is a self-contained set of consecutive time points or a single time point from a single protocol. Self-contained means that data collected for all subjects at all time points within that epoch allows to draw a complete conclusion. 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 9.3 for details on criteria for evaluability).
Impartial witness:	A person, who is independent of the trial, who cannot be unfairly influenced by people involved with the trial, who attends the informed consent process (and assent process if applicable) if the subject of the subject's legally acceptable representative cannot read, and who reads the informed consent form (and assent form if applicable) and any other written information supplied to the subject.
Interventional Human Subject Research:	Studies in which participants are administered medical care, medicinal products and/or medical/scientific procedures as described in a research protocol.
Legally Acceptable Representative (LAR):	An individual or juridical or other body authorised under applicable law to consent, on behalf of a prospective subject, to the subject's participation to in the clinical trial.
Primary completion date:	The date that the final subject was examined or received an intervention for the purpose of final collection of data for the primary outcome, whether the clinical trial/pharmaco-epidemiological study concluded according to the pre-specified protocol or was terminated prematurely for any reason.
Prospective study:	A study in which the subjects/cases are identified and then followed forward in time in order to address one or more study objectives.
Research protocol:	A document that describes the objective(s), design, methodology, statistical considerations, and organisation of a study. The protocol usually also gives the background and rationale for the study, but these could be provided in other protocol referenced documents.

Self-contained study:	Study with objectives not linked to the data of another study.
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, subjects files, and records kept at the pharmacy at the laboratories and at medico-technical departments involved in the clinical trial).
Study population:	Sample of population of interest.
Study staff:	All study personnel, including study coordinators, field workers, nurses, investigators, technicians and other study functions.
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 (or assenting when applicable) 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, the most common arthropod-borne viral disease worldwide, is caused by four types of dengue viruses (DENV 1-4) and transmitted primarily by *Aedes aegypti*, a mosquito that is highly adapted to urban environments. Dengue infection can cause a range of clinical manifestations from febrile illness to potentially fatal dengue shock syndrome (DSS).

It is estimated that between 2.5 to 3.5 billion people, or 40 to 55 % of the world's population, live in areas where there is a risk of transmission of dengue fever [Kyle, 2008; WHO, 2009; Guzman, 2010]. Dengue is endemic in at least 100 countries in Asia, the Pacific, the Americas, Africa, and the Caribbean. New estimates indicate approximately 390 million (284-528) dengue infections occur per year, of which 96 million (67-136) manifest apparently (any level of disease severity) [Bhatt, 2013]. The global dengue burden is believed to increase due to increasing mobility, increasing urbanisation, ecological changes, and the inability to sustain effective vector control.

Globally, 70% of the estimated 96 million apparent dengue infections are thought to occur in Asia.

Sero-surveillance data from Colombo, Sri Lanka, indicate that over 70% of children experienced at least 1 dengue infection by the age of 12 years, and the median age at infection there is 4.7 years. The risk of primary infection in children below 12 years of age was 14% per year [Tam, 2013]. In 2012, the reported dengue fever incidence cases per 100,000 people were 200 for Sri Lanka [NVBDCP, 2017; Epidemiology Unit, 2017] and reports of dengue outbreaks and fatal cases frequently made headline news in South Asia.

In Sri Lanka, dengue surveillance and training of doctors in the management of dengue illness is a top priority of the Ministry of Health, especially because the rate of severe disease in Sri Lanka is one of the highest national rates reported. DENV infection and disease incidence in children in Colombo, Sri Lanka, have been reported to exceed 8% and 3% per year, respectively [Tissera, 2014].

(Amended 01 March 2017)

There are no licensed antivirals or vaccines to treat or prevent dengue. The development and widespread use of a safe and efficacious dengue vaccine is required to significantly reduce the global dengue burden. Estimating the incidence of clinically significant dengue fever is critical to the design of a dengue vaccine efficacy trial. In a given city or region, dengue incidence is highly variable from one year to another and cannot be reliably predicted. In a dengue vaccine efficacy trial, volunteers would be recruited from geographically distinct sites. Some of these sites would experience high dengue transmission during the trial duration while some other may have limited transmission. Therefore, it is important to make a valid assumption regarding the average incidence

across many sites in order to accurately calculate the sample size for a vaccine efficacy trial. In addition, a critical factor for the success of efficacy trials is the training of the various sites to perform febrile illness detection, dengue laboratory diagnosis and data collection in a cohort of subjects.

1.2. Rationale for the study

This study aims to estimate the burden of dengue illness in selected sites in South Asia and to prepare sites for the conduct of future vaccine efficacy trials.

Operational goals include:

- Build long-term collaboration with sites in dengue-endemic regions of South Asia where the incidence of clinical dengue illness can be studied.
- Establish dengue surveillance cohorts that can be followed long-term.
- Establish operational feasibility of future Phase III studies with regard to recruitment, case capture and sampling procedures.
- Prepare sites for participation in Phase III clinical endpoint studies

2. OBJECTIVES

2.1. Primary objective

- To determine the incidence of acute febrile illness (AFI) due to Laboratory Confirmed Dengue (LCD) in the study population.

Refer to Section 9.1.1 for the definition of the primary endpoint.

2.2. Secondary objectives

- To determine the incidence of AFI due to non-LCD in the study population.
- To describe the signs and symptoms of AFI due to LCD and of AFI due to non-LCD.
- To estimate the incidence of AFI due to LCD by Dengue Virus (DENV) type, study site, and age group.

Refer to Section 9.1.2 for the definition of the secondary endpoints.

2.3. Tertiary (research) objectives

(**Amended 01 March 2017**)

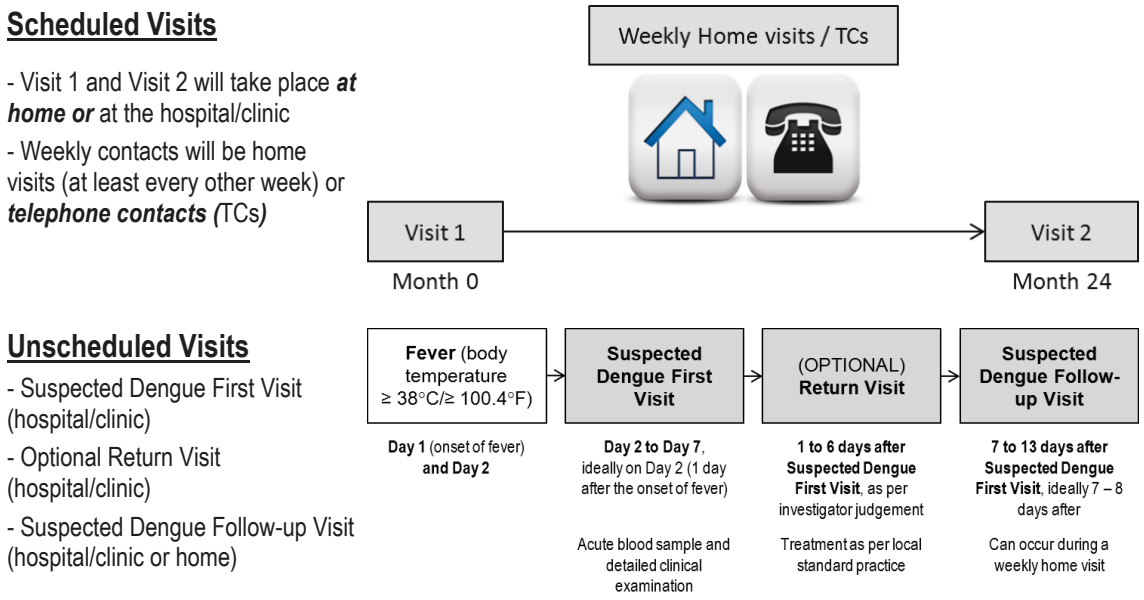
- To describe DENVand Japanese Encephalitis Virus (JEV) antibody profiles in subset*s* of subjects with AFI.
- To assess the concordance between dengue Reverse transcriptase-quantitative Polymerase Chain Reaction (RT-qPCR) and Non-Structural protein 1 (NS1)-antigen ImmunoChromaTographic (ICT) test and/or Enzyme-Linked Immunosorbent Assay (ELISA) in the assessment of dengue infection.
- To gather clinical data that will support the definition of clinical endpoints for future efficacy trials.
- To describe spatio-temporal clustering of LCD cases.
- To describe entomological characteristics at selected site(s).
- To estimate the prevalence and incidence of tuberculosis diagnosis in the study population.
- To describe the incidence of hospitalisation and discharge diagnosis in the study population.
- To isolate and characterise infectious agents from a subset of subjects with AFI.
- To evaluate the antibody response to infectious agents in a subset of subjects with AFI.

Refer to Section 9.1.3 for the definition of the tertiary (research) endpoints.

3. STUDY DESIGN OVERVIEW

Protocol waivers or exemptions are not allowed. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures (Section 6.4), are essential and required for study conduct.

Figure 1 Study design overview



Notes: **(Amended 01 March 2017)**

The weekly contact may be a face to face visit outside the home in certain cases (i.e. at hospital during suspected dengue visit)

An unscheduled visit (either for first, return, or follow-up dengue visit) will be considered as the weekly contact for the week in which it occurs.

- **Type of design**: Prospective, community-based, cohort study, household-sampling**,** ***single country***. **(Amended 01 March 2017)**
- **Study population:** Approximately ***2000*** subjects aged between 6 months and 50 years at the time of enrolment living in randomly selected households in geographically-defined communities. Households including at least one member aged less than 18 years will be considered eligible if at least one adult (aged no more than 50 years) and one child (aged less than 18 years) consent (and assent if applicable) to participate in the study. **(Amended 01 March 2017)**

- **Study visits**: The study will consist of scheduled visits (the enrolment visit [Visit 1], weekly contacts, and the close-out visit [Visit 2]) and, in case of AFI*, unscheduled Suspected Dengue Visits:
  - Visit 1 and Visit 2 will take place at home or at the hospital/clinic, in accordance with local ethics committee requirements and/or national laws or regulations
  - Weekly contacts will be home visits (at least every other week) or telephone contacts (TCs). ***The weekly contact may be a face to face visit outside the home in certain cases (i.e. at hospital during suspected dengue visit)*** **(Amended 01 March 2017)**
  - Unscheduled Suspected Dengue Visits will take place in case of AFI*

  *AFI is defined as fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days, measured at least twice, at least 8 hours apart.
- **Biological samples**: Blood samples will be collected at each Suspected Dengue First Visit.
- **Type of study**: self-contained.
- **Data collection**: Electronic Case Report Form (eCRF).
- **Duration of the study**: approximately 2 years per subject:
- **Epoch 001**: prospective data collection starting at Visit 1 (Month 0) and ending at Visit 2 (Month 24).

Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)	Epoch 001
Prospective	***≈2000***	6 months to 50 years	x

(Amended 01 March 2017)

3.1. Discussion of study design

This prospective cohort study including children aged less than 18 years and adults aged up to 50 years is designed to support the selection and definition of clinical and laboratory endpoints for future dengue vaccine efficacy trials and to provide incidence data for sample size calculation. In addition, the study aims to train investigators and their teams on protocol specific processes and procedures, in preparation for the conduct of anticipated future vaccine efficacy studies. (**Amended 01 March 2017**)

4. CASE DEFINITION

4.1. Acute febrile illness due to LCD

ALL of the following findings must be met for an AFI due to LCD (see Figure 2):

- Fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days**,** measured at least twice, at least 8 hours apart, and
- Laboratory confirmation of dengue through dengue RT-qPCR on the acute serum sample taken during the 7-day period (Days 2-7) from the onset of fever (body temperature ≥ 38°C/≥ 100.4°F)

4.2. Acute febrile illness due to non-LCD

ALL of the following findings must be met for an AFI due to non-LCD (see Figure 2):

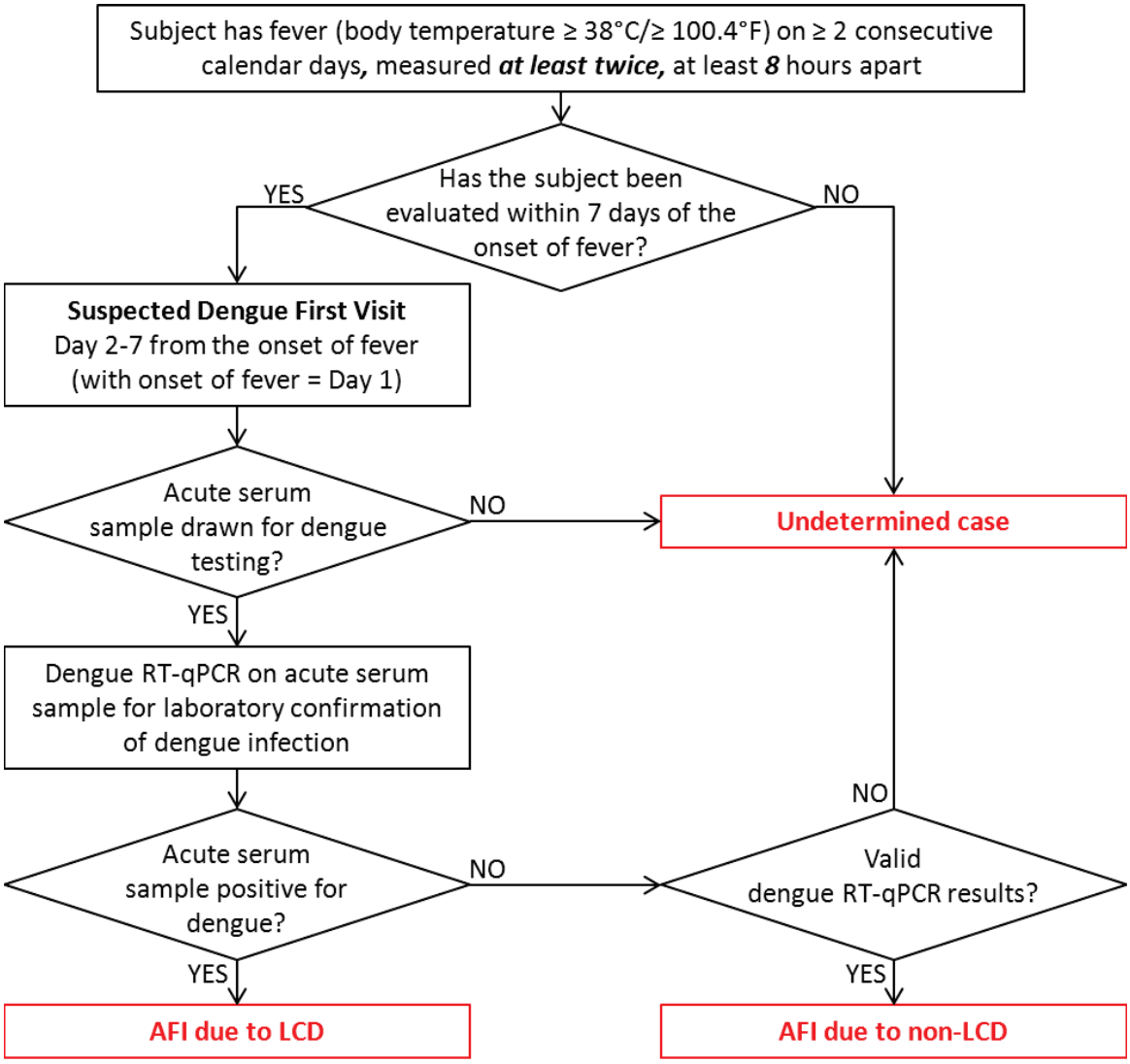
- Fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days**,** measured at least twice, at least 8 hours apart, and
- Dengue RT-qPCR result on the acute serum sample taken during the 7-day period (Days 2-7) from the onset of fever (body temperature ≥ 38°C/≥ 100.4°F) is (valid but) negative for dengue

4.3. Undetermined cases

At least ONE of the following findings must be met for an undetermined case (see Figure 2)

- Fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days**,** measured at least twice, at least 8 hours apart in a subject who has not been evaluated (at a Suspected Dengue First Visit or directly in a hospital/clinic) within the 7-day period (Days 2-7) from the onset of fever or who had an obvious alternative diagnosis other than dengue (i.e., an identified focus of fever), and therefore dengue RT-qPCR was not requested, or
- Dengue RT-qPCR result on the acute serum sample taken during the 7-day period (Days 2-7) from the onset of fever (body temperature ≥ 38°C/≥ 100.4°F) is invalid or missing

Figure 2 Schematic overview of different cases



AFI: Acute febrile illness; **LCD**: Laboratory confirmed dengue; **RT-qPCR**: Reverse transcriptase quantitative polymerase chain reaction (**Amended 01 March 2017**)

Note: Two consecutive calendar days without fever (body temperature ≥ 38°C/≥ 100.4°F), in the absence of antipyretic medication, are required to separate two episodes of fever.

Note: Subjects with fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days**,** measured at least twice, at least 8 hours who fail to attend a Suspected Dengue First Visit as they were directly hospitalised will be recorded as LCD, non-LCD or undetermined cases depending on the availability and result of the dengue RT-qPCR test. Refer to the Study Procedures Manual (SPM) for more information.

5. STUDY POPULATION

5.1. Number of subjects / centres

This study will be a multi-centre, household-based study conducted in dengue-endemic regions of South Asia.

The target is to enrol approximately ***2,000*** eligible subjects in order to reach approximately ***3300*** person-years evaluable overall at the time of final analysis. Refer to Section 9.2 for a detailed description of the criteria used in the estimation of sample size. (**Amended 01 March 2017**)

One or more members of each household (preferably all eligible household members) will be enrolled.

5.1.1. Selection of communities and households

This study will enrol subjects in selected communities based on the following non-exclusive characteristics:

- preferably in areas where access to the community is already established (previous community-based surveillance studies)
- in areas with a high population density
- in areas with low out-migration rate
- in areas with preferably a single point-of-care for the community
- preferably in areas where there is a confirmation of dengue endemicity
- in areas where children are likely to constitute at least 30% of the study population per site
- in households with at least one child (less than 18 years of age)
- in households with at least one adult (aged no more than 50 years) and one child (aged less than 18 years) consenting (and assenting if applicable) to participate in the study

Refer to Section 5.1.2 for an overview of the recruitment plan.
(**Amended 01 March 2017**).

A list of all households in the sampling area will be generated by each site. Simple random sampling will be performed to select households for enrolment. If the sampling area is too large to compile a list of all households, a least eight clusters of similar population size (blocks or streets) will be selected by simple random sampling. Households in the selected clusters will then be listed and selected by simple random sampling.

5.1.2. Overview of the recruitment plan

Enrolment will ***continue*** until a total of approximately ***2,000*** subjects are recruited. Subjects lost to follow-up (i.e., with no contact for 4 consecutive weeks) during the enrolment period will be replaced (Refer to Section 8.2). **(Amended 01 March 2017)**

SBIR (GSK Biologicals' randomisation system on internet) will be used for this study. All details pertaining to user access and specific procedures can be found in the SBIR version 8 User Guide and the SPM.

Recruitment will be organised by study staff at participating sites. Community health workers or equivalent will serve to the liaison, and may accompany study staff during the visits. New target households will be visited until the enrolment target for the study area is reached. The study will be explained to the individuals living in the household, and if any eligible individual is interested in study participation, informed consent (and assent if applicable) will be obtained at the hospital/clinic or during a home visit, where eligibility criteria will be checked and subjects will be enrolled and interviewed for potential study participation.

Refusal (including reasons for refusal) of a household to participate will be documented. Refusal (including reasons for refusal) of individual household members will be documented as well. Household visits may be scheduled to occur during weekends if necessary.

Possession of a phone/cell phone will be documented.

- If a site decides to perform home visits only (i.e., no TC contacts), the possession of a phone/cell phone is not mandatory.
- If a site decides to perform both home visits and TC contacts, the access to a phone/cell phone will be ensured.

Infants who become eligible (i.e., reach the age of 6 months) will be offered enrolment in the study. These children, if enrolled, will be followed from that time until the last adult household member has completed the study.

5.2. Inclusion criteria for enrolment

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

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

- Subject and/or subject's parent(s)/legally acceptable representative(s) (LAR[s]) who, in the opinion of the investigator, can and will comply with the requirements of the protocol (e.g., willingness to go to the hospital/clinic for visit[s] in case of AFI, able to observe the signs of dengue and to understand how to take and report body temperature, etc.).

- Signed/thumb-printed (and video recorded if required by law) informed consent (and assent if applicable) must be obtained from the subject/subject's parent(s)/LAR(s) at the hospital/clinic or during a home visit. If the subject/subject's parent(s)/LAR(s) are illiterate, the informed consent form (ICF) (or informed assent form [IAF] when applicable) will be countersigned by an impartial witness.
- Subject is part of a household with at least one child (aged less than 18 years) and in which informed consent (and assent if applicable) to study participation was obtained from at least one adult and one child.
- Male or female aged between and including 6 months and 50 years at the time of enrolment.
- Subject who plans, at the time of enrolment, to remain at same residence/study area during the two-year study period.

5.3. Exclusion criteria for enrolment

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

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the subject must not be included in the study:

- 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 based on investigator's judgement.
- Mental incapacity based on investigator's judgement.

6. CONDUCT OF THE STUDY

6.1. Regulatory and ethical considerations, including the informed consent process (and assent process if applicable)

The study will be conducted in accordance with all applicable regulatory requirements. GSK will obtain the favourable approvals from Ethics committees and other appropriate authorities prior to initiating a site.

The study will be conducted in accordance with the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice (GCP) and other applicable local guidelines, all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

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

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

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

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favourable opinion/approval of study protocol and any subsequent amendments.
- Subject/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, at the hospital/clinic or during a home visit prior to participation in the study.

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

In accordance with the ICH Harmonised Tripartite Guidelines for Good Clinical Practice, a subject who can only be enrolled in the study with the consent of his/her parent(s)/LAR(s) (e.g., a minor below the age of consent) but at the age for assent, should be informed about the study to the extent compatible with his/her understanding and, if capable, the subject should sign or thumb-print and personally date a written IAF (while being video recorded if required by law). 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(s)/LAR(s). It should be assessed whether an assent is required depending on the age of the study population and the local requirements.

The consenting process will be video recorded if required by law. If the subject/subject's parent(s)/LAR(s) are illiterate, the ICF (and IAF if applicable) will be countersigned by an impartial witness. Informed consent/assent can be obtained at the study clinic or during a home visit. In either case, medically and GCP trained study staff will follow an SOP for the consenting/assenting process.

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

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

6.2. Subject identification

Subject numbers will be assigned sequentially to subjects consenting (or assenting when applicable) to participate/to be included in the study, according to the range of subject numbers allocated to each study centre.

Recruitment in this study will be monitored using SBIR.

6.3. General study aspects

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

6.4. Outline of study procedures

Table 2 List of study procedures for scheduled visits and contacts

Epoch	Epoch 001		
Visit/contact	**Visit 1**	**Home visits or TCs**	**Visit 2**
Time point	**Month 0**	**Weekly between visits [1]**	**Month 24**
Informed consent (and assent if applicable)	●		
Check inclusion/exclusion criteria	●		
Subject number attribution	O		
Household number attribution	●		
Record socio-demographic data	●		
Record medical history (including history of dengue illness, diagnosis of tuberculosis and JEV vaccination history)	●		
Update medical history			●
Subject card distribution	O		
Distribute dengue kits to subjects/subject's parent(s)/LAR(s), including diary cards to be filled out in case of fever [2]	O	O	
Instruct/remind subjects/subject's parent(s)/LAR(s) to contact study staff and to report symptoms in diary card in case of fever [3]	O	O	
Contact subjects regarding any AFI and/or hospitalisation [4]		●	
Collect and verify diary cards (if applicable) [5]		O [6]	O
Record data ***(solicited symptoms, medication)*** from diary cards (if applicable)		● [6]	●
Record SAEs related to study participation	●	●	●
Study conclusion [7]			●

● 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.
AFI: acute febrile illness
eCRF: electronic Case Report Form
JEV: Japanese encephalitis virus
LAR: legally acceptable representative
SAE: serious adverse event
TC: telephone contact

1) Weekly contacts will be home visits (at least every other week) or TCs. ***The weekly contact may be a face to face visit outside the home in certain cases (i.e. at hospital during suspected dengue visit)*** **(Amended 01 March 2017)**
2) Diary cards will be distributed at the first scheduled visit. A new diary card will be issued by the study staff whenever necessary (i.e., if the subject no longer has one, e.g., the previous one was lost or used).
3) The subject/subject's parent(s)/LAR(s) will be instructed to start completing a diary card in the event of fever (i.e., body temperature ≥ 38°C/≥ 100.4°F). Body temperature and the intensity of each of the solicited symptoms should be recorded on the diary cards for the next 6 subsequent days. After 7 days, only the end date for any ongoing solicited symptoms will be recorded on the diary cards. Please refer to Table 6 for the list of solicited symptoms to be recorded. Any medications (e.g., antipyretics) taken during this 7-day period will also be recorded. Two consecutive calendar days without fever, in the absence of antipyretic medication, are required to separate two episodes of fever. Signs and symptoms prior to the onset of fever will not be recorded.
4) Including recording of discharge diagnosis for hospitalised subjects.
5) The subject/subject's parent(s)/LAR(s) will bring the diary card with him/her to each Suspected Dengue Visit (see Table 3). The investigator or the appointed study personnel will record the diary card information in the eCRF ***if a*** temperature of ≥ 38°C (≥ 100.4°F) was recorded on ≥ 2 consecutive calendar days, measured at least twice, at least 8 hours apart. The subject/subject's parent(s)/LAR(s) will return the diary card when completed at the next visit or contact (i.e., at the next home visit or at a Suspected Dengue Visit).
6) Only applicable for home visits.
7) Only one study conclusion will be conducted, either at Visit 2, or if a dengue case is ongoing at Visit 2, at the last Suspected Dengue Follow-up Visit for the dengue case.

Table 3 List of study procedures for *unscheduled* Suspected Dengue Visits (Amended 01 March 2017)

Epoch	Epoch 001		
Visit	**Suspected Dengue First Visit**	**Return Visit [1]**	**Suspected Dengue Follow-up Visit [2]**
Time point	**Day 2 to Day 7, ideally on Day 2 (1 day after the onset of fever)**	**1 to 6 days after Suspected Dengue First Visit**	**7 to13 days after Suspected Dengue First Visit**
Specific physical examination	●	●	
Update medical history	●	●	●
Blood sampling*			
Dengue NS1-antigen ICT test and/or ELISA (0.5 mL)	●		
Dengue RT-qPCR (2.5 mL)	●		
for haematology (2 mL) [3]	●		
for other tertiary (research) endpoints related tests (***3.5*** mL) [4]	●		
Safety follow-up:			
Collect and verify diary cards (if applicable) [5]	O	O	O
Record data (solicited symptoms, medication) from diary cards (if applicable) [5]	●	●	●
Issue diary cards to subjects/subject's parent(s)/LAR(s) to be filled out in the event of fever [6]	O	O	O
Record SAEs related to study participation	●	●	●
Study conclusion [7]			●

● 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.
µPRN: micro-plaque-reduction neutralisation; **eCRF**: electronic Case Report Form; **ELISA**: enzyme-linked immunosorbent assay; **ICT**: immunochromatographic; **JEV**: Japanese encephalitis virus; **LAR**: legally acceptable representative; **NS1**: non-structural protein 1; **RT-qPCR**: reverse transcriptase quantitative polymerase chain reaction; **SAE**: serious adverse event. (**Amended 01 March 2017**

* Blood samples should only be drawn in case of AFI (i.e., fever [body temperature ≥ 38°C/≥ 100.4°F] on ≥ 2 consecutive calendar days***,*** measured ***at least twice,*** at least ***8*** hours apart)) during the Suspected Dengue First Visit from the second day of fever (i.e., from Day 2, the second calendar day with fever) and onwards, up to Day 7.

1) A Return Visit is a visit linked to the Suspected Dengue First Visit and should take place 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.
2) A Suspected Dengue Follow-up Visit may occur during a weekly home visit. This visit should be performed by a medically trained site staff member. ***Note that when a subject comes in for a suspected dengue follow-up visit, this will replace the regularly scheduled weekly home visit.*** (**Amended 01 March 2017**)

3) For all subjects ≥ 5 years of age. For subjects < 5 years of age only if clinically indicated, as per investigator judgement. Complete blood count (White blood cells [WBC], red blood cells [RBC], platelets and haemoglobin).

4) For subjects ≥ 18 years of age only. Including, but not limited to, neutralising antibody against JEV and Chikungunya virus. A subset of samples will be tested.

5) The subject/subject's parent(s)/LAR(s) will bring the diary card with him/her to each Suspected Dengue Visit. The investigator or appointed study personnel will record the diary card information in the eCRF if a temperature of ≥ 38°C (≥ 100.4°F) was recorded on ≥ 2 consecutive calendar days, measured at least twice, at least 8 hours apart. The subject/subject's parent(s)/LAR(s) will return the diary card when completed at the next scheduled visit or contact (i.e., at the next home visit or at a Suspected Dengue Visit).

6) A new diary card will be issued by the study staff whenever necessary (i.e., if the subject no longer has one, e.g., the previous one was lost or used).

7) Only one study conclusion will be conducted, either at Visit 2, or if a suspected dengue case is ongoing at Visit 2, at the last Suspected Dengue Follow-up Visit for the suspected dengue case.

Table 4 Intervals between study visits/contacts

Interval	Optimal length of interval	Allowed interval *
Scheduled Visits:		
Visit 1 (Month 0) → Visit 2 (Month 24)	24 months	23 months - 25 months
Weekly contacts	7 days	6 – 8 days
Unscheduled Visits:		
Onset of fever (body temperature ≥ 38°C/≥ 100.4°F) (Day 1) → Suspected Dengue First Visit	1 day (on Day 2)	up to 6 days (Day 2 to Day 7) **
Suspected Dengue First Visit → (optional) Return Visit (as per investigator judgement)	1 – 6 days	1 – 6 days
Suspected Dengue First Visit → Suspected Dengue Follow-up Visit	7 – 8 days (may occur during a weekly home visit)	7 – 13 days

*Whenever possible the investigator should arrange study visits/contacts within this interval.
** If the subject has not been evaluated within the 7-day period (Days 2-7) from the onset of fever, the Suspected Dengue First Visit is no longer required and the case will be considered as an undetermined case.

6.5. Detailed description of study procedures

6.5.1. Procedures at scheduled Visit 1 prior to enrolment

The enrolment visit (Visit 1) will take place at home or at the hospital/clinic.

6.5.1.1. Informed consent (and assent if applicable)

The signed/thumb printed (and video recorded, if required by law) informed consent of the subject/subject's parent(s)/LAR(s) must be obtained before study participation at the hospital/clinic or during a home visit. The signed/thumb printed informed assent of a subject below the age of consent (i.e., minor) but at the age for assent should also be obtained in addition to the signed/thumb printed informed consent by his/her parent(s)/LAR(s) according to local rules and regulations. If the subject/subject's parent(s)/LAR(s) are illiterate, the ICF (and IAF if applicable) will be countersigned by an impartial witness. Refer to Section 6.1 for the requirements on how to obtain informed consent and assent, as appropriate.

The initial contact and consenting process (and assenting process if applicable) will take place at the hospital/clinic or during a home visit.

6.5.1.2. Check inclusion and exclusion criteria

Check all applicable inclusion and exclusion criteria as described in Sections 5.2 and 5.3 before enrolment.

6.5.2. Procedures at scheduled Visit 1, after enrolment

6.5.2.1. Subject and household number attribution

A subject number and household number will be attributed. The household number will be allocated by each site, according to their local practice. Existing system of house numbering or census method will be used. The household number will be recorded in the eCRF.

6.5.2.2. Record socio-demographic data

Socio-demographic data such as date of birth, gender, education, occupation and socio-economic status will be recorded in the eCRF.

A Geographic Information System (GIS) will be used to map the location of each study household. Based on these data, a map of study households without GIS coordinates will be generated and transferred to GSK Biologicals for analysis of spatio-temporal clustering of cases. The individual GIS coordinates, which are considered as personal identifiable information (PII), will be maintained at local sites only. They will not be recorded in the eCRF and will not be transferred to GSK Biologicals.

The location of each study hospital/clinic will also be mapped.

Refer to the SPM for more information.

6.5.2.3. Record medical history (including history of dengue illness, diagnosis of tuberculosis and JEV vaccination history)

Medical history (e.g., diabetes, cardiovascular diseases, asthma, cancers, haematologic diseases, genetic disorders), including history of dengue illness, tuberculosis, and JEV vaccination will be taken and results will be recorded in the eCRF.

A JEV vaccination record is preferable but self-reported history will be allowed if no vaccination record is available.

6.5.2.4. Subject card distribution

All subjects/subjects' parent(s)/LAR(s) will be provided with the address and telephone number of the main contact for information about the study.

6.5.2.5. Distribution of dengue kits and instruction of subjects/subject's parent(s)/LAR(s) to contact study staff in case of acute febrile illness

Each household will be given at least one dengue kit, which includes one thermometer per subject, study contact information (phone numbers), and diary cards.

Study personnel will instruct subject/subject's parent(s)/LAR(s) to contact the study personnel or come to a designated study hospital/clinic for medical evaluation at the occurrence of fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days, measured at least twice, at least 8 hours apart.

Diary cards

Diary cards will be given to each of the subjects in the household to be used in the event of fever (body temperature ≥ 38°C/≥ 100.4°F). Diary cards will be distributed at the first scheduled visit. New diary cards will be issued by the study staff whenever necessary (i.e., if the subject no longer has one, e.g., the previous one was lost or used).

The subject/subject's parent(s)/LAR(s) will be instructed to start completing a diary card in the event of fever (body temperature ≥ 38°C/≥ 100.4°F). If necessary, assistance to complete the diary cards will be provided to the subject/subject's parent(s)/LAR(s).

Body temperature and the intensity of each of the solicited symptoms should be recorded on the diary cards for the next 6 subsequent days (with Day 1 the day of the onset of fever and Day 7 the last day of recording solicited symptoms). After 7 days, only the end date for any ongoing solicited symptoms will be recorded on the diary cards. Please refer to Table 6 for the list of solicited symptoms to be recorded. Any medications (e.g., antipyretics) taken during this 7-day period will also be recorded. Two consecutive calendar days without fever, in the absence of antipyretic medication, are required to separate two episodes of fever. Symptoms prior to the onset of fever will not be recorded on the diary card.

The subject/subject's parent(s)/LAR(s) will bring the diary card with him/her to each Suspected Dengue Visit. The investigator or appointed study personnel will review the diary card information with the subject and will record the information in the eCRF if a temperature of ≥ 38°C (≥ 100.4°F) was recorded on ≥ 2 consecutive calendar days, measured at least twice, at least 8 hours apart. The subject/subject's parent(s)/LAR(s) will return the diary card when completed at the next visit or contact (i.e., at the next home visit or at a Suspected Dengue Visit).

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

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

6.5.2.6. Recording of serious adverse events related to study participation

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

6.5.3. Procedures during scheduled weekly contacts

Weekly contacts will be home visits (at least every other week) or telephone contacts (TCs). The weekly contact may also be a face to face visit outside the home in certain cases (i.e. at hospital during suspected dengue visit) **(Amended 01 March 2017)**

During the weekly contacts, an adult household member enrolled in the study may respond for all enrolled members of the household. If no adult household member can be reached during the weekly contact (home visit or TC), 3 additional attempts to contact the household will be made on the 3 days following the initial attempted contact (see Section 8.2).

6.5.3.1. Distribution of dengue kits and instruction of subjects/subject's parent(s)/LAR(s) to contact study staff in case of acute febrile illness

Each household will be given at least one dengue kit, which includes one thermometer per subject, study contact information (phone numbers), and diary cards.

- During the weekly contact (home visit or TC), study personnel will ask about AFI (fever [body temperature ≥ 38°C/≥ 100.4°F] on ≥ 2 consecutive calendar days**,** measured at least twice, at least 8 hours apart) in the past week and will instruct/remind subject/subject's parent(s)/LAR(s) to contact the study personnel or come to a designated study hospital/clinic for medical evaluation at the occurrence of AFI and solicited symptoms.

6.5.3.2. Collection and verification of diary cards, and recording of diary cards data from diary cards to the eCRF

- During the weekly contact (home visit or TC), a structured check list will be used to inquire about any AFI since the last contact (home visit or TC). Refer to the SPM for the structured check list. This will include inquiring about any hospitalisation that may have occurred and hospitalisation discharge diagnosis for all cases of hospitalisation. If necessary, assistance to complete the diary cards will be provided to the subject/subject's parent(s)/LAR(s). If the subject is hospitalised at the study health care facility, information will be available via the hospital record. Information from both the diary card and hospital/clinic records can be used to update clinical data in the eCRF.
- If a subject or subject's parent/LAR reports fever (body temperature ≥ 38°C/≥ 100.4°F) during any home visit or TC, the local study coordinator will arrange for a Suspected Dengue First Visit at the designated study hospital/clinic for medical evaluation and blood sampling (refer to Section 6.5.5.1). The Suspected Dengue First Visit should be scheduled within 7 days from the onset of fever (Days 2-7) and should ideally take place on the second day of fever (Day 2). If the visit has not taken place within the 7-day period (Days 2-7) from the onset of fever, it is no longer required and the case will be considered as an undetermined case.
- Collection and distribution of diary cards as needed (only applicable for home visits).
- Recording of diary cards information by the investigator.

6.5.3.3. Recording of serious adverse events related to study participation

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

6.5.4. Procedures at scheduled Visit 2

The close-out visit (Visit 2) will take place at home or at the hospital/clinic.

6.5.4.1. Update medical and JEV vaccination history

Update medical history, including tuberculosis diagnosis and JEV vaccination.

6.5.4.2. Collection and verification of diary cards, and recording of diary cards data from diary cards to the eCRF

- Collection and recording of diary card if needed.
- Recording of SAEs related to study participation.

6.5.4.3. Recording of serious adverse events related to study participation

Refer to Section 6.5.2.6.

6.5.5. Procedures at Suspected Dengue Visits

6.5.5.1. Procedures at Suspected Dengue First Visit

6.5.5.1.1. Important general remarks

All study subjects with AFI (fever [body temperature ≥ 38°C/≥ 100.4°F] on ≥ 2 consecutive calendar days**,** measured at least twice, at least 8 hours apart) should be seen at a designated study hospital/clinic by the study physician. The Suspected Dengue First Visit should be scheduled within 7 days from the onset of fever (Days 2-7) and should ideally take place on the second day of fever (Day 2).

If a subject with fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days**,** measured at least twice, at least 8 hours apart has not been evaluated at a Suspected Dengue First Visit (or directly in a hospital/clinic) within the 7-day period (Days 2-7) from the onset of fever, the Suspected Dengue First Visit is no longer required and the case will be considered as an undetermined case. If a diary card has been filled, the data needs to be recorded in the eCRF.

Blood samples for study procedures should not be drawn from subjects who attend a study healthcare facility on the first day of fever (body temperature ≥ 38°C/≥ 100.4°F), i.e., on Day 1. Medical assistance can be provided to these subjects but they will be asked to come back the following day should the fever persist. Blood samples for study procedures should only be drawn from the second day of fever (i.e., from Day 2, the second calendar day with fever) and onwards, up to Day 7.

Although subjects will be instructed to contact the study staff in the event of AFI (fever [body temperature ≥ 38°C/≥ 100.4°F] on ≥ 2 consecutive calendar days**,** measured at least twice, at least 8 hours apart), there may be cases where the subject is taken directly to a hospital/clinic (study or non-study health care facility). If this occurs, a family member should inform the study staff as early as possible, and the study staff should contact the hospital/clinic where the subject is hospitalised. In addition, weekly contacts will also be used to identify subjects who have been hospitalised with AFI where study staff has not been informed. In such case, clinical data will be retrospectively collected in the eCRF. The study physician will be responsible for collecting the retrospective data and informing the local study coordinator for appropriate follow-up.

All subjects with AFI who fail to attend a Suspected Dengue Visit as they were directly hospitalised will be recorded as LCD, non-LCD, or undetermined cases depending on the availability and result of the dengue RT-qPCR test. Refer to the SPM for more information.

6.5.5.1.2. Physical examination

A detailed physical examination to assess the subject's general condition and any dengue associated clinical signs/symptoms as well as relevant clinical signs/symptoms will be performed (see Table 5). Collected information needs to be recorded in the "Suspected Dengue First Visit" section of the eCRF.

Table 5 Physical examination during Suspected Dengue First Visit

Physical examination *(all subjects)*	• body temperature and route, • height and weight, • cardiac and respiratory rates, • blood pressure, • dengue-like rash, • abdominal tenderness • liver enlargement > 2 cm, • tourniquet test (positive/negative), ***only pertains to subjects ≥5 years of age*** **(Amended 01 March 2017)** • haemorrhagic manifestations; petechiae, bleeding from mucosa, gastrointestinal tract, injection sites or other locations, • Injected pharynx, • clinical signs of plasma leakage: oedema, pleural effusion, ascites
Additional information *(subjects with severe disease and suspected Dengue Shock Syndrome [DSS] - according to investigator's judgement)*	• narrow pulse pressure (< 30 mm Hg) • hypotension • cold, clammy skin • restlessness

Treatment of any condition observed during the physical examination will be performed according to local medical practice.

6.5.5.1.3. Record medical history

Update medical history in the eCRF (in relation to what was encoded during the Suspected Dengue First Visit).

6.5.5.1.4. Blood sampling

Blood samples should only be drawn in case of AFI (i.e., fever [body temperature ≥ 38°C/≥ 100.4°F] on ≥ 2 consecutive calendar days**,** measured at least twice, at least 8 hours apart) during the Suspected Dengue First Visit from the second day of fever (i.e., from Day 2, the second calendar day with fever) and onwards, up to Day 7.

The following blood samples should be collected unless an obvious alternative diagnosis, i.e., an obvious cause of fever other than dengue, has been identified and is documented **(Amended 01 March 2017)**:

- Whole blood for laboratory confirmation of dengue by NS1-antigen (ICT test and/or ELISA): 0.5 mL, from all subjects (any age). The results of this/these test(s) should be recorded in the eCRF.
- Whole blood for laboratory confirmation of dengue infection by (generic and/or serotype-specific) RT-qPCR: 2.5 mL, from all subjects (any age).

- Whole blood for haematology assessment (complete blood count): 2 mL, from all subjects ≥ 5 years of age and from subjects < 5 years of age only if clinically indicated, as per investigator judgement or local recommendations. The results of these tests should be recorded in the eCRF.
- Whole blood for other tertiary (research) endpoints related tests (including, but not limited to, neutralising antibody against JEV and Chikungunya virus, tissue culture to isolate infectious agents, and sequencing to characterise infectious agents): ***3.5 mL***, from all subjects ≥ 18 years of age. A subset of these samples will be tested.

6.5.5.1.5. Collection and recording of solicited symptoms from diary card

- During the Suspected Dengue First Visit, the diary card (partially completed) is reviewed by the physician and data recorded in the "solicited symptoms" section of the eCRF if the subject meets AFI criteria. Refer to the Section 6.6 for a description of the solicited symptoms to be recorded.
- After the Suspected Dengue First Visit, the subject/subject's parent(s)/LAR(s) continues to complete the diary card until resolution of all symptoms. If necessary, assistance to complete the diary cards will be provided to the subject/subject's parent(s)/LAR(s).
- Once completed, the diary card is collected and the data are reviewed with the subject and encoded in the eCRF, either by the physician during a Return or Suspected Dengue Follow-up Visit at hospital/clinic or by the study staff during a home visit.

6.5.5.1.6. Recording of serious adverse events related to study participation

Refer to Section 6.5.3.3.

6.5.5.2. Procedures at Return Visit (optional)

Based on the physician's assessment during the Suspected Dengue First Visit, a **Return Visit** may be needed and will be conducted as directed as per local medical practices.

A Return Visit is a visit linked to the Suspected Dengue First Visit and should take place 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.

- Update physical examination: medical data related to any suspected dengue case will be collected for hospitalised study subjects and for those followed in the outpatient setting at the Return Visit. Diagnostic and treatment intervention will be conducted as per local medical practices.
- Verify diary cards and record any new data in the eCRF.
- Record any SAEs related to study participation. Refer to Section 6.5.3.3.

6.5.5.3. Procedures at Suspected Dengue Follow-up Visit

All AFI cases need to be followed up until resolution of fever (body temperature ≥ 38°C/≥ 100.4°F) and/or resolution of symptoms and signs are reported. The signs and symptoms should be recorded on the **Suspected Dengue Follow-up (convalescent) Visit** page of the eCRF or on the weekly contact page (in case of home visit). This information may be collected during a home visit by medically trained member of the study personnel.

- Verify diary cards and record any new data in the eCRF.
- Record any SAEs related to study participation. Refer to Section 6.5.3.3.

6.5.6. Study conclusion

The Study Conclusion screen in the eCRF will be completed at the last study contact. This last contact could occur at Visit 2 or at the last Suspected Dengue Follow-up Visit if dengue is suspected at Visit 2.

The study staff will review data collected to ensure accuracy and completeness and will complete the Study Conclusion screen in the eCRF.

The sponsor may decide to continue to follow up subjects for a specified time period. This would be detailed in a protocol amendment and subject will be asked to sign a new ICF (and IAF if applicable).

If so, at the end of the study (study conclusion visit/contact), the investigator will ask each subject/subject's parent(s)/LAR(s) if they are interested in participating/allowing the subject to participate in a long-term (follow-up) study. If a subject/subject's parent(s)/LAR(s) is/are not interested in participating in the long-term study the reason for refusal will be documented in the subject's eCRF.

6.6. Solicited symptoms in case of acute febrile illness (suspected dengue infection)

Solicited symptoms will be collected in case of fever (body temperature ≥ 38°C/≥ 100.4°F). The Diary card should be completed from the first day of fever.

The following solicited symptoms will be recorded on the diary cards in case of fever:

Table 6 Solicited symptoms in case of suspected dengue infection

Signs and symptoms
body temperature and route * _ (minimum of two measurements)
headache / irritability **
eye pain ***
myalgia (muscle pain) ***
arthralgia (joint pain) ***
abdominal pain ***
nausea ***
vomiting
rash
any bleeding (skin, mouth, anus)
loss of appetite
Fatigue/decrease in normal activity **
reduced fluid intake **

* Refer to Table 10
** Wording intended for infants and toddlers.
*** When possible (depending on age in young children).

6.6.1. Assessment of the intensity of solicited symptoms

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

Table 7 Intensity scales for solicited symptoms (excluding bleeding and reduced fluid intake, and body temperature)

Grade		
0 (none)	=	No discomfort
1 (mild)	=	Any solicited symptom which is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
2 (moderate)	=	Any solicited symptom which is sufficiently discomforting to interfere with normal everyday activities.
3 (severe)	=	Any solicited symptom which prevents normal, everyday activities. (in a young child, such a solicited symptom would, for example, prevent attendance at school/kindergarten/day-care centre and would cause the parent(s)/LAR(s) to seek medical advice. In adults/adolescents, such a solicited symptom would, for example, prevent attendance at work/school and would necessitate the administration of corrective therapy.)

Table 8 Intensity scales for bleeding

Grade		
0 (none)	=	No bleeding or bruising
1 (mild)	=	Small skin bleed or easy bruising
2 (moderate)	=	Nose bleed or gum bleed not requiring medical attention
3 (severe)	=	Bleeding that requires medical attention

Table 9 Intensity scales for reduced fluid intake

Grade		*
0 (none)	=	Normal feeding
1 (mild)	=	Drinks a little less than usual
2 (moderate)	=	Does not drink well
3 (severe)	=	Lethargic, does not drink

* Wording intended for infants.

Table 10 Grading scale for body temperature

Grade		Temperature (°C)	Temperature (°F)
0	=	< 38.0	< 100.4
1	=	[38.0 - 38.4]	[100.4 – 101.2]
2	=	[38.5 - 38.9]	[101.3 – 102.0]
3	=	> 38.9	> 102.0

The preferred route for recording temperature in this study will be axillary for infants and younger children, and oral for older children and adults. For all age groups, tympanic measurements are not recommended, although they may be used for screening when followed up by another route of measurement.

Note: Ideally, body temperature should be measured twice a day.

Any symptom that is assessed as Grade 3 (severe) should not be confused with an SAE. Grade 3 is a category used for rating the intensity of an event; and both symptoms and SAEs can be assessed as Grade 3.

6.7. Biological sample handling and analysis

Biological samples will only be taken in case of AFI (i.e., fever [body temperature ≥ 38°C/≥ 100.4°F] on ≥ 2 consecutive calendar days**,** measured at least twice, at least 8 hours apart), during the Suspected Dengue First Visit.

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

Samples will not be labelled with information that directly identifies the subjects but will be coded with the identification number of the subject (subject number and Visit 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 (and assent if applicable) 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 except those described in the protocol or its amendment(s).

Collected samples will be stored for a maximum of 20 years (counting from when the last subject performed the last study visit/ contact), unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subject consent (or assent when applicable). These extra requirements need to be communicated formally to and discussed and agreed with GSK Biologicals.

For routine sample collection, when a coded sample/aliquot is shipped to GSK Biologicals and needs to be linked to coded subject data, (re-) consent (and assent if applicable) from the subject/subject's parent(s)/LAR(s) and/or IRB/IEC approval will be sought.

6.7.1. 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 according-to-protocol (ATP) analysis (See Section 9.3 for the definition of study cohorts to be analysed). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals does not provide material for collecting and storing samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM.

6.7.2. Biological samples

Table 11 Biological samples (Amended 01 March 2017)

Purpose	Collected sample type	Quantity	Unit	Tested sample type	Sub-cohort	Time point
Haematology assessment	Whole blood	2	mL	Whole blood	Suspected dengue infection – all subjects ≥ 5 years of age – subjects < 5 years of age only if clinically indicated, as per investigator judgement	***At each occurrence of AFI, i.e., each Suspected Dengue First Visit***
Laboratory confirmation of dengue by RT-qPCR	***Whole blood***	***2.5***	***mL***	***Serum****	***Suspected dengue infection – all subjects (any age)***	
Tertiary endpoint laboratory confirmation of dengue by NS1-antigen ICT test and/or ELISA	***Whole blood***	***0.5***	***mL***	***Whole Blood/Serum*/Plasma****		
Other tertiary (research) endpoint related tests (including, but not limited to, neutralising antibody against JEV)	Whole blood	***3.5***	mL	Serum*	Suspected dengue infection – all subjects ≥ 18 years of age	At each occurrence of AFI, i.e., each Suspected Dengue First Visit

ELISA: enzyme-linked immunosorbent assay
ICT: immunochromatographic
JEV: Japanese encephalitis virus
NS1: non-structural protein 1
RT-qPCR: reverse transcriptase quantitative polymerase chain reaction
***** Assumption: 1 mL of whole blood usually allows a yield of between 30% and 40% of serum/plasma.

6.8. Laboratory assays

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

Table 12 Laboratory confirmation of dengue infection (Amended 01 March 2017)

System	Component *	Method	Kit/Manufacturer	Laboratory†
Whole Blood/Serum/ Plasma	NS1-antigen	***ICT test***	***SD Bioline Dengue Duo (or other manufacturer)*** **	***Local***
Serum	DENV RNA	RT-qPCR	Dengue Simplexa kit or comparable assay	***Q²Solutions -Focus Diagnostics or validated laboratory designate by GSK Biologicals††***

ELISA: enzyme-linked immunosorbent assay
ICT: immunochromatographic
NS1: non-structural protein 1
RT-qPCR: reverse transcriptase quantitative polymerase chain reaction
* Refer to the SPM and the central laboratory manual for more information.
** GSK Biologicals recommends SD Bioline Dengue Duo.
† Refer to the APPENDIX B for the laboratory addresses.
†† GSK Biologicals laboratory refers to the ***Clinical Laboratory Sciences (CLS)*** in Rixensart, Belgium, Wavre, Belgium, or validated laboratory designated by GSK Biologicals.

Table 13 Haematology

System	Component	Method	Laboratory
Whole blood	Complete blood count (CBC)*	Per local standard practice	Local

* **CBC**: Complete blood count: white blood cells (WBC), red blood cells (RBC), platelets and haemoglobin.

Table 14 Other exploratory (tertiary, research) assays

System	Component *	Method	Kit/Manufacturer	Laboratory
Serum	Viral serology (e.g., JEV, Chikungunya virus)	TBD	TBD	TBD **
Serum	Isolation of infectious agents †	Tissue culture	NA	TBD **
	Characterisation of infectious agents †	Sequencing	TBD	TBD **

AFI: acute febrile illness
JEV: Japanese encephalitis virus
TBD: to be determined
* A subset of samples will be tested.
† Additional potential exploratory tests (tertiary endpoints related tests) for the diagnosis of other common causes of AFI, such as malaria, typhoid, scrub typhus, ***influenza***, ***viral*** hepatitis, etc. (**Amended 01 March 2017**)
** The ***testing*** laboratory(ies) that will perform the additional potential exploratory (tertiary, research) tests have not been identified yet.

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. The local clinical laboratories must also have a Quality System established and supported by procedures.

7. SAFETY

In this prospective cohort study no test product/vaccine will be given. Blood samples will be collected during each Suspected Dengue First Visit.

SAEs related to study participation will be recorded throughout the study.

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

7.1. Safety definitions

7.1.1. Definition of an adverse event

An AE is any untoward medical occurrence in a 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.

7.1.2. Definition of a serious adverse event

Only SAEs related to study participation will be recorded during this study.

An SAE is any untoward medical occurrence that:

a. Results in death,

b. Is life-threatening,

   Note: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, had it been more severe.

c. Requires hospitalisation or prolongation of an existing hospitalisation,

   Note: In general, hospitalisation signifies that the subject has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or in an out-patient setting.

   Complications that occur during hospitalisation are also considered AEs. If a complication prolongs hospitalisation or fulfils any other serious criteria, the event will also be considered serious. When in doubt as to whether 'hospitalisation' occurred or was necessary, the AE should be considered serious.

Hospitalisation for elective treatment of a pre-existing condition (known or diagnosed prior to informed consent signature) that did not worsen from baseline is NOT considered an SAE.

d. Results in disability/incapacity,

   Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza like illness, and accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

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.

7.1.3. 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 related to study participation if they meet the definition of an SAE (refer to Section 7.1) and if deemed by the investigator to be causally related to study participation. 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, if deemed by the investigator to be causally related to study participation. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with dengue will not be reported as SAEs but will be reported in Medical history section during Suspected Dengue Visits.

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

7.2. Detecting and recording SAEs related to study participation

7.2.1. Time periods for detecting and recording SAEs

The time period for collecting and recording SAEs related to study participation will begin at the time the subject consents (or assents when applicable) to participate in the study until he/she is discharged from the study. See Section 7.3 for instructions on reporting of SAEs related to study participation.

An overview of the protocol-required reporting periods for SAEs related to study participation is given in Table 15.

Table 15 Reporting periods for SAEs related to study participation

Study activity	Visit 1 * (Month 0)	Home visits/TCs/ any Suspected Dengue Visits	Visit 2 (Month 24)**
SAEs related to study participation			

SAE: serious adverse event
TC: telephone contact
* i.e., at the time consent (or assent when applicable) is obtained.
** Only one study conclusion will be conducted, either at Visit 2, or if a dengue case is ongoing at Visit 2, at the last Suspected Dengue Follow-up Visit for the dengue case.

7.2.2. Evaluation of SAEs related to study participation

7.2.2.1. Active questioning to detect SAEs

Each subject/subject's parents/LAR(s) will be instructed to contact the investigator immediately should the subject manifest any signs and symptoms he/she 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) 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 related to study participation 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.

7.2.2.2. Assessment of the intensity SAEs

The investigator will assess the maximum intensity that occurred over the duration of the event for all SAEs related to study participation 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/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.)

A symptom that is assessed as Grade 3 (severe) should not be confused with an SAE. Grade 3 is a category utilised for rating the intensity of an event; and both symptoms and SAEs can be assessed as Grade 3. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 7.1.2.

7.2.2.3. 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 an SAE related to study participation has occurred and the investigator has minimal information to include in the initial report to GSK Biologicals. However it is very important that the investigator always makes an assessment of causality for every event prior to submission of the SAE report to GSK Biologicals. The investigator may change his/her opinion of causality in light of follow-up information and update the SAE information accordingly.

If an event meets the criteria to be considered as 'serious' (see Section 7.1), additional examinations/tests will be performed by the investigator in order to determine ALL possibly contributing factors to each SAE related to study participation.

Possibly contributing factors include:

- Medical history.
- Concurrent medication.
- Protocol required procedure.
- Other procedure not required by the protocol.
- Other cause (specify).

7.2.2.4. Assessment of outcomes

The investigator will assess the outcome of all SAEs related to study participation recorded during the study as:

- Recovered/resolved.
- Recovering/resolving.
- Not recovered/not resolved.
- Recovered with sequelae/resolved with sequelae.
- Fatal.

7.3. Reporting of SAEs related to study participation

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

SAEs related to study participation that occur in the time period defined in Section 7.2.1 will be reported promptly to GSK within the timeframes described in Table 16 once the investigator determines that the event meets the protocol definition of an SAE.

Table 16 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*	electronic Expedited Adverse Event Report	24 hours*	electronic Expedited Adverse Event Report

SAE: serious adverse event
* Timeframe allowed after receipt or awareness of the information.
** Timeframe allowed after the diagnosis is established and known to the investigator.

7.3.2. Contact information for reporting SAEs related to study participation to GSK

Back-up Study Contact for Reporting SAEs related to study participation
24/24 hour and 7/7 day availability: GSK Biologicals Clinical Safety & Pharmacovigilance Fax: +PPD or +PPD

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

Once an investigator becomes aware that an SAE related to study participation 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.

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

If the electronic reporting system does not work, the investigator (or designee) must complete, then date and sign a paper Expedited Adverse Event Report and fax it to the GSK Biologicals Clinical Safety and Pharmacovigilance department within 24 hours.

This back-up system should only be used if the electronic reporting system is not working and NOT if the system is slow. As soon as the electronic reporting system is working again, the investigator (or designee) must complete the electronic Expedited Adverse Event Report within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic reporting system.

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

When additional SAE information is received after freezing of the subject's eCRF, new or updated information should be recorded on a paper Expedited Adverse Event Report, with all changes signed and dated by the investigator. The updated report should be faxed to the GSK Biologicals Clinical Safety and Pharmacovigilance department or to the Study Contact for Reporting SAEs (see the Sponsor Information) within the designated reporting time frames specified in Table 16.

7.3.5. Regulatory reporting requirements for SAEs

The investigator will promptly report SAEs related to study participation to GSK Biologicals in accordance with the procedures detailed in Section 7.3.1. GSK Biologicals has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies. Prompt notification of SAEs related to study participation by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

7.4. Follow-up of SAEs related to study participation

7.4.1. Follow-up of SAEs related to study participation

7.4.1.1. 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 16).

All SAEs related to study participation 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.

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

The investigator will follow-up subjects with SAEs related to study participation, until the event has resolved, subsided, stabilised, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up.

If the investigator receives additional relevant information on a previously reported SAE, he/she will provide this information to GSK Biologicals using a paper Expedited Adverse Event Report.

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

8. SUBJECT COMPLETION AND WITHDRAWAL

8.1. Subject completion

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

8.2. 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 related to study participation until resolution of the event (see Section 7.4.1.2).

Withdrawals will not be replaced.

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

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

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

If no adult household member can be reached during a weekly contact (home visit or TC), 3 additional attempts to contact the household should be made on the 3 days following the initial attempted contact. If after a period of 4 weeks, the household can still not be contacted, its members will be considered as a lost to follow-up (see below). Each attempt to contact a household should be documented.

Investigators will make an attempt to contact those subjects who do not return for Visit 2 or for Suspected Dengue Follow-up Visits.

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)/LAR(s), or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- SAE*
- Protocol violation (specify)
- Consent (or assent when applicable) withdrawal, not due to an SAE**
- Moved from the study area
- Lost to follow-up***
- Other (specify)

* Subjects who are withdrawn from the study because of SAEs related to study participation 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 related to study participation until resolution of the event (see Section 7.4.1.2).

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

*** Subjects who cannot be contacted (via home visits/TCs or visits at study hospital/clinic) during 4 consecutive weeks will be considered as lost to follow-up. Subjects lost to follow-up will only be replaced during the enrolment period.

8.3. Extension study

The sponsor may decide to extend the study for a specified time period. This would be detailed in a protocol amendment and the subject will be asked to sign a new ICF (or IAF when applicable).

If so, at the end of the study (study conclusion visit/contact), the investigator will ask each subject/subject's parent(s)/LAR(s) if they are interested in participating/allowing the subject to participate in future studies. If a subject/subject's parent(s)/LAR(s) is/are not interested in participating in future studies the reason for refusal will be documented in the subject's eCRF.

9. STATISTICAL METHODS

9.1. Endpoints

9.1.1. Primary endpoint

- Occurrence of AFI due to LCD.

Please refer to Section 4 for the case definitions of AFI due to LCD.

9.1.2. Secondary endpoints

- Occurrence of AFI due to non-LCD.
- Occurrence and intensity of signs and symptoms of interest, during the 7-day period following the onset of each episode of AFI due to LCD and due to non-LCD.
- Occurrence of AFI due to LCD by DENV type, study site, and age group.

Please refer to Section 4 for the case definitions of AFI due to LCD and due to non-LCD.

9.1.3. Tertiary (research) endpoints

During the Suspected Dengue First Visit, additional blood volumes will be collected for tertiary study endpoint assays. These tertiary study endpoints include, but are not limited to, determination of neutralising antibody titres against dengue virus (DENV), Japanese encephalitis virus (JEV), and chikungunya virus, tissue culture to isolate infectious agents, and sequencing to characterise infectious agents. (**Amended 01 March 2017**)

Tertiary study endpoint assays ***may*** be performed for a subset of samples (for example, JEV assays will only be conducted if additional studies indicate that JEV serology is needed to interpret the DENV antibody response), i.e., not all of these endpoint assays will be performed on all of the sera collected from adult study subjects. (**Amended 01 March 2017**)

Research objectives may be assessed based on, but not limited to, the following endpoints: (**Amended 01 March 2017**)

- Neutralising antibody titres against JEV.
- Neutralising antibody titres against other viruses, including but not limited to, Chikungunya virus.
- Incidence of NS1-antigen (ICT test and/or ELISA)-positive AFI by DENV type.
- Concordance between dengue RT-qPCR and NS1-antigen (ICT and/or ELISA) assays.
- Occurrence of AFI due to LCD and due to non-LCD having (≥ 3 days of fever (body temperature (≥ 38°C/≥ 100.4°F).
- Occurrence of AFI due to LCD and due to non-LCD resulting in hospitalisation.
- Occurrence of combinations of signs and symptoms during the 7-day period following the onset of each episode of AFI due to LCD and due to non-LCD.
- Occurrence of AFI due to LCD within 2 weeks from an index case, in the same household or within 50 metres of the household of the index case.
- Entomological characteristics for LCD cases (e.g., vector species, density and number of breeding sites) at selected site(s).
- Occurrence (by medical history only) of a diagnosis of tuberculosis, from birth up to study conclusion, in the study population.
- Occurrence of hospitalisation and discharge diagnosis.
- Identification and characterisation of infectious agents isolated from blood (e.g., DENV, influenza viruses, chikungunya viruses), in subjects with AFI.

Please refer to Section 4 for the case definitions of AFI due to LCD and due to non-LCD.

9.2. Sample size considerations

A ***maximum of 2,000*** subjects will be enrolled for a 2-year follow-up period. Assuming a drop-out rate of 10% the first year and a drop-out rate of 20% the second year, the final analysis will be done on approximately ***3300*** person-years overall.

In order to evaluate the precision with a sample size of ***3,000*** subjects, the 95% confidence interval (CI) (Poisson exact distribution and normal approximation of the Poisson distribution accounting for a design effect) for a range of expected incidences rates of AFI due to LCD were computed. The 95% CI for a range of expected incidences rates for DENV specific LCD were also presented (see Table 17).

(Amended 01 March 2017)

Estimating design effect and precision

The normal approximation of the Poisson distribution was used for calculating 95% CI for this cluster design. The variance was adjusted for a design effect of 2.6 to account for the between-cluster variability. The design effect measures the increase in the standard error of the estimate due to the sampling design used and is given by: D = 1 + (b – 1) rho, where rho is the rate of homogeneity (a measure of variability and equivalent to the "intra-cluster correlation") and b is the average number of subjects sampled per household. Here we assumed b to be 5. Although, in theory rho can have a value up to 1, in practice values higher than 0.4 are uncommon [Bennett, 1991]. We used a conservative estimate of 0.4 for this study. The design effect is then estimated to 2.6.

Table 17 95% CI with design effect of 2.6

	Number of cases	Number of person-years	Incidence of LCD (per 100 person-years)	95% CI Poisson exact				95% CI Normal approx with design effect of 2.6			
					LL		UL		LL		UL
Overall	10	3300	0.30	[	0.15	;	0.56]	[	0.00	;	0.61]
	20	3300	0.61	[	0.37	;	0.94]	[	0.18	;	1.03]
	30	3300	0.91	[	0.61	;	1.30]	[	0.38	;	1.43]
	40	3300	1.21	[	0.87	;	1.65]	[	0.61	;	1.82]
	50	3300	1.52	[	1.12	;	2.00]	[	0.84	;	2.19]
	60	3300	1.82	[	1.39	;	2.34]	[	1.08	;	2.56]
DENV specific	4	3300	0.12	[	0.03	;	0.31]	[	0.00	;	0.31]
	8	3300	0.24	[	0.10	;	0.48]	[	0.00	;	0.51]
	12	3300	0.36	[	0.19	;	0.64]	[	0.03	;	0.70]
	16	3300	0.48	[	0.28	;	0.79]	[	0.10	;	0.87]

(Amended 01 March 2017)

CI: confidence interval
DENV: dengue virus
LCD: laboratory Confirmed Dengue
LL lower limit;
UL: upper limit

At time of analysis, the design effect of this study will be estimated using the following formula [Bennett, 1991]:

$$DE = \frac{s^2_{CLUSTER\ SAMPLE}}{s^2_{SIMPLE\ RANDOM\ SAMPLE}}$$

With

$$s^2_{CLUSTER\ SAMPLE} = (c/\sum x_i)^2(\sum y_i^2 - 2p\sum x_i y_i + p^2\sum x_i^2)/[c(c-1)]$$

$$s^2_{SIMPLE\ RANDOM\ SAMPLE} = p(1-p)/n$$

Where, c is the number of clusters, x_i and y_i are the number of person-years and the number of cases, respectively in the i^{th} cluster, n is the total number of person-years and p is the overall proportion $\sum y_i / \sum x_i$.

The intra-cluster correlation ρ may then be estimated as:

$$\rho = (DE - 1)/[(\sum x_i/c) - 1]$$

9.3. Cohorts for analyses

All the analyses of objectives and of demographics will be performed on the total cohort.

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

9.4. Derived and transformed data

- An AFI case will be defined as:
  - LCD, if fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days ***is*** measured ***at least twice,*** at least ***8*** hours apart and if dengue RT-qPCR result on a blood sample taken during the 7-day period (Days 2-7) from the onset of fever is positive.
  - Non-LCD, if fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days is measured at least twice, at least 8 hours apart and if dengue RT-qPCR result on a blood sample taken during the 7-day period (Days 2-7) from the onset of fever is negative.
  - Undetermined, if fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days is measured at least twice, at least 8 hours apart but is not evaluated within the 7-day period (Days 2-7) from the onset

of fever and/or if dengue RT-qPCR result on a blood sample taken during the 7-day period (Days 2-7) from the onset of fever is invalid or missing.

- DENV-1 LCD, if fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days is measured at least twice, at least 8 hours apart in diary card and if positive result for DENV-1 by serotype-specific RT-qPCR on a blood sample taken within the 7-day period (Days 2-7) from the onset of fever is positive.
- DENV-2 LCD, if fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days is measured at least twice, at least 8 hours apart in diary card and if positive result for DENV-2 by serotype-specific RT-qPCR on a blood sample taken within the 7-day period (Days 2-7) from the onset of fever is positive.
- DENV-3 LCD, if fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days is measured at least twice, at least 8 hours apart in diary card and if positive result for DENV-3 by serotype-specific RT-qPCR on a blood sample taken within the 7-day period (Days 2-7) from the onset of fever is positive.
- DENV-4 LCD, if fever (body temperature ≥ 38°C/≥ 100.4°F) on ≥ 2 consecutive calendar days is measured at least twice, at least 8 hours apart in diary card and if positive result for DENV-4 by serotype-specific RT-qPCR on a blood sample taken within the 7-day period (Days 2-7) from the onset of fever is positive.

- Age in the study will be computed as the difference between the date of enrolment (i.e., date of ICF [and IAF if applicable]) and the date of birth. The age will be expressed in years.
- The subject age at time of AFI will be computed as the difference between the date of the onset of fever (body temperature ≥ 38°C/≥ 100.4°F) completed on diary card and the date of birth.
- The follow-up time for estimation of incidence of AFI due to LCD, will be computed, for each subject, as the difference between the date of first occurrence of AFI due to LCD (by serotype) or the date of last contact if no LCD occurred (i.e., last weekly Suspected Dengue Follow-up or last Visit) and the date of enrolment.
- The follow-up time for estimation of incidence of AFI due to non-LCD, will be computed, for each subject, as the difference between the date of first occurrence of AFI due to non-LCD or the date of last contact if any (i.e., last weekly Suspected Dengue Follow-up or last Visit) and the date of enrolment.
- The follow-up time for estimation of incidence of AFI due to LCD by DENV-1 type, will be computed, for each subject, as the difference between the date of first occurrence of AFI due to DENV-1 LCD or date of last contact if any (i.e., last weekly Suspected Dengue Follow-up or last Visit) and the date of enrolment. The same computations will be done for DENV-2-3 and 4 specific cases.

- The total person-years at risk will be computed as the sum of the follow-up times in year for each individual.
- The number of consecutive calendar days with fever (body temperature ≥ 38°C/≥ 100.4°F) used for definition of cases will be computed as the number of consecutive calendar days with fever during the 7-day period following the onset of fever.
- The duration of symptoms will be computed as the number of days with grade 1, 2 or 3 symptoms during the 7 days following the onset of fever.
- For the analysis of solicited symptom, missing or non-evaluable measurements will not be replaced. Therefore the analysis of the solicited symptoms will include only episodes of AFI with documented safety data (i.e., diary card completed).
- The geometric mean titre (GMT) calculations are performed by taking the anti-log of the mean of the $\log_{10}$ titre transformations. Antibody titres below the cut-off of the assay will be given an arbitrary value of half the cut-off for the purpose of GMT calculation. For a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, an analysis will exclude subjects with missing or non-evaluable measurements.

9.5. Analysis of demographics / baseline characteristics

Socio-demographic and patient characteristics (e.g., age at study enrolment, gender, household conditions, medical history and vaccination history) will be summarised overall and for LCD cases and by site using descriptive statistics.

9.6. Analysis of primary and secondary objectives

The following analyses will be performed:

- Computation of incidence rate of AFI due to LCD and due to non-LCD with 95% CI: the numerator will be the number of subjects with AFI due to LCD and due to non-LCD during the study period. The denominator will be the total person-years at risk.
- Description of signs and symptoms of AFI due to LCD and due to non-LCD will include the percentage of AFI presenting each sign or symptom (any intensity and grade 3) during the 7-day period from the onset of fever (body temperature ≥ 38°C/≥ 100.4°F). Duration of signs and symptoms will also be described for LCD and non-LCD episodes. Analysis will be done overall and then separately for episodes of AFI occurring in subjects < 5 years of age, in subjects 5 to 11 years of age, and in subjects ≥ 12 years of age.
- Computation of incidence rates of AFI due to LCD with 95% CI by type (DENV 1-4), study site and age group (6 months to 4 years, ≥ 5 years-11 years, ≥ 12 years to 17 years, ≥ 18 years): the numerator will be the number of subjects with AFI due to LCD in the category. The denominator will be the total person-years at risk in the category.

9.7. Analysis of tertiary (research) objectives

Some tertiary (research) analyses may be done including:

(Amended 01 March 2017)

- Neutralising antibody titres against JEV: the seropositivity rates to JEV with exact 95% CI and GMTs with 95% CI and range of antibody titres will be tabulated.
- A concordance analysis between dengue virus detection by RT-qPCR and by NS1-antigen (***ICT test***) may be performed. This analysis may be stratified by day of sampling if sufficient data are available. Sensitivity, specificity, positive predictive value and negative predictive value of NS1-antigen (***ICT test***) in prediction of RT-qPCR test results will be computed. (**Amended 01 March 2017**)
- Computation of incidence rate of AFI due to dengue confirmed by NS1-antigen (ICT test and/or ELISA) with 95% CI: the numerator will be the number of subjects with AFI due to dengue confirmed by NS1-antigen (ICT test and/or ELISA) during the study period. The denominator will be the total person-years at risk.
- Evaluation of different case definitions by:
- Computation of incidence rates of AFI due to LCD having ≥ 3 consecutive calendar days of fever (body temperature ≥ 38°C/≥ 100.4°F) or incidence of AFI due to LCD resulting in hospitalisation with 95% CI.
- Descriptive analysis of the signs and symptoms associated with AFI due to LCD having ≥ 3 consecutive calendar days of fever (body temperature ≥ 38°C/≥ 100.4°F) or resulting in hospitalisation.
- Multivariate analyses could be done to identify categories of medically relevant signs and symptoms associated with high probability of LCD (any severity, having ≥ 3 consecutive calendar days of fever (body temperature ≥ 38°C/≥ 100.4°F) and resulting in hospitalisation) *versus* non-LCD episodes using logistic regressions and/or classification and regression tree analysis to provide support for the definition of a moderate-to-severe dengue case for future clinical endpoint trials. Same analyses may be done to identify category of signs and symptoms associated with high probability of LCD having ≥ 3 consecutive calendar days of fever (body temperature ≥ 38°C/≥ 100.4°F) or resulting to hospitalisation.
- Values of signs and symptoms in predicting LCD in AFI will be computed (sensitivity, specificity, positive predictive value and negative predictive value). Same computations may be done in predicting LCD having ≥ 3 consecutive calendar days of fever (body temperature ≥ 38°C/≥ 100.4°F) or resulting to hospitalisation.
- Descriptive analysis of the spatio-temporal clustering of cases using a map of study households. Occurrence of AFI due to LCD within 2 weeks from an index case, in the same household or within 50 metres of the household of the index case will be analysed.
- Descriptive analysis of incidence and reason for hospitalisation in the study population, throughout the study period.

- Description of entomological characteristics such as vector species, vector density and breeding sites at selected site(s).

9.8. Analysis of SAEs related to study participation

The verbatim reports of SAEs related to study participation will be reviewed by a physician and the signs and symptoms will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). Every verbatim term will be matched with the appropriate PT. SAEs related to study participation reported during the entire study period will be described in individual data listings.

9.9. Interpretation of analyses

There are no confirmatory objectives with a pre-defined success criterion.

The exploratory (tertiary, research) analyses are not controlled for Type 1 error.

9.10. Conduct of analyses

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

9.10.1. Sequence of analyses

The final analysis will be performed when all prospective data have been collected and cleaned. A final study report containing data from the entire study will be written at this time.
(Amended 01 March 2017)

10. ADMINISTRATIVE MATTERS

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

10.1. Electronic Case Report Form instructions

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

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

While completed eCRFs are reviewed by a GSK Biologicals' Site Monitor at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate clarification or correction of omissions or inconsistencies with documentation and approval by the investigator or appropriately qualified designee. In all cases, the investigator remains accountable for the study data.

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.

10.2. Study monitoring by GSK Biologicals

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

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

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

The investigator must ensure provision of reasonable time, space and qualified personnel for monitoring visits.

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform 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.

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.

In accordance with applicable regulations, GCP and GSK procedures, GSK monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and GSK requirements. When reviewing data collection procedures, the discussion will also include identification, agreement and documentation of data items for which the eCRF entries will serve as the source document.

10.3. Record retention

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

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

10.4. Quality assurance

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

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

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

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

10.6. Provision of study results to investigators

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

Not applicable.

12. REFERENCES

(Amended 01 March 2017)

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APPENDIX A LABORATORY ASSAYS

NS1-antigen ICT test

The Dengue NS1 ICT test will be a commercial individual test for the qualitative detection of Dengue virus NS1 antigen in human serum, plasma or on whole blood. Performance characteristics of the assay are described in the manufacturer package insert.

NS1-antigen ELISA

The NS1 antigen in serum or plasma samples will be detected by using a commercial assay. Performance characteristics of the assay are described in the manufacturer package insert.

Dengue RT-qPCR assay

The detection of the dengue virus in human serum is done by reverse transcription of viral RNA, followed by amplification and quantification of the resultant cDNA using a real-time RT-qPCR assay, such as the Focus Diagnostics Simplexa™ Dengue RT-PCR assay. 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. Performance characteristics of the assay are described in the manufacturer package insert and in a recent publication [Sasmono, 2014].

(**Amended 01 March 2017**)

Neutralising antibody against JEV and Chikungunya virus

To be determined. A testing laboratory has not been identified yet.

Virus isolation and characterisation

To be determined. A testing laboratory has not been identified yet.

APPENDIX B CLINICAL LABORATORIES

Table 18 GSK Biologicals' laboratories

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

Table 19 Outsourced laboratories

Laboratory	Address
Focus (US)	***Q²Solutions -Focus Diagnostics***, Inc. (d/b/a Quest Diagnostics) 33608 Ortega Highway San Juan Capistrano, CA 92675-2042 USA

(Amended 01 March 2017)

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	200274 (DPIV-021 EXPLO)
Amendment number:	Amendment 1
Amendment date:	16 December 2014
Co-ordinating author:	PPD, 4Clinics Belgium, contractor for GSK Biologicals

Rationale/background for changes:
Dengue virus RNA and NS1 detection assays will be performed for all suspected dengue cases. During the same study visit and the same procedure, additional blood volumes will be collected for tertiary study endpoints:

- for dengue serology (sites in India only since seroprevalence is well defined for sites in Sri Lanka), at the first Suspected Dengue First Visit only, from all subjects ≥ 5 years of age, and
- for tertiary study endpoint assays other than dengue serology* (in both India and Sri Lanka), at each Suspected Dengue First Visit, from adult study subjects only.

* including, but not limited to, determination of neutralising antibody titres against Japanese encephalitis virus (JEV), and chikungunya virus, tissue culture to isolate infectious agents, and sequencing to characterise infectious agents.

All of the dengue serology specimens collected from children aged 5 to 17 years will be tested for dengue neutralising antibody titres. In addition, sera collected from adult study subjects who tested positive for dengue by RT-qPCR, and up to three matched controls, will be tested for dengue serology. For tertiary study endpoints other than dengue serology, only a subset of samples will be tested. The current amendment clarifies that all of the endpoint assays will be performed on all of the sera collected from children while not all of the endpoint assays will be performed on all of the sera collected from adult study subjects.

Clarification was added regarding laboratory assay methodologies and responsible laboratories.

The listing of contributing authors has been updated and typographical errors have been corrected.

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

Cover page

Contributing authors

- PPD , ***Project Statistician***
- PPD , ***Study Delivery Manager***
- PPD , ***Clinical Laboratories Representative***

Synopsis

Rationale for the study	This study aims to estimate the burden of dengue illness in selected sites in ~~India and Sri Lanka~~ ***South Asia*** and to prepare sites for the conduct of future vaccine efficacy trials.

Scientific Objectives	**Tertiary (Research)** • To describe DENV and Japanese Encephalitis Virus (JEV) antibody profiles ***in a subset of subjects with AFI***. • To assess the concordance between dengue Reverse transcriptase-quantitative Polymerase Chain Reaction (RT-qPCR) and Non-Structural protein 1 (NS1)-antigen Enzyme-Linked Immunosorbent Assay (ELISA) and/or ImmunoChromaTographic (ICT) test in the ~~diagnosis~~ ***assessment*** of dengue infection. • To isolate and characterise infectious agents from ***a subset of*** subjects with AFI. • ***To evaluate the antibody response to infectious agents in a subset of subjects with AFI.***

Endpoints	**Tertiary (Research)** ***During the Suspected Dengue First Visit, additional blood volumes will be collected for tertiary study endpoint assays. These tertiary study endpoints include, but are not limited to, determination of neutralising antibody titres against dengue virus (DENV), Japanese encephalitis virus (JEV), and chikungunya virus, tissue culture to isolate infectious agents, and sequencing to characterise infectious agents.*** ***Dengue serology (sites in India only since seroprevalence is well defined for sites in Sri Lanka) will be performed on samples collected at the first Suspected Dengue First Visit only, from all children aged 5 to 17 years, as well as from adult study subjects who tested positive for Dengue RT-qPCR and up to three matched controls. Matching will be done by study site, age and gender.*** ***Tertiary study endpoint assays other than dengue serology will be performed for a subset of samples (for example, JEV assays will***

	only be conducted if additional studies indicate that JEV serology is needed to interpret the DENV antibody response), i.e., not all of these endpoint assays will be performed on all of the sera collected from adult study subjects. Research objectives may be assessed based on, but not limited to, the following endpoints: • ***Neutralising antibody titres against other viruses, including but not limited to, Chikungunya virus.***

Section 1.1 Background

In 2012, the Government of India convened a meeting of dengue experts to address the growing burden of disease and to form a task force on dengue, indicating a growing sense of urgency and increasing awareness of the importance of this disease. In Sri Lanka, dengue surveillance and training of doctors in the management of dengue illness is a top priority of the Ministry of Health, especially because the rate of severe disease in Sri Lanka is one of the highest national rates reported. ***DENV infection and disease incidence in children in Colombo, Sri Lanka, have been reported to exceed 8% and 3% per year, respectively [Tissera, 2014].***

Section 1.2 Rationale for the study

This study aims to estimate the burden of dengue illness in selected sites in ~~India and Sri Lanka~~ ***South Asia*** and to prepare sites for the conduct of future vaccine efficacy trials.

Section 2.3 Tertiary (research) objectives

- To describe DENV and Japanese Encephalitis Virus (JEV) antibody profiles ***in a subset of subjects with AFI***.
- To assess the concordance between dengue Reverse transcriptase-quantitative Polymerase Chain Reaction (RT-qPCR) and Non-Structural protein 1 (NS1)-antigen Enzyme-Linked Immunosorbent Assay (ELISA) and/or ImmunoChromaTographic (ICT) test in the ~~diagnosis~~ ***assessment*** of dengue infection.
- To isolate and characterise infectious agents from ***a subset of*** subjects with AFI.
- ***To evaluate the antibody response to infectious agents in a subset of subjects with AFI.***

Section 5.1 Number of subjects/centres

One or more ~~(preferably all eligible)~~ members of each household ***(preferably all eligible household members)*** will be enrolled.

Section 6.3 General study aspects

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying Study Procedures Manual (SPM ***and central laboratory manual***).

Section 6.4 Outline of study procedures

Table 3 List of study procedures for Suspected Dengue Visits

Blood sampling*
~~for d~~***D***engue ~~infection diagnosis by~~ NS1-antigen ELISA and/or ICT test (0.5 mL)
~~for d~~***D***engue ~~infection diagnosis by (generic and/or serotype-specific)~~ RT-qPCR (2.5 mL)
~~for dengue infection diagnosis by anti-~~DENV 1-4 ~~µPRN, microneutralisation or comparable assay~~ ***serology*** (4 mL) [3]
for ***other*** tertiary (research) endpoints related tests (4 mL) [5]

**** Blood samples should only be drawn in case of AFI (i.e., fever [body temperature ≥ 38°C/≥ 100.4°F] on ≥ 2 consecutive calendar days measured at least 12 hours apart) during the Suspected Dengue First Visit from the second day of fever (i.e., from Day 2, the second calendar day with fever) and onwards, up to Day 7.***

3) For subjects ≥ 5 years of age only ***at the first Suspected Dengue First Visit only and in India only***.

5) For subjects ≥ 18 years of age only. Including, but not limited to, neutralising antibody against JEV ***and Chikungunya virus***. ***A subset of samples will be tested.***

Section 6.5.5.1.4 Blood sampling

Blood samples should only be drawn in case of AFI (i.e., fever [body temperature ≥ 38°C/≥ 100.4°F] on ≥ 2 consecutive calendar days measured at least 12 hours apart) during the Suspected Dengue First Visit from the second day of fever (i.e., from Day 2, the second calendar day with fever) and onwards, up to Day 7.

The following blood samples should be collected unless an obvious alternative diagnosis, i.e., an obvious cause of fever other than dengue, has been identified and is documented:

- Whole blood for ***laboratory confirmation of*** dengue ~~infection diagnosis~~ by NS1-antigen (ELISA and/or ICT test): 0.5 mL, from all subjects (any age). The results of this/these test(s) should be recorded in the eCRF.
- Whole blood for ***laboratory confirmation of*** dengue ~~infection diagnosis~~ by (generic and/or serotype-specific) RT-qPCR: 2.5 mL, from all subjects (any age).
- Whole blood for haematology assessment (complete blood count): 2 mL, from all subjects ≥ 5 years of age and from subjects < 5 years of age only if clinically indicated, as per investigator judgement ***or local recommendations***. The results of these tests should be recorded in the eCRF.
- Whole blood for dengue ***serology*** ~~infection~~ by anti-DENV 1-4 µPRN, microneutralisation or comparable assay: ***(India only)*** 4 mL, from all ~~subjects~~ ***children aged*** ~~≥~~5 ***to 17*** years ~~of age~~***, as well as from adult study subjects who tested positive for Dengue RT-qPCR and up to three matched controls.***
- Whole blood for ***other*** tertiary (research) endpoints related tests (including, but not limited to, neutralising antibody against JEV ***and Chikungunya virus, tissue culture to isolate infectious agents, and sequencing to characterise infectious agents)***: 4 mL, from all subjects ≥ 18 years of age. ***A subset of these samples will be tested.***

Section 6.7.2 Biological samples

Table 11 Biological samples

Purpose	Time point
Laboratory confirmation of ~~Dd~~engue ~~infection diagnosis~~ by NS1-antigen ELISA and/or ICT test	***At each occurrence of AFI, i.e., each*** Suspected Dengue First Visit
Laboratory confirmation of ~~Dd~~engue ~~infection diagnosis~~ by ~~(generic and/or serotype-specific)~~ RT-qPCR	***At each occurrence of AFI, i.e., each Suspected Dengue First Visit***
Haematology assessment	***At each occurrence of AFI, i.e., each Suspected Dengue First Visit***
Dengue ***serology*** ~~infection diagnosis~~ by anti-DENV 1-4 µPRN, microneutralisation or comparable assay *	***Only at the first occurrence of AFI, i.e., during the first Suspected Dengue First Visit only***
Tertiary (research) endpoint related tests (including, but not limited to, neutralising antibody against JEV) **	***At each occurrence of AFI, i.e., each Suspected Dengue First Visit***

**** For subjects ≥ 5 years of age only at the first Suspected Dengue First Visit only and in India only.***
***** For subjects ≥ 18 years of age only.***

Section 6.8 Laboratory assays

Table 12 ~~Diagnosis~~ *Laboratory confirmation* of dengue infection

System	Component [1]	Method	Kit/Manufacturer	Laboratory*
Whole Blood/Serum/Plasma	NS1-antigen	ICT test	***SD Bioline Dengue Duo*** ~~Bio-Rad~~ (or other manufacturer) [2]	Local
Serum	DENV RNA	~~Generic RT-qPCR [2] and/or serotype-specific~~ RT-qPCR [3]	***Dengue Simplexa kit o comparable assay*** ~~In-house~~	~~Fiocruz and/or GSK Biologicals** and/or Local~~ ***Quest and/or Focus***
Serum	Neutralising antibodies against DENV 1-4	Micro-plaque-reduction neutralisation (µPRN), microneutralisation or comparable assay [3]	In-house	~~Fiocruz and/or~~ GSK Biologicals**

1) ***Refer to the SPM and the central laboratory manual for more information.*** ~~Generic RT-qPCR will be run at Fiocruz and/or GSK Biologicals but may not be used in Local lab~~.
2) ~~As per local practices.~~ GSK Biologicals recommends ***SD Bioline Dengue Duo*** ~~DENGUE NS1 AG STRIP, Bio-Rad~~.
3) ~~If serotype-specific testing is done at Fiocruz and/or GSK Biologicals, only samples tested positive with dengue generic RT-qPCR will be tested on serotype-specific RT-qPCR. If testing done locally, serotype-specific RT-qPCR can be used only.~~ ***Dengue serology (India only) will be performed on samples collected at the first Suspected Dengue First Visit only, from all children aged 5 to 17 years, as well as from adult study subjects who tested positive for Dengue RT-qPCR and up to three matched controls. Matching will be done by study site, age and gender.***

** GSK Biologicals laboratory refers to the Global Vaccines Clinical Laboratories (GVCL) in Rixensart, Belgium; Wavre, Belgium; Laval, Canada***, or validated laboratory designated by GSK Biologicals***.

Table 14 Other exploratory (tertiary, research) assays

System	Component	Method	Kit/Manufacturer	Laboratory
Serum	***Viral serology (e.g.,*** ~~Neutralising antibodies against~~ JEV, ***Chikungunya virus)***	TBD	***TBD*** ~~In-house~~	TBD **
Serum	Characterisation of infectious agents [T]	Sequencing ~~of PCR product~~	TBD	TBD **

**** A subset of samples will be tested.***
** The laboratory(ies) that will perform the additional potential exploratory (tertiary, research) tests is/are not yet identified and will be defined as soon as available.

The GSK Biologicals' ~~and Fiocruz~~ clinical laboratories have established a Quality System supported by procedures.

Section 9.1.3 Tertiary (research) endpoints

During the Suspected Dengue First Visit, additional blood volumes will be collected for tertiary study endpoint assays. These tertiary study endpoints include, but are not limited to, determination of neutralising antibody titres against dengue virus (DENV), Japanese encephalitis virus (JEV), and chikungunya virus, tissue culture to isolate infectious agents, and sequencing to characterise infectious agents.

Dengue serology (sites in India only since seroprevalence is well defined for sites in Sri Lanka) will be performed on samples collected at the first Suspected Dengue First Visit only, from all children aged 5 to 17 years, as well as from adult study subjects who tested positive for Dengue RT-qPCR and up to three matched controls. Matching will be done by study site, age and gender.

Tertiary study endpoint assays other than dengue serology will be performed for a subset of samples (for example, JEV assays will only be conducted if additional studies indicate that JEV serology is needed to interpret the DENV antibody response), i.e., not all of these endpoint assays will be performed on all of the sera collected from adult study subjects.

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

- ***Neutralising antibody titres against other viruses, including but not limited to, Chikungunya virus.***

Section 9.10.2 Statistical considerations for interim analyses

At time of interim analysis, all primary and secondary endpoints will be assessed to monitor the quality of data collected. The statistical report will then include the incidence of AFI due to LCD and due to non-LCD overall, by ~~DENV type,~~ study site and age group as well as a description of signs and symptoms of AFI due to LCD and due to non-LCD cases.

Section 12 References

Tissera H, Amarasinghe A, De Silva AD, et al. Burden of dengue infection and disease in a pediatric cohort in urban Sri Lanka. Am J Trop Med Hyg. 2014;91:132-7.

Section APPENDIX A. LABORATORY ASSAYS

Dengue generic RT-qPCR

The detection of the dengue virus in human serum is done by the amplification and quantification of its genetic material using a real time RT-qPCR assay.

Viral RNA is extracted from serum samples and reverse-transcribed into cDNA. The resulting cDNA is ~~detected~~ ***amplified and quantified*** by real time ***q***PCR ~~in one amplification reaction~~ using ~~specific~~ ***generic*** primers and fluorogenic probes ~~selected from 3' untranslated highly conserved region of~~ ***targeting*** the dengue viral genome.

Dengue serotype-specific RT-qPCR

The detection and typing of the dengue virus 1,2,3 and 4 in human serum is done by the amplification of each serotype RNA using a real time RT-qPCR assay.

~~The dengue serotype-specific RT-qPCR assay has been developed at GSK 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~~ ***amplified*** by real time ***q***PCR using ***type-***specific ~~DNA~~ primers ***and fluorogenic probes targeting*** the ***dengue viral genome*** ~~DENV capsid gene. PCR is performed as 2 duplex amplification (DENV-1/-3 and DENV-2/-4)~~.

Neutralising antibody against JEV *and Chikungunya virus*

Section APPENDIX B. CLINICAL LABORATORIES

Table 19 Outsourced laboratories

Laboratory	Address
~~Fiocruz~~	~~Laboratório de Tecnologia Virológica, Bio-Manguinhos Rio de Janeiro~~
QUEST (India)	***Quest Diagnostics Private Limited A-17 Info City, Sector 34 Gurgaon-122001 Haryana INDIA***
Focus (US)	***Focus Diagnostics, Inc. (d/b/a Quest Diagnostics) 33608 Ortega Highway San Juan Capistrano, CA 92675-2042 USA***

GlaxoSmithKline Biologicals

Vaccine Value & Health Science (VVHS)

Protocol Amendment 2

eTrack study number and Abbreviated Title	200274 (DPIV-021 EXPLO)
Amendment number:	Amendment 2
Amendment date:	02 April 2015
Co-ordinating author:	PPD, 4Clinics Belgium, contractor for GSK Biologicals

Rationale/background for changes:
The location where the scheduled enrolment visit (Visit 1) and the scheduled close-out visit (Visit 2) will take place has been extended, i.e., both visits will take place either at home or at the hospital/clinic, in accordance with local ethics committee requirements and/or national laws or regulations.

The testing schedule of dengue serology in adults has been aligned to the one of children 5 to 17 years of age, i.e., all subjects 5 years of age and above will be tested for dengue serology (in India only) on samples collected at the first Suspected Dengue First Visit.

The definition of acute febrile illness has been further clarified.

Detailed description of the quantification process of the dengue viral genome has been provided.

Typographical errors have been corrected and table legends reformatted

Synopsis

Scientific Objectives

Tertiary (Research)

- To describe DENV and Japanese Encephalitis Virus (JEV) antibody profiles in ~~a~~ subset***s*** of subjects with AFI.
- To assess the concordance between dengue Reverse transcriptase-quantitative Polymerase Chain Reaction (RT-qPCR) and Non-Structural protein 1 (NS1)-antigen ***ImmunoChromaTographic (ICT) test and/or*** Enzyme-Linked Immunosorbent Assay (ELISA) ~~and/or ImmunoChromaTographic (ICT) test~~ in the assessment of dengue infection.

All occurrences of "NS1-antigen ELISA and/or ICT test" have been updated to "NS1-antigen ICT test and/or ELISA": as the ICT test is the preferred test, and the ELISA is an optional test. Changes have been implemented in the following sections: synopsis (scientific objectives, endpoints), 2.3, 6.4 (Table 3), 6.5.5.1.4, 6.7 (Table 11), 6.8 (Table 12), 9.1.3, and 9.7. The order of presentation of both assays has also been modified in Appendix A.

Study design	• **Study visits:** The study will consist of ~~2~~ scheduled visits ~~and~~ ***(the enrolment visit [Visit 1],*** weekly contacts***, and the close-out visit [Visit 2]) and, in case of AFI*, unscheduled Suspected Dengue Visits:*** – ***Visit 1 and Visit 2 will take place at home or at the hospital/clinic, in accordance with local ethics committee requirements and/or national laws or regulations*** – ***Weekly contacts will be*** ~~i.e.,~~ home visits (at least every other week) or telephone contacts (TCs) ~~over a period of approximately two years per subject.~~ – ~~In addition, study subjects will be asked to attend~~ Unscheduled Suspected Dengue Visits ***will take place in case of AFI**** ****AFI is defined as*** ~~if~~ fever (body temperature ≥ 38°C/≥ 100.4°F) ~~is recorded or reported~~ on ≥ 2 consecutive calendar days***,*** ~~and~~ measured ***at least twice,*** at least ***8***~~12~~ hours apart.

The definition of AFI has been updated accordingly throughout the whole document. Changes have been implemented in the following sections: synopsis (study design/study visits, case definitions), 3, 4 (Figure 2), 4.1, 4.2, 4.3, 6.4 (Table 3), 6.5.2.5, 6.5.3.1, 6.5.5.1.1, 6.5.5.1.4, 6.7, and 9.4,

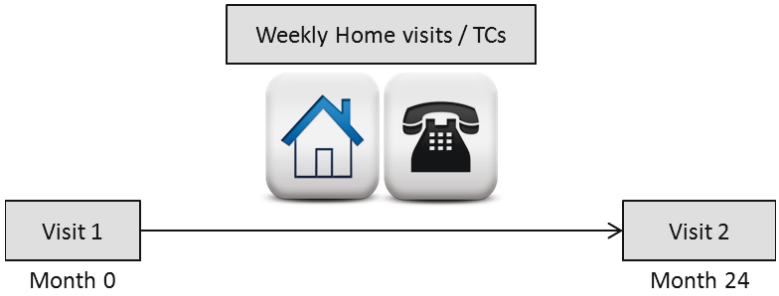
Scientific Objectives	**Tertiary (Research)** Dengue serology (sites in India only since seroprevalence is well defined for ~~sites in~~ ***the*** Sri Lanka ***site***) will be performed on samples collected at the first Suspected Dengue First Visit only, from all ***subjects*** ~~children aged~~ 5 ~~to 17~~ years ***of age and above***~~, as well as from adult study subjects who tested positive for Dengue RT-qPCR and up to three matched controls. Matching will be done by study site, age and gender~~.
Endpoints	**Tertiary (Research)** • ***Occurrence (by medical history only) of a*** ~~Confirmation of~~ diagnosis of tuberculosis, from birth up to study conclusion, in the study population.

Section 2.3 Tertiary (research) objectives

- To describe DENV and Japanese Encephalitis Virus (JEV) antibody profiles in ~~a~~ subset***s*** of subjects with AFI.

Section 3 Study design overview

Figure 1 Study design overview



- **Study visits:** The study will consist of ~~2~~ scheduled visits ~~and~~ ***(the enrolment visit [Visit 1],*** weekly contacts***, and the close-out visit [Visit 2]) and, in case of AFI*, unscheduled Suspected Dengue Visits:***
  - ***Visit 1 and Visit 2 will take place at home or at the hospital/clinic, in accordance with local ethics committee requirements and/or national laws or regulations***
  - ***Weekly contacts will be*** ~~i.e.,~~ home visits (at least every other week) or telephone contacts (TCs) ~~over a period of approximately two years per subject.~~
  - ~~In addition, study subjects will be asked to attend~~ Unscheduled Suspected Dengue Visits ***will take place in case of AFI****

    ****AFI is defined as*** ~~if~~ fever (body temperature ≥ 38°C/≥ 100.4°F) ~~is recorded or reported~~ on ≥ 2 consecutive calendar days***,*** ~~and~~ measured ***at least twice,*** at least ~~12~~***8*** hours apart.

Section 4 Case definition

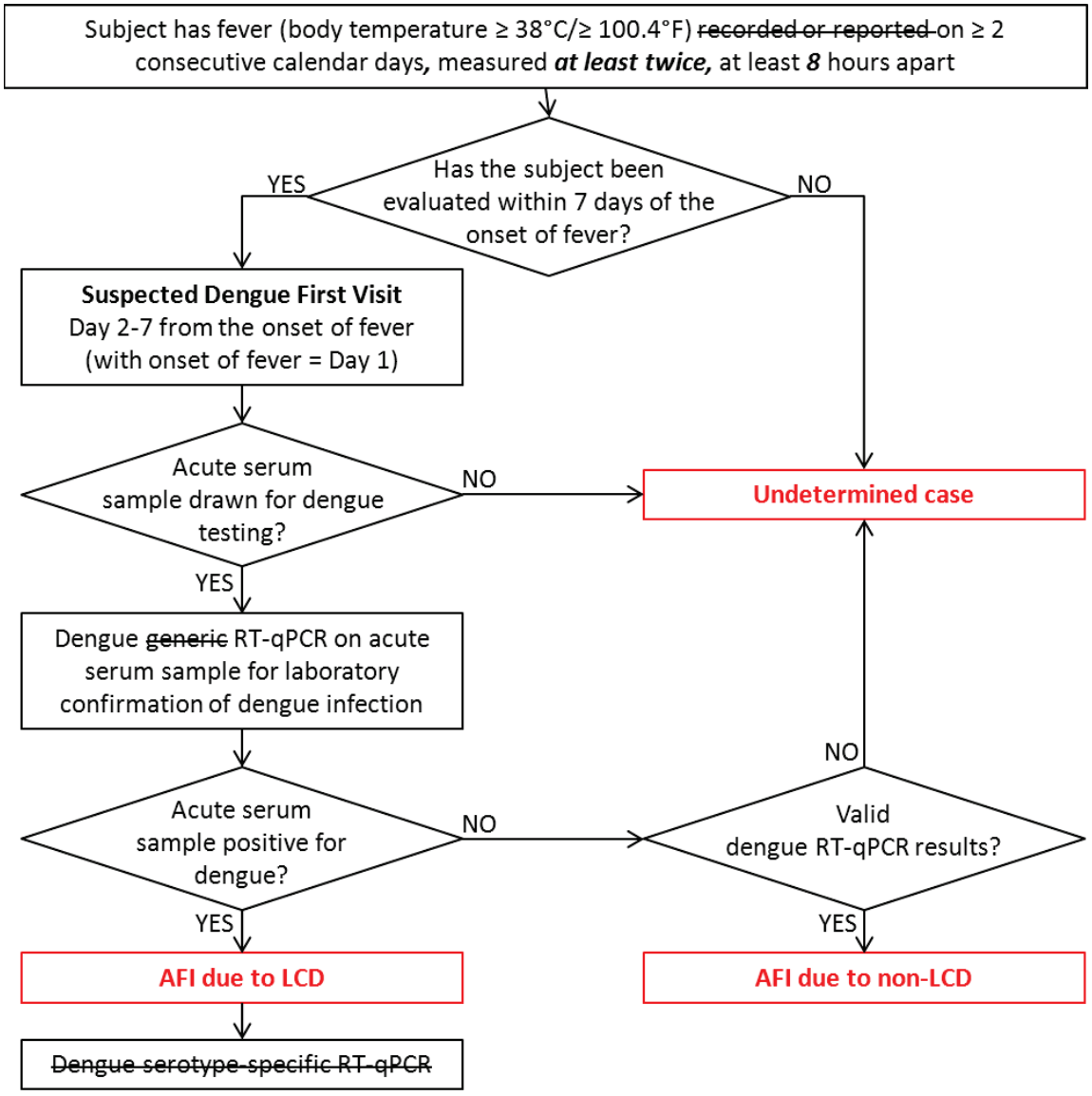
Section 4.1 Acute febrile illness due to LCD

ALL of the following findings must be met for an AFI due to LCD (see ~~also~~ Figure 2):

Section 4.2 Acute febrile illness due to non-LCD

ALL of the following findings must be met for an AFI due to non-LCD (see ~~also~~ Figure 2)

Figure 2 Schematic overview of different cases



AFI: Acute febrile illness; **LCD**: Laboratory Confirmed Dengue; ~~NS1-antigen test: Non-Structural protein 1 antigen test by ELISA or ICT;~~ RT-qPCR: reverse transcriptase quantitative polymerase chain reaction

Section 6.1 Regulatory and ethical considerations, including the informed consent process (and assent process if applicable)

The consenting process will be video recorded if required by law. If the subject/subject's parent(s)/LAR(s) are illiterate, the ICF (and IAF if applicable) will be countersigned by an impartial witness. Informed consent/assent can be obtained at the study clinic or during a home visit. In either case, medically ~~trained~~ and GCP trained study staff will follow an SOP for the consenting/assenting process.

Section 6.4 Outline of study procedures

Table 2 List of study procedures for scheduled visits and contacts

Epoch	Epoch 001		
Visit/contact	Visit 1	Home visits or TCs	Visit 2
Time point	Month 0	Weekly between visits [1]	Month 24
Record data ***(solicited symptoms, medication)*** from diary cards (if applicable)		● [6]	●

5) The subject/subject's parent(s)/LAR(s) will bring the diary card with him/her to each Suspected Dengue Visit (see Table 3). The investigator or the appointed study personnel will record the diary card information in the eCRF ***if a temperature of ≥ 38°C (≥ 100.4°F) was recorded on ≥ 2 consecutive calendar days, measured at least twice, at least 8 hours apart.***. The subject/subject's parent(s)/LAR(s) will return the diary card when completed at the next visit or contact (i.e., at the next home visit or at a Suspected Dengue Visit).

Table 3 List of study procedures for *unscheduled* Suspected Dengue Visits

6) The subject/subject's parent(s)/LAR(s) will bring the diary card with him/her to each Suspected Dengue Visit. The investigator or appointed study personnel will record the diary card information in the eCRF ***if a temperature of ≥ 38°C (≥ 100.4°F) was recorded on ≥ 2 consecutive calendar days, measured at least twice, at least 8 hours apart***. The subject/subject's parent(s)/LAR(s) will return the diary card when completed at the next scheduled visit or contact (i.e., at the next home visit or at a Suspected Dengue Visit).

Section 6.5.1 Procedures at scheduled Visit 1 prior to enrolment

The enrolment visit (Visit 1) will take place at home or at the hospital/clinic.

Section 6.5.2.2 Record socio-demographic data

Socio-demographic data such as date of birth, gender***, education, occupation and socio-economic status*** ~~and household/living conditions~~ will be recorded in the eCRF.

Section 6.5.2.5 Distribution of dengue kits and instruction of subjects/subject's parent(s)/LAR(s) to contact study staff in case of acute febrile illness

Diary cards

The subject/subject's parent(s)/LAR(s) will bring the diary card with him/her to each Suspected Dengue Visit. The investigator or appointed study personnel will review the diary card information with the subject and will record the information in the eCRF ***if a temperature of ≥ 38°C (≥ 100.4°F) was recorded on ≥ 2 consecutive calendar days, measured at least twice, at least 8 hours apart***.

Section 6.5.3 Procedures at scheduled weekly contacts

Weekly contacts will be home visits (at least every other week) or telephone contacts (TCs).

Section 6.5.4 Procedures at scheduled Visit 2

The close-out visit (Visit 2) will take place at home or at the hospital/clinic.

Section 6.5.5.1.1 *Important g*~~G~~eneral remarks

Blood samples ***for study procedures*** should **not** be drawn from subjects who attend a study healthcare facility on the first day of fever (body temperature ≥ 38°C/≥ 100.4°F), i.e., on Day 1. Medical assistance can be provided to these subjects but they will be asked to come back the following day should the fever persist.

Section 6.5.5.1.2 Physical examination

Table 5 Physical examination during Suspected Dengue First Visit

Additional information *(subjects with severe disease and suspected Dengue Shock Syndrome [DSS] - according to investigator's judgement)*	– narrow pulse pressure (< ~~20~~ ***30*** mm Hg) – hypotension – cold, clammy skin – restlessness

Section 6.5.5.1.4 Blood sampling

- Whole blood for dengue serology by anti-DENV 1-4 µPRN, microneutralisation or comparable assay: (India only) 4 mL ***collected at the first Suspected Dengue First Visit only***, from all ***subjects*** ~~children aged~~ 5 ~~to 17~~ years ***of age and above***~~, as well as from adult study subjects who tested positive for Dengue RT-qPCR and up to three matched controls~~.

Section 6.5.5.1.5 Collection and recording of solicited symptoms from diary card

1. During the Suspected Dengue First Visit, the diary card (partially completed) is reviewed by the physician and data recorded in the "solicited symptoms" section of the eCRF ***if the subject meets AFI criteria***.

Section 6.6 Solicited symptoms in case of acute febrile illness (suspected dengue infection)

Table 6 Solicited symptoms in case of suspected dengue infection

Signs and symptoms
abdominal pain ***

*** When possible (depending on age in young children).

Section 6.6.1 Assessment of the intensity of solicited symptoms

Note: Ideally, body temperature should be measured twice a day. ~~Should additional temperature measurements be performed at other times of day, the highest temperature will be recorded in the eCRF.~~

Section 6.7.2 Biological samples

Table 11 Biological samples

Purpose	Collected sample type	Quantity	Unit	Tested sample type	Sub-cohort	Time point
~~Laboratory confirmation of dengue by NS1-antigen ELISA and/or ICT test~~	~~Whole blood~~	~~0.5~~	~~mL~~	~~Whole Blood/Serum/Plasma~~	~~Suspected dengue infection – all subjects (any age)~~	~~At each occurrence of AFI, i.e., each Suspected Dengue First Visit~~
~~Laboratory confirmation of dengue by RT-qPCR~~	~~Whole blood~~	~~2.5~~	~~mL~~	~~Serum~~		~~At each occurrence of AFI, i.e., each Suspected Dengue First Visit~~
Haematology assessment	Whole blood	2	mL	Whole blood	Suspected dengue infection – all subjects ≥ 5 years of age – subjects < 5 years of age only if clinically indicated, as per investigator judgement	***At each occurrence of AFI, i.e., each Suspected Dengue First Visit***
Laboratory confirmation of dengue by RT-qPCR	***Whole blood***	***2.5***	***mL***	***Serum****	***Suspected dengue infection – all subjects (any age)***	
Tertiary endpoint Laboratory confirmation of dengue by NS1-antigen ICT test and/or ELISA	***Whole blood***	***0.5***	***mL***	***Whole Blood/Serum*/Plasma****		
Tertiary endpoint Dengue serology by anti-DENV 1-4 µPRN, microneutralisation or comparable assay	Whole blood	4	mL	Serum*	Suspected dengue infection – all subjects ≥ 5 years of age ***in India only***	Only at the first occurrence of AFI, i.e., during the first Suspected Dengue First Visit only
Other tertiary (research) endpoint related tests (including, but not limited to, neutralising antibody against JEV)	Whole blood	4	mL	Serum *	Suspected dengue infection – all subjects ≥ 18 years of age	At each occurrence of AFI, i.e., each Suspected Dengue First Visit

~~* For subjects ≥ 5 years of age only at the first Suspected Dengue First Visit only and in India only.~~
~~** For subjects ≥ 18 years of age only.~~
* Assumption: 1 mL of whole blood usually allows a yield of between 30% and 40% of serum/plasma.

Section 6.8 Laboratory assays

Table 12 Laboratory confirmation of dengue infection

System	Component [1]*	Method	Kit/Manufacturer	Laboratory ~~*~~†
Whole Blood/Serum/Plasma	NS1-antigen	***ICT test***	***SD Bioline Dengue Duo (or other manufacturer)*** [2]**	***Local***
		ELISA	PLATELIA™ Dengue NS1 Ag (or other manufacturer)	Local
Serum	DENV RNA	RT-qPCR	Dengue Simplexa kit o comparable assay	Quest and/or Focus ***or validated laboratory designated by GSK Biologicals***
Serum	Neutralising antibodies against DENV 1-4	Micro-plaque-reduction neutralisation (µPRN), microneutralisation or comparable assay [3]***	In-house	GSK Biologicals ~~**~~††

* ~~1)~~ Refer to the SPM and the central laboratory manual for more information.

** ~~2)~~ GSK Biologicals recommends SD Bioline Dengue Duo.

*** ~~3)~~ Dengue serology (India only) will be performed on samples collected at the first Suspected Dengue First Visit only, from all ***subjects*** ~~children aged~~ 5 ~~to 17~~ years ***of age and above***~~, as well as from adult study subjects who tested positive for Dengue RT-qPCR and up to three matched controls. Matching will be done by study site, age and gender~~.

† ~~*~~ Refer to the APPENDIX B for the laboratory addresses.

†† ~~**~~ GSK Biologicals laboratory refers to the Global Vaccines Clinical Laboratories (GVCL) in Rixensart, Belgium~~;~~, Wavre, Belgium~~;~~ ~~Laval, Canada,~~ or validated laboratory designated by GSK Biologicals.

Table 14 Other exploratory (tertiary, research) assays

** The ***testing*** laboratory(ies) that will perform the additional potential exploratory (tertiary, research) tests ~~is/ (are) not yet identified and will be defined as soon as available~~ ***have not been identified yet***.

Section 9.1.3 Tertiary (research) endpoints

Dengue serology (sites in India only since seroprevalence is well defined for ~~sites in~~ ***the*** Sri Lanka ***site***) will be performed on samples collected at the first Suspected Dengue First Visit only, from all ***subjects*** ~~children aged~~ 5 ~~to 17~~ years ***of age and above***~~, as well as from adult study subjects who tested positive for Dengue RT-qPCR and up to three matched controls. Matching will be done by study site, age and gender~~.

- ***Occurrence (by medical history only) of a*** ~~Confirmation of~~ diagnosis of tuberculosis, from birth up to study conclusion, in the study population.

Section 12. REFERENCES

Sasmono RT, Aryati A, Wardhani P, et al. Performance of Simplexa dengue molecular assay compared to conventional and SYBR Green RT-PCR for detection of dengue infection in Indonesia. PLoS One. 2014;9:e103815

Section APPENDIX A. LABORATORY ASSAYS

NS1-antigen ICT test

The Dengue NS1 ICT test will be a commercial individual test for the qualitative detection of Dengue virus NS1 antigen in human serum, plasma or on whole blood. Performance characteristics of the assay are described in the manufacturer package insert.

NS1-antigen ELISA

The NS1 antigen in serum or plasma samples will be detected by using a commercial ~~CE labelled~~ assay. Performance characteristics of the assay are described in the manufacturer package insert.

~~NS1-antigen ICT test~~

~~The Dengue NS1 ICT test will be a commercial CE labelled individual test for the qualitative detection of Dengue virus NS1 antigen in human serum, plasma or on whole blood. Performance characteristics of the assay are described in the manufacturer package insert.~~

Dengue ~~generic~~ RT-qPCR *assay*

The detection of the dengue virus in human serum is done by ***reverse transcription of viral RNA, followed by*** the amplification and quantification of ~~its genetic material~~ ***the resultant cDNA*** using a real-time RT-qPCR assay***, such as the Focus Diagnostics Simplexa™ Dengue RT-PCR assay***. ~~Viral RNA is extracted from serum samples and reverse-transcribed into cDNA. The resulting cDNA is amplified and quantified by real time qPCR using generic primers, and fluorogenic probes targeting the dengue viral genome~~.

~~Dengue serotype-specific RT-qPCR~~

~~The detection and typing of the dengue virus 1,2,3 and 4 in human serum is done by the amplification of each serotype RNA using a real time RT-qPCR assay.~~

~~Viral RNA is extracted from serum samples and reverse-transcribed into cDNA. The resulting cDNA is amplified by real time qPCR using type-specific primers targeting and fluorogenic probes targeting the dengue viral genome.~~

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. Performance characteristics of the assay are described in the manufacturer package insert and in a recent publication [Sasmono, 2014].

Neutralising antibody against JEV and Chikungunya virus

To be determined. ***A testing laboratory has not been identified yet.***

Virus isolation and characterisation

To be determined. ***A testing laboratory has not been identified yet.***

Section APPENDIX B. CLINICAL LABORATORIES

Table 18 GSK Biologicals' laboratories

Laboratory	Address
~~GSK Biologicals Global Vaccine Clinical Laboratory, North America- Laval~~	~~Biospecimen Reception - Clinical Serology 525 Cartier blvd West - Laval - Quebec – Canada - H7V 3S8~~

GlaxoSmithKline Biologicals

Vaccine Value & Health Science (VVHS)

Protocol Amendment 3

eTrack study number and Abbreviated Title	200274 (DPIV-021 EXPLO)
Amendment number:	Amendment 3
Amendment date:	01 March 2017
Co-ordinating author:	PPD, contractor for GSK Biologicals

Rationale/background for changes:

After study enrolment was initiated, the 3 sites in India were still delayed due to the inability to export the blood samples for dengue testing. Following Sponsor review, the India portion of the study was canceled due to logistical difficulties. Therefore, sites in India will no longer be included this study protocol. Among the 5000 subjects planned in the study, 3000 were to come from India. The planned number of study subjects changed from 5000 to 2000 and all subjects will be enrolled from Sri Lanka.

Sample size calculations were adjusted accordingly.

Added text to clarify that an unscheduled visit (either for first, return, and follow-up dengue visits) will be considered as the weekly contact for the week in which it occurs.

Title A cohort study to determine the incidence of dengue fever and to build capacity for dengue vaccine trials in ~~dengue-endemic regions of~~ South Asia.

Detailed Title A prospective, ~~multi-centre,~~ cohort study to determine the incidence of acute febrile dengue illness and to build capacity for dengue vaccine clinical endpoint trials in South Asian communities.

Co-ordinating authors

- PPD (4Clinics Belgium for GSK Biologicals) / PPD / PPD /PPD ***(XPE Belgium for GSK Biologicals***

Contributing authors

- PPD***, Clinical and Epidemiology Project Leader***
- PPD***, Senior Epidemiologist***
- PPD ***Project Statistician***
- PPD, PPD**,** Study Delivery Lead
- PPD, PPD**,** Clinical Safety ~~Representative~~ ***Physician***
- PPD, PPD, Study Data Manager
- PPD, ~~Clinical Laboratories Representative~~ ***Clinical Laboratory Science Clinical Readout Team Leader***

PPD **, Global Regulatory Leader**

- PPD , ~~Global Vaccines Clinical Laboratories~~ ***Clinical Laboratory Sciences (CLS)*** Study Manager
- PPD , ***CLS*** ~~GVCL~~ Study Manager
- PPD ***, Oversight Data Manager***

Synopsis:
Detailed Title: A prospective, ~~multi-centre~~, cohort study to determine the incidence of acute febrile dengue illness and to build capacity for dengue vaccine clinical endpoint trials in South Asian communities

Tertiary Research Objective: ~~To describe neutralising antibody titre profiles in a subset of LCD and non-LCD subjects.~~

- **Study design: Type of design** Prospective, ~~multi-centre~~, community-based, cohort study, household-sampling, ***single country*** multi-country
- **Study population:** approximately ***2,000*** ~~5,000~~ subjects ...
- Unscheduled Suspected Dengue Visits will take place in case of AFI*

  Note that an unscheduled visit (either for first, return, or follow-up dengue visits) will be considered as the weekly contact for the week in which it occurs.

Synopsis Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects
Prospective	~2000-***≈2000***

Discussion of study design: This prospective ~~multiple-site~~ cohort study including children aged less than 18 years and adults aged up to 50 years...

Number of subjects: Approximately ~~5,000~~ ***2000***

Endpoint: Tertiary (Research)

~~Dengue serology (sites in India only since seroprevalence is well defined for ***the*** Sri Lanka ***site***) will be performed on samples collected at the first Suspected Dengue First Visit only, from all ***subjects*** 5 years ***of age and above***. (**Amended 01 March 2017**)~~

Introduction, Background:
Deleted text regarding India
Globally, 70% of the estimated 96 million apparent dengue infections are thought to occur in Asia. ~~India alone accounts for approximately 34% (33 million [22-44] infections) of the global total. Current national and global estimations of the disease burden of dengue in India are considered uncertain. Underreporting and misdiagnoses are major obstacles to understanding the full burden, and active surveillance studies in prospective cohorts are thought to be needed to estimate the burden of the disease more accurately [Gupta, 2006; Kakkar, 2012; Gupta, 2013]. Dengue fever became a notifiable disease in 2005, and the Government of India has established a network of Sentinel Surveillance Hospitals linked to referral Laboratories. In 2006, India's National Vector~~

~~Control Program reported an incidence of 1.2 cases/100,000 people per year and a case fatality rate of 1.5%. This incidence rate is much lower than the expected rate reported from other dengue endemic regions, and most reported cases in India lack laboratory confirmation [Kanungo, 2012], indicating that there is a pressing need for active surveillance studies. Even though solid incidence rate estimates for dengue fever are not available for India, surveillance data indicate that dengue is a substantial contributor to febrile illness and that all 4 dengue virus serotypes co-circulate in large parts of India [Bharaj, 2008; Chakravarti, 2012]. A recent study in Kolkata found that 8% of all acute febrile illness (AFI) cases were due to dengue [Kanungo, 2012]. The number of reported cases has increased considerably over the past decade and hyper-endemicity seems to contribute to increased disease severity.~~

~~The reported age-distribution of dengue in India suggests that dengue illness is most common in young adults and teenagers [Chakravarti, 2012], with a tendency for more severe disease in younger people. In contrast,~~ sero-surveillance data from Colombo, Sri Lanka, indicate that over 70% of children experienced at least 1 dengue infection by the age of 12 years, and the median age at infection there is 4.7 years. The risk of primary infection in children below 12 years of age was 14% per year [Tam, 2013]. In 2012, the reported dengue fever incidence cases per 100,000 people were 200~~, 20 and 4~~ for Sri Lanka~~, the state of Tamil Nadu, and all of India, respectively~~ [NVBDCP, 2014; Epidemiology Unit, 2014] and reports of dengue outbreaks and fatal cases frequently made headline news in South Asia.

~~In 2012, the Government of India convened a meeting of dengue experts to address the growing burden of disease and to form a task force on dengue, indicating a growing sense of urgency and increasing awareness of the importance of this disease.~~

2.3 Tertiary objectives

- ~~To describe neutralising antibody titre profiles in a subset of LCD and non-LCD subjects.~~

3 Study Design Overview

Figure1 Study design overview

Added footnote:
Notes:

The weekly contact may be a face to face visit outside the home in certain cases (i.e. at hospital during suspected dengue visit)

An unscheduled visit (either for first, return, or follow-up dengue visit) will be considered as the weekly contact for the week in which it occurs.

Type of design: Prospective, ~~multi-centre~~, community-based, cohort study, household-sampling***, single country***.

- **Study population:** approximately ~~5,000~~ ***2000*** subjects aged

Study Visits

Weekly contacts will be home visits (at least every other week) or telephone contacts (TCs) ***The weekly contact may be a face to face visit outside the home in certain cases (i.e. at hospital during suspected dengue visit)***

Table1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)	Epoch 001
Prospective	***≈2000*** ~~5000~~	6 6 months to 50 years	x

3.1 Discussion of study design

This prospective, ~~multi-center~~ cohort study including children aged less than 18 years and adults aged up to 50 years is designed to support the selection and definition of clinical.....

5.1 Number of subjects / centres

The target is to enrol approximately ***2,000*** ~~5,000~~ eligible subjects in order to reach approximately ***3300*** ~~8,000~~ person-years evaluable overall at the time of final analysis.

5.1.1. Selection of communities and households

~~A minimum of 500 subjects will be recruited per site.~~

5.1.2. Overview of the recruitment plan

~~Each site will have approximately 4 months from global study start to reach the minimum target of 500 subjects. Thereafter~~ **e *E*nrolment will** ~~be competitive~~ **continue** until a total of approximately **2,000 ~~5,000~~** subjects are recruited**.**

6.4. Outline of study procedures

Table 2 List of study procedures for scheduled visits and contacts

Table footnote text added: 1) Weekly contacts will be home visits (at least every other week) or TCs. ***The weekly contact may be a face to face visit outside the home in certain cases (i.e. at hospital during suspected dengue visit)***

Deleted amendment reference after row entitled “record data” and moved notes regarding abbreviations to occur adjacent to other notes in order to have table appear on a single page

Table 3 List of study procedures for unscheduled Suspected Dengue Visits

Epoch	Epoch 001		
Visit	**Suspected Dengue First Visit**	**Return Visit [1]**	**Suspected Dengue Follow-up Visit [2]**
Time point	**Day 2 to Day 7, ideally on Day 2 (1 day after the onset of fever)**	**1 to 6 days after Suspected Dengue First Visit**	**7 to13 days after Suspected Dengue First Visit**
Blood sampling*			
Dengue NS1-antigen ***ICT test and/or*** ELISA (0.5 mL) (**Amended 01 March 2017**)	●		
Dengue RT-qPCR (2.5 mL)	●		
~~DENV 1-4 serology (4 mL)~~ [3]	●		
for haematology (2 mL) [4 3]	●		
for other tertiary (research) endpoints related tests ~~(≈4~~ ***3.5*** mL) [5 4]	●		
Safety follow-up:			

~~**µPRN**: micro-plaque-reduction neutralisation;~~ ~~**DENV**: dengue virus;~~ **eCRF**: electronic Case Report Form;

2) A Suspected Dengue Follow-up Visit may occur during a weekly home visit. This visit should be performed by a medically trained site staff member. ***Note that when a subject comes in for a suspected dengue follow-up visit, this will replace the regularly scheduled weekly home visit.***

~~3) For subjects ≥ 5 years of age only at the first Suspected Dengue First Visit only and in India only.~~

4 ***3***) For all subjects ≥ 5 years of age. For subjects < 5 years of age only if clinically indicated, as per investigator judgement. Complete blood count (White blood cells [WBC], red blood cells [RBC], platelets and haemoglobin).

~~5~~ ***4***) For subjects ≥ 18 years of age only. Including, but not limited to, neutralising antibody against JEV and ....

~~6~~ **5**) The subject/subject's parent(s)/LAR(s) will bring the diary card with him/her to each Suspected Dengue ....

~~7~~ ***6***) A new diary card will be issued by the study staff whenever necessary (i.e., if the subject no longer ...

~~8~~ ***7***) Only one study conclusion will be conducted, either at Visit 2, or if a suspected dengue case is....

6.5.3 Procedures during scheduled weekly contacts (Amended 01 March 2017)

Weekly contacts will be home visits (at least every other week) or telephone contacts (TCs). (Amended 02 April 2015). ***The weekly contact may also be a face to face visit outside the home in certain cases (i.e. at hospital during suspected dengue visit)***

Table 5 Physical examination during Suspected Dengue First Visit

Physical examination *(all subjects)*	• tourniquet test (positive/negative), ***only pertains to subjects ≥5 years of age*** **(Amended 01 March 2017)** • haemorrhagic manifestations; petechiae, bleeding from mucosa, gastrointestinal tract, injection sites or other locations, • Injected pharynx, • clinical signs of plasma leakage: oedema, pleural effusion, ascites

6.7.2 Biological samples

Table 11 Biological samples

Purpose	Collected sample type	Quantity	Unit	Tested sample type	Sub-cohort	Time point
Laboratory confirmation of dengue by RT-qPCR	***Whole blood***	***2.5***	***mL***	***Serum****	***Suspected dengue infection – all subjects (any age)***	
Tertiary endpoint laboratory confirmation of dengue by NS1-antigen ICT test and/or ELISA	***Whole blood***	***0.5***	***mL***	***Whole Blood/Serum*/Plasma****		
~~***Tertiary endpoint*** Dengue serology * by anti-DENV 1-4 µPRN, microneutralisation or comparable assay~~	~~Whole blood~~	~~4~~	~~mL~~	~~Serum*~~	~~Suspected dengue infection – all subjects ≥ 5 years of age ***in India only***~~	~~Only at the first occurrence of AFI, i.e., during the first Suspected Dengue First Visit only~~
Other tertiary (research) endpoint related tests (including, but not limited to, neutralising antibody against JEV)~~**~~	Whole blood	~~4~~ ***3.5***	mL	Serum*	Suspected dengue infection – all subjects ≥ 18 years of age	At each occurrence of AFI, i.e., each Suspected Dengue First Visit

~~**µPRN**: micro-plaque-reduction neutralisation~~
~~**DENV**: dengue virus~~

6.8 Laboratory Assays

Table 12 Laboratory confirmation of dengue infection

System	Component *	Method	Kit/Manufacturer	Laboratory†
Whole Blood/Serum/Plasma	NS1-antigen	***ICT test***	***SD Bioline Dengue Duo (or other manufacturer)*** **	***Local***
		~~ELISA~~	~~PLATELIA™ Dengue NS1 Ag (or other manufacturer)~~	~~Local~~
Serum	DENV RNA	RT-qPCR	Dengue Simplexa kit o comparable assay	~~Quest and/or Focus~~ ***Q²Solutions -Focus Diagnostics or validated laboratory designated by GSK Biologicals††***
~~Serum~~	~~Neutralising antibodies against DENV 1-4~~	~~Micro-plaque-reduction neutralisation (µPRN), microneutralisation or comparable assay ***~~	~~In-house~~	~~GSK Biologicals††~~

~~**DENV**: dengue virus~~

†† GSK Biologicals laboratory refers to the ~~Global Vaccines Clinical Laboratories (GVCL)~~ ***Clinical Laboratory Sciences (CLS)*** in Rixensart, Belgium**,** Wavre, Belgium**,** or validated laboratory designated by GSK Biologicals.

Table 14 Other exploratory (tertiary, research) assays

System	Component *	Method	Kit/Manufacturer	Laboratory
Serum	Isolation of infectious agents †	Tissue culture	NA	TBD **
	Characterisation of infectious agents †	Sequencing	TBD	TBD **

† Additional potential exploratory tests (tertiary endpoints related tests) for the diagnosis of other common causes of AFI, such as malaria, typhoid, scrub typhus, ~~flu~~ ***influenza***, ***viral*** hepatitis ~~A~~, etc.

9.1.3 Tertiary (research) endpoints

During the Suspected Dengue First Visit, additional blood volumes will be collected for tertiary study endpoint assays. These tertiary study endpoints include, but are not limited to, determination of neutralising antibody titres against dengue virus (DENV), Japanese encephalitis virus (JEV), and chikungunya virus, tissue culture to isolate infectious agents, and sequencing to characterise infectious agents.

~~Dengue serology (sites in India only since seroprevalence is well defined for ***the*** Sri Lanka ***site***) will be performed on samples collected at the first Suspected Dengue First Visit only, from all ***subjects*** 5 years ***of age and above***. (**Amended 01 March 2017**)~~

Tertiary study endpoint assays ***may*** be performed for a subset of samples (for example, JEV assays will only be conducted if additional studies indicate that JEV serology is needed to interpret the DENV antibody response), i.e., not all of these endpoint assays will be performed on all of the sera collected from adult study subjects.

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

- ~~Neutralising antibody titres against DENV 1-4.~~

9.2 Sample size considerations

A ***maximum of 2,000*** ~~5,000~~ subjects will be enrolled for a 2-year follow-up period. Assuming a drop-out rate of 10% the first year and a drop-out rate of 20% the second year, the final analysis will be done on approximately ***3300*** ~~8,000~~ person-years overall.

In order to evaluate the precision with a sample size of ***3,000*** ~~5,000~~ subjects, the 95% confidence interval (CI) (Poisson exact distribution and normal approximation of the Poisson distribution accounting for a design effect) for a range of expected incidences rates of AFI due to LCD were computed. The 95% CI for a range of expected incidences rates for DENV specific LCD were also presented (see Table 17).

New table

Table 17 95% CI with design effect of 2.6

	Number of cases	*Number of person-years*	*Incidence of LCD (per 100 person-years)*	*95% CI Poisson exact*		*95% CI Normal approx with design effect of 2.6*	
				LL	*UL*	*LL*	*UL*
Overall	***10***	***3300***	***0.30***	***[0.15 ; 0.56]***		***[0.00 ; 0.61]***	
	20	***3300***	***0.61***	***[0.37 ; 0.94]***		***[0.18 ; 1.03]***	
	30	***3300***	***0.91***	***[0.61 ; 1.30]***		***[0.38 ; 1.43]***	
	40	***3300***	***1.21***	***[0.87 ; 1.65]***		***[0.61 ; 1.82]***	
	50	***3300***	***1.52***	***[1.12 ; 2.00]***		***[0.84 ; 2.19]***	
	60	***3300***	***1.82***	***[1.39 ; 2.34]***		***[1.08 ; 2.56]***	
DENV specific	***4***	***3300***	***0.12***	***[0.03 ; 0.31]***		***[0.00 ; 0.31]***	
	8	***3300***	***0.24***	***[0.10 ; 0.48]***		***[0.00 ; 0.51]***	
	12	***3300***	***0.36***	***[0.19 ; 0.64]***		***[0.03 ; 0.70]***	
	16	***3300***	***0.48***	***[0.28 ; 0.79]***		***[0.10 ; 0.87]***	

Deleted table:

	Number of cases	Number of person-years	Incidence of LCD (per 100 person-years)	95% CI Poisson exact		95% CI Normal approx with design effect of 2.6	
				LL	UL	LL	UL
Overall	20	8000	0.25	[0.15 ; 0.39]		[0.07 ; 0.43]	
	40	8000	0.50	[0.36 ; 0.68]		[0.25 ; 0.75]	
	60	8000	0.75	[0.57 ; 0.97]		[0.44 ; 1.06]	
	80	8000	1.00	[0.79 ; 1.24]		[0.65 ; 1.35]	
	100	8000	1.25	[1.02 ; 1.52]		[0.85 ; 1.65]	
	120	8000	1.50	[1.24 ; 1.79]		[1.07 ; 1.93]	
DENV specific	4	8000	0.05	[0.01 ; 0.13]		[0.00 ; 0.13]	
	8	8000	0.10	[0.04 ; 0.20]		[0.00 ; 0.21]	
	12	8000	0.15	[0.08 ; 0.26]		[0.01 ; 0.29]	
	16	8000	0.20	[0.11 ; 0.32]		[0.04 ; 0.36]	
Site specific	5	2000	0.25	[0.08 ; 0.58]		[0.00 ; 0.60]	
	10	2000	0.50	[0.24 ; 0.92]		[0.00 ; 1.00]	
	15	2000	0.75	[0.42 ; 1.24]		[0.14 ; 1.36]	
	20	2000	1.00	[0.61 ; 1.54]		[0.29 ; 1.71]	
	25	2000	1.25	[0.81 ; 1.85]		[0.46 ; 2.04]	
	30	2000	1.50	[1.01 ; 2.14]		[0.63 ; 2.37]	

CI: confidence interval
DENV: dengue virus
LCD: laboratory Confirmed Dengue
LL lower limit;
UL: upper limit

9.7. Analysis of tertiary (research) objectives

Some tertiary (research) analyses may be done including:

~~• Neutralising antibody titres against DENV 1-4 on serum: the seropositivity rates for each DENV serotype with exact 95% CI and GMTs with 95% CI and range of antibody titres for each DENV serotype will be tabulated.~~

• A concordance analysis between dengue virus detection by RT-qPCR and by NS1-antigen (ICT test ~~and/or ELISA~~) may be performed. This analysis may be stratified by day of sampling if sufficient data are available. Sensitivity, specificity, positive predictive value and negative predictive value of NS1-antigen (ICT test ~~and/or ELISA~~) in prediction of RT-qPCR test results will be computed.

9.10.1 Sequence of analyses

- ~~An interim analysis will be performed (on as clean as possible data) once 1,500 subjects have been followed up for at least 1 year. If the observed incidence of cases is lower than expected, possible causes will be explored, including quality of surveillance (weekly contacts and suspected dengue visits, diary card completion), time from fever onset to blood draw, sample management and laboratory assay performance. The outcome of this analysis could lead to process clarification or even a protocol amendment to improve surveillance and or case confirmation.~~

The final analysis will be performed when all prospective data have been collected and cleaned. A final study report containing data from the entire study will be written at this time. ~~Depending on whether or not some of the processes of surveillance before / after the interim analysis have occurred, the new data may be analysed separately.~~

~~9.10.2 Statistical considerations for interim analyses~~

~~At time of interim analysis, all primary and secondary endpoints will be assessed to monitor the quality of data collected. The statistical report will then include the incidence of AFI due to LCD and due to non-LCD overall, by study site and age group as well as a description of signs and symptoms of AFI due to LCD and due to non-LCD cases. Additionally, the number of subjects withdrawn from the study and the compliance in returning diary card in case of AFI will also be generated.~~

Section 12 References

~~Bharaj P, Chahar HS, Pandey A, *et al*. Concurrent infections by all four dengue virus serotypes during an outbreak of dengue in 2006 in Delhi, India. *Virol J.* 2008;5:1.~~

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APPENDIX B CLINICAL LABORATORIES

Table 19 Outsourced laboratories

Laboratory **Address**
QUEST (India) Quest Diagnostics Private Limited A-17 Info City, Sector 34 Gurgaon-122001 Haryana INDIA
Focus (US) ***Q²Solutions -Focus Diagnostics*** Focus Diagnostics, Inc. (d/b/a Quest Diagnostics) 33608 Ortega Highway San Juan Capistrano, CA 92675-2042 USA