Study Protocol
Sponsor:
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Title:	An epidemiological surveillance study to evaluate the incidence of dengue in endemic regions of Brazil.
Detailed Title:	A prospective, multicenter, multiyear, cohort study to determine dengue incidence in children and adults in communities from endemic regions in Brazil.
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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 13.2

Protocol Amendment 4 Sponsor Signatory Approval

eTrack study number and Abbreviated Title:	116606 (EPI-DENGUE-006 BOD BR)
Date of Protocol Amendment	Protocol Amendment 4 Final: 16 October 2017
Detailed Title:	A prospective, multicenter, multiyear, cohort study to determine dengue incidence in children and adults in communities from endemic regions in Brazil.
Sponsor signatory	***Robert Paris,*** ***CEPL, Early Development,*** ***Vaccines R&D,*** ***GSK Biologicals US***
Signature	_____
Date	_____

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Protocol Amendment 4 Rationale

Rationale/background for changes:

Additional exploratory objectives have been added in this protocol amendment to explore the seroprevalence of antibodies to Zika and chinkungunya viruses in the study population, as well as the potential aetiological role of these viruses in episodes of febrile illness detected during follow-up .

Zika is a *flavivirus* and chikungunya virus is a member of the *alphavirus* genus, and *Togaviridae* family. These cause emerging mosquito-borne viral diseases in Latin America especially in Brazil. Zika virus infections have been laboratory confirmed in 19 states in Brazil between April and December 2015 [Boletim Epidemiológico n5, 2016]. In 2016, until epidemiological week 47, 196.976 cases were reported and 101,851 were confirmed [Boletim epidemiológico n 33, 2016].

In 2015, 20,661 autochthonous suspected chikungunya cases were reported in Brazil [Boletim Epidemiológico n5, 2016]. In 2016, 216,102 reported cases and 102,638 were confirmed up to epidemiological week 47 [Boletim epidemiológico n33, 2016]. Zika and chikungunya viruses cause symptoms often similar to dengue during the acute phase of illness.

The interpretation of the serological tests used to determine the seroprevalence of dengue antibodies or for diagnostic of acute dengue infection should take into account the possibility of cross-reaction with antibodies elicited by exposure to the Zika virus. The public health importance of Zika and chikungunya virus emergence, as well as the potential impact of these viruses on the interpretation of the laboratory assays, supports the inclusion of the exploratory objectives related to Zika and chikungunya infection.

Fever is the triggering event for a subject to be evaluated as a suspect dengue case in the study. While fever is common in suspected chikungunya cases, fever does not always present in suspected Zika cases. For this reason, rash is being added as a triggering event, in addition to fever, for Zika virus disease evaluation. A prospective surveillance will be maintained for febrile cases and added for rash cases. Also, considering the epidemiological situation in Brazil for both Zika and chikungunya infection, a retrospective testing for these infections in subjects considered as suspect dengue case since the beginning of the study will also be done.

The current case definition for suspected dengue does not include a criteria specifying the maximum interval between fever onset and the initial medical visit. This is specified in section 5.7.1.1.

Currently, haematology testing (HCT, CBC) is planned to be performed for all suspected dengue cases at the initial visit, regardless of the time between fever onset and the visit. However, for patients presenting more than 14 days after fever and/or rash onset, the relevance of these tests is low. Therefore, a maximum interval will be defined in this amendment.

The geographical distribution of the participants may impact the risk of infection. Therefore, updating information on the household of the study participants and their location at yearly visits is important during the follow-up period (which may extend to 4 years). Hence, socio-demographic and household information will be collected at yearly visits.

Some sites may have more than 500 active participants enrolled in the penultimate year of the study. However, their site may have experience with high drop-out rate and can expect to drop below 500 participants during the last year of the study. To address this challenge, criteria to allow for replacement cohort recruitment was clarified.

Also, the NS1 test has been removed for the late presenters since this test does not have clinical relevance in the case of late presenters.

Collection of dengue vaccination history has been added since a competitor dengue vaccine is currently available in the Brazilian market.

Protocol Amendment 4 Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any mutually agreed future protocol amendments or protocol administrative changes, and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline Biologicals (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 authorized 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:	116606 (EPI-DENGUE-006 BOD BR)
Date of Protocol Amendment	Protocol Amendment 4: 16 October 2017
Detailed Title	A prospective, multicenter, multiyear, cohort study to determine dengue incidence in children and adults in communities from endemic regions in Brazil.
Investigator name	_____
Signature	_____
Date	_____

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Sponsor Information

1. **Sponsor**

GlaxoSmithKline Biologicals, Belgium

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

Refer to the local study contact information document.

3. **Sponsor Study Monitor**

Refer to the local study contact information document.

4. **Sponsor Study Contact for Reporting of a Serious Adverse Event**

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to protocol Section 6.2.3

GSK Biologicals Central Safety Physician and Back-up Phone contact: refer to protocol Section 6.2.3

SYNOPSIS

Detailed Title: A prospective, multicenter, multiyear, cohort study to determine dengue incidence in children and adults in communities from endemic regions in Brazil.

Rationale for the study: The estimation of dengue incidence is critical when designing an efficacy trial for a dengue vaccine. In a given city or region, dengue incidence is highly variable from one year to another (variations in geography, season, year-to-year variations in mosquito vector populations in different areas, etc.) and cannot yet be predicted with sufficient accuracy in many locations being considered for clinical trials. In a dengue vaccine efficacy trial, volunteers are recruited from geographically distinct sites. Some of these sites may experience high dengue transmission during the trial duration while others may have limited transmission. Therefore, ***making*** a valid assumption regarding the average incidence over time and across sites in order to assess the efficacy of an investigational vaccine ***is important***.

In addition, a critical factor for the success of an efficacy trial is training staff at the various sites to perform study procedures such as febrile illness detection, dengue laboratory diagnosis and data collection in a cohort of subjects.

The proposed study will estimate the incidence of dengue infection and disease across geographically distinct locations over time in order to validate attack rate assumptions in future efficacy trials. We will enrol individuals from a random sample of households from selected communities of geographically different sites in Brazil and assess incidence of dengue infection and disease, by conducting scheduled serosurveys and an enhanced surveillance of febrile disease. The study will be conducted in at least three cities.

This study will also prepare potential sites for future clinical trials by setting up the logistics and training staff on site to enrol a cohort of subjects, perform dengue surveillance and other study procedures.

Finally, in line with the overarching strategy of building a strong partnership with the Ministry of Health (MoH), this study will serve as a useful comparison for evaluation of dengue surveillance systems.

Objectives

Primary

- To estimate the incidence of laboratory-confirmed symptomatic dengue infection in the study population by year/season.

Secondary

- To estimate the serotype-specific incidence of virologically-confirmed symptomatic dengue infection in the study population overall and by season.
- To estimate the incidence of symptomatic dengue infection (including laboratory-confirmed and probable cases) in the study population by study site, gender, age-group, previous dengue exposure (primary or secondary), overall and by season.
- To estimate the prevalence of previous dengue infection (dengue seroprevalence) in the study population overall and by study site, gender and age-group at enrolment.
- To estimate the incidence of primary inapparent, dengue infection, in the study population overall and by study site, gender age-group and by season.
- To describe symptoms and spectrum of dengue disease in the study population.

Tertiary (optional) objectives (Amended 16 October 2017)

- To investigate potential risk factors for symptomatic dengue infection.
- To investigate the role of dengue neutralizing antibodies elicited through natural infection in protection against subsequent infection.
- To estimate the degree of underreporting of dengue cases using the passive national notification surveillance.
- ***To estimate the incidence of symptomatic Zika virus infection (including laboratory-confirmed and probable cases) in the study population by study site, gender, age-group, previous flavivirus exposure, overall and by season.***
- ***To estimate the incidence of symptomatic chikungunya virus infection (including laboratory-confirmed and probable cases) in the study population by study site, gender, age-group, overall, and by season.***
- To describe previous Yellow Fever (YF) antibody profile in selected dengue cases to investigate the role of previous YF immunity on the dengue neutralizing antibody profile following dengue infection.

- To describe the spatial and temporal distribution of dengue cases among cohort participants in the study areas.
- ***To describe the spatial and temporal distribution of Zika and chikungunya cases among cohort participants in the study areas.***
- ***To describe other infectious aetiologies related to differential diagnosis of dengue (chikungunya and Zika) in subjects with episodes of febrile illness referred to as "suspected dengue case".***
- ***To estimate the seroprevalence of antibodies against Zika and chikungunya virus at selected timepoints.***

Study design

- **Type of design:** Prospective, multicenter, community-based, household-sampling, cohort study in Brazil.
- **Study population:** Subjects six months of age and older at the time of enrolment who live in the selected study sites in Brazil, covering different regions of the country.
- There will be 2 waves of enrolment for recruiting 3600 subjects:
  - Initial Cohort = about 1800 subjects – up to 6 visits (Amendment 2) – INITIAL sites
  - Expansion Cohort = about 1800 subjects – up to 4 visits – NEW sites
- The study period, initially planned to be one year, has been extended by additional three years (overall 4 years) to cover three additional dengue seasons (Amendment 2).
- Initial cohort subjects were invited to extend their participation.

  (See glossary of terms for the definition of initial and expansion cohort subjects)

  In each cohort: replacement subjects may be enrolled in order to compensate for subjects who did not wish to extend their participation for three additional years (for subjects from the initial cohort – Amendment 2), subjects who prematurely terminate participation or subjects who are lost to follow-up. This will be done to maintain a cohort size of at least 500 subjects per site, in the Initial and Expansion cohorts, at the beginning of each additional study year/season.

  At the end of the penultimate year of the study, the cohort size will be reviewed to address the potential need for

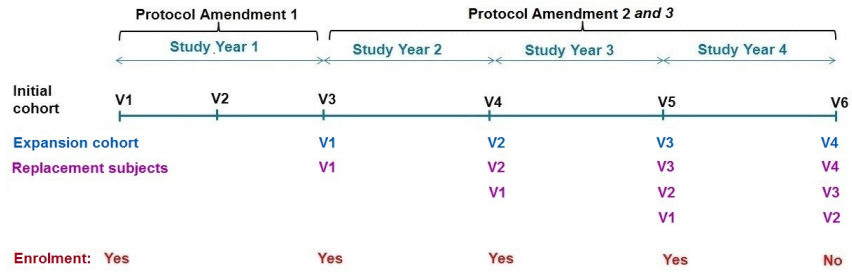
replacement cohort recruitment.

(See glossary of terms for the definition of replacement subjects, subjects who prematurely terminate participation and subjects lost to follow-up)

- Informed consent (and assent, if applicable) will be obtained:
  - From the subject (and parents/LAR of the subjects, if applicable) at the study start for the initial cohort.
  - From the subject (and parents/LAR of the subjects, if applicable) of the initial cohort prior to participation in the additional three years of the study.
  - From the subject (and parents/LAR of the subjects, if applicable) at the study start for the expansion cohort.
  - From the subject (and parents/LAR of the subjects, if applicable) prior to enrolment in the study for the replacement subjects.
- **Study visits:** The visits will be as follows and shown in Synopsis Figure 1:
  - Initial cohort subjects consenting to continue participation for the three additional years will have:
    - three scheduled visits in the first year (Visit 1, Visit 2 and Visit 3) at six months intervals ***(+/- 28 days)***,
    - and thereafter, one scheduled visit per year during a period of low dengue transmission for the three additional years; i.e; one visit at study Year 2, Year 3 and Year 4 (Visit 4, Visit 5 and Visit 6 respectively).
  - Initial cohort subjects who do not consent to extend participation for three additional years will:
    - have three scheduled visits (Visit 1, Visit 2 and Visit 3) at six months interval ***(+/- 28 days)***.
    - conclude their participation in the study at Visit 3 or the last follow up visit if the subject is suspected of having a dengue case on-going at Visit 3.
  - Subjects from the expansion cohort will have only three years of follow-up, with at most 4 scheduled visits.
  - For replacement subjects, the number of scheduled visits will depend on the study year in which they are enrolled. Replacement subjects will not be enrolled until after the third visit of the first study year is concluded for the initial cohort. The number of visits for the replacement subjects will range between two and four.
    The yearly scheduled visits for the initial and expansion cohorts and replacement subjects will preferably occur

during the period of usually low dengue transmission.

Synopsis Figure 1 Schematic representation of the scheduled visits



V= Visit
The scheduled visits for initial cohort subjects are shown in black.
New enrolments will be done yearly (as needed) till the end of Study Year 3.
Enrollment of expansion subjects will start preferably during study Year 2.
For replacement subjects, the number of visits will depend on the year enrolled.

- **Suspected dengue, *chikungunya and/or Zika* case detection (Amended 16 October 2017):** Suspected dengue, ***Zika and/or chikungunya*** cases will be detected using three sources:

  1. referred by study personnel during scheduled home visits;
  2. through enhanced passive surveillance; and
  3. as a result of active surveillance between scheduled visits; (see Section 5.5.3 for details).

- If dengue, ***Zika and/or chikungunya*** is/***are*** suspected, a medical appointment with a physician at a designated study hospital/clinic will be arranged (see Section 5.6 for management of suspected dengue, ***Zika and chikungunya*** cases).

- If the suspicion is confirmed during the visit:

i. A blood sample for dengue, ***Zika and/or chikungunya*** diagnosis and for haematology (CBC and HCT) ***(mandated procedure for subjects presenting within the first 14 days following onset of fever and/or rash)*** will be collected at the first visit for suspected dengue, ***Zika and/or chikungunya*** (acute blood sample) and

ii. a second blood sample (convalescent blood sample) will be collected approximately 21 (maximum 28 days) days later.

iii. In between, a return visit may be scheduled as needed.

- **Data collection:** eCRF
- **Type of study:** self-contained

- **Duration of the study:** Four years for the overall study. For an individual subject, it will range between one and four years.
  - **Epoch 001:** prospective data collection starting at Visit 1 Day 0 and ending at the last subject last visit.

Synopsis Table 1 Study group and epoch foreseen in the study

Study Group	Number of subjects*	Age (Min/Max)	Epoch
Prospective study cohort	Initial Cohort: 1800 at enrolment (first year) At least 1500 in each subsequent year	6 months of age and older	Epoch 001
	Expansion Cohort: 1800 at enrolment (first year). At least 1500 in each subsequent year		

* The final number of participants will take into account the active subjects, subjects who prematurely terminate participation and subjects lost to follow-up.

Discussion of study design

Selection of study sites

At least six sites in Brazil will be selected for this study. Selection criteria include support from the Family Health Physician Program (FHP), the Larval Index Rapid Assay (LIRA) or with field research experience in the community. The FHP is a Brazilian government public health strategy that consists of multidisciplinary teams of health care workers that cover geographically defined areas to provide basic/preventive care to community members. The teams include a doctor, a nurse, a nurse auxiliary, other health professionals and community health care workers who pay regular visits to the household under its responsibility to deliver health promotion activities and facilitate triage and referral into the health unit, among other activities. Community health care workers are local residents who are trained for basic tasks.

LIRA is an alternative vector surveillance strategy that consists of random sampling of a number of dwellings in which surveillance of aedes-positive breeding places is carried out, and has been widely implemented in Brazil (Pontes, 2000).

At least one community of households within each site will be selected.

Number of subjects

Approximately 3600 subjects will be recruited in the study in 2 waves of enrolment. Each wave, initial and expansion cohorts, will enrol about 1800 subjects. Replacement subjects may be recruited afterwards to compensate for subjects not willing to extend participation for the three additional years, subjects who prematurely terminate participation and subjects lost to follow-up. The recruitment of replacement subject aims at maintaining the number of subjects at a minimum of 500 subjects/site at the beginning of study Year 2, 3 and 4. The total number of subjects enrolled in the study may exceed 3600.

In order to reach the Brazilian population distribution, subjects enrolled in the expansion cohort should be composed of at least 30% of subjects younger than 18 years old and should not exceed 20 % of adults 50 years or older. Subjects enrolled as replacement subjects for the initial and expansion cohort will be recruited to approach as much as possible a minimal representation of 30% of children and a maximal representation of 20% for adults 50 years or older.

Endpoints

Primary

- Laboratory-confirmed symptomatic dengue infection (all DENV types)

Symptomatic dengue infection is defined in Section 5.7.2

Secondary

- DENV-type specific primary laboratory-confirmed symptomatic dengue infection
- DENV-type specific secondary laboratory-confirmed symptomatic dengue infection
- Primary symptomatic dengue infection (including laboratory-confirmed and probable cases)
- Secondary symptomatic dengue infection (including laboratory-confirmed and probable cases)
- Previous dengue infection(s) (dengue seroprevalence) at baseline. (defined in Section 5.7.5)
- Primary inapparent dengue infection
- Severity of symptoms of symptomatic dengue (using the 2009 WHO guidelines)

Tertiary (optional) (Amended 16 October 2017)

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

- ***Symptomatic laboratory-confirmed Zika virus infection***
- ***Symptomatic Zika virus infection (including laboratory-confirmed and probable cases).***
- ***Symptomatic laboratory-confirmed chikungunya infection***
- ***Symptomatic chikungunya infection (including laboratory-confirmed and probable cases).***
- Risk factors for dengue infection and disease
- Neutralizing antibodies titers against DENV 1-4
- Neutralizing antibody titers against YF virus
- Spatial and temporal distribution of dengue cases (confirmed and probable symptomatic cases and inapparent infections)
- ***Spatial and temporal distribution of laboratory confirmed and probable Zika virus cases***
- ***Spatial and temporal distribution of patients with laboratory confirmed and probable chikungunya virus infection***
- ***Occurrence of laboratory confirmed or probable Zika and/or chikungunya infection in suspected dengue cases (differential diagnosis of dengue), retrospectively***
- ***Antibody titre against Zika and chikungunya virus at the scheduled visits***

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LIST OF ABBREVIATIONS

ATP	According-To-Protocol
CBC	Complete blood count
CI	Confidence interval
CRADA	Cooperative Research and Development Agreement
DENV	Dengue virus
eCRF	electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
FHP	Family Health Physician Program
Fiocruz	Fundação Oswaldo Cruz
GCP	Good Clinical Practice
GSK	GlaxoSmithKline
HCT	Hematocrit
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IgG	Immunoglobulin type G
IgM	Immunoglobulin type M
IRB	Institutional Review Board
LIRA	Larval Index Rapid Assay
LAR	Legally Acceptable Representative
MoH	Ministry of Health
NS1	Non Structural 1
RDE	Remote Data Entry
RT-qPCR	Reverse Transcriptase quantitative Polymerase Chain Reaction
SAE	Serious Adverse Event
SDV	Source Document Verification
SPM	Study Procedures Manual
WHO	World Health Organization
YF	Yellow Fever

GLOSSARY OF TERMS

Anonymization:	Information that identifies a specific individual (including, but not limited to name, address and national identification number such as social security number, date of birth) has been removed and no link to the donor, through a code number for example, is maintained.
Block	A group of dwellings (buildings or residential units); dwellings may include one or more households. Blocks are used as sampling units in the LIRA strategy and serve as unit for spatial analysis.
Child in care:	A child who has been placed under the control or protection of an agency, organization, 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:	Information is associated with a subject number i.e. a code number. Coded information can only be linked back to the individual via a key code i.e. a listing of the research participants and their code. Within the pharmaceutical industry coding data is the usual mechanism used for protecting an individual's research data. The key code is kept secure, usually by the investigator, and GSK researchers cannot identify the research individual other than in exceptional and controlled circumstances.
Cohort study:	A form of epidemiology 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).

Diary log:	A diary log will be used in this study. The diary log is given to subjects to record body temperatures and symptoms in the event that a suspected dengue, ***Zika and/or chikungunya*** symptom occurs (see Section 5.7.1.1). The information collected in the diary log will be provided to the study physician as a tool in the medical evaluation of the subject. If the physician considers that the subject meets the criteria for suspected dengue, ***Zika and/or chikungunya*** he/she will use the information contained in the diary log to complete the description of first clinical symptoms in the eCRF.
Early presenter	A suspected dengue, ***Zika and/or chikungunya*** case presenting at the health care facility within 5 days following the onset of fever ***and/or rash (for Zika virus)***.
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
Epidemiology study:	An observational study or an interventional study without administration of medicinal product(s) as described in a research protocol.
Epoch:	An epoch is a well-defined part of a protocol that covers a set of consecutive time points. Generally, an epoch is self-contained and allows to perform a data analysis to address some of the trial objectives (e.g. primary, booster, yearly follow-ups, retrospective data collection, prospective data collection).
eTrack:	GSK's tracking tool for clinical/epidemiology trials.
Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, and, therefore, included in the according-to-protocol (ATP) analysis (see Section 8.3 for details on criteria for evaluability).
Expansion cohort subjects	Subjects recruited in the additional sites for study expansion
Initial cohort subjects	Subjects enrolled at the start of the study.
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.

Late presenter	A suspected dengue, ***Zika and/or chikungunya*** case presenting at the health care facility 6 to 30 days after the onset of fever ***and/or rash (for Zika virus).***
Lost to follow-up	A subject ***who*** moved outside study area or cannot be reached after several attempts and cannot perform the scheduled visit within the low dengue season period.
Prematurely terminate participation	A subject who is withdrawn from the study by the investigator or who decides to stop participation before the time he/she had to be in the study.
Primary dengue infection	The first dengue infection experienced by a subject is qualified as primary while subsequent infections (second, third or fourth) are qualified as secondary.
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 endpoints
Replacement subjects	Subjects ***who*** will be enrolled to compensate for subjects: • from ***the*** initial cohort: who will not want to extend participation for three additional years or • from ***the*** initial and expansion cohorts: who prematurely terminate participation or subjects lost to follow-up.
Research protocol:	A document that describes the objective(s), design, methodology, statistical considerations, and organization of a trial. The protocol usually also gives the background and rationale for the trial, but these could be provided in other protocol referenced documents
Seroconversion:	***Documented positive antibody test with previous documented negative antibody test.***
Site Monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of clinical/ epidemiology studies at one or more investigational sites
Study population:	***Enrolled members of a*** sample of ***the*** population of interest

Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the clinical/ epidemiology study, or a person about whom some medical information has been recorded in a database
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study
Surveillance:	Surveillance is defined as the on-going 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 (Amended 16 October 2017)

Dengue, the most common arthropod-borne viral disease worldwide, is caused by four types of dengue viruses (DENV 1-4), transmitted primarily by *Aedes aegypti*, a mosquito that is highly adapted to urban environments. Dengue infection can cause a range of clinical illnesses, from inapparent to a life threatening hemorrhagic disease, often associated with pre-existing heterotypic dengue virus antibodies.

An estimated 2.5 billion people are at risk of infection in tropical and subtropical countries worldwide (WHO, 2009) where major urban epidemics are responsible for substantial social and economic burden. Dengue has drastically increased in the last two decades in Central and South America with Brazil being one of the most affected countries (Siqueira-Junior, 2008). In Central Brazil, an almost 20-fold increase in reported cases of dengue have been reported from 1990 (1,660 cases) to 2000 (20,552 cases) (Siqueira, 2004). In all of Brazil, the number of yearly reported cases increased from 124,827 in 1995 to more than 1 million in 2010 (PAHO, 2011)

Currently, strategies to reduce disease burden rely mainly on mosquito control and human preventive behaviour modification, as treatment is limited to supportive care. The World Health Organization (WHO) has, therefore, considered the development of a vaccine a priority research area (Brandt, 1990), and a number of vaccine candidates are under development or being tested in clinical trials (Thomas, 2011).

In preparation for phase III trials, the WHO recommends that the primary efficacy endpoint be the presence of DENV in a patient with signs and/or symptoms of dengue disease (WHO, 2009; Edelman, 2008). However, mild cases of dengue are largely underreported, as shown in dengue cohort studies and capture-recapture studies (Wichmann, 2011; Vong, 2011; Suaya, 2007). For example, in Nicaragua an active dengue surveillance system in children was able to detect approximately 14 to 28 (average 21.3) times more dengue cases each year per 100,000 persons than passive surveillance among similar paediatric populations (Standish, 2010).

Zika is a flavivirus and chikungunya virus is a member of the alphavirus genus, and Togaviridae family. These are emerging mosquito-borne viral diseases in Latin America. Zika virus infections have been laboratory confirmed in 19 states in Brazil between April and December 2016 [Boletim Epidemiológico, 2017]. In 2016, until epidemiological week 52, 215,319 cases were reported and 130,701 were confirmed [Boletim Epidemiológico, 2017].

In 2015, 20,661 autochthonous suspected chikungunya cases were reported in Brazil [Boletim Epidemiológico, 2017]. In 2016, 271,824 reported cases and 151,318 were confirmed up to epidemiological week 47 [Boletim Epidemiológico, 2017]. Zika and chikungunya viruses cause symptoms often similar to dengue during the acute phase of illness.

Another challenge when conducting vaccine trials is to select geographic settings where the incidence of dengue would allow for a reliable assessment of vaccine efficacy. ***Endemicity for more than one dengue virus type is also*** highly desirable. Thus, selection of geographically diverse sites can potentially maximize the likelihood of having a sufficient number of exposed individuals for the conduct of a vaccine trial. Finally, ***identifying*** all other coexisting flaviviruses circulating at potential study sites ***is important***, as infection by such viruses might, in theory, modulate immune response and clinical course of dengue infection.

In this study, we will estimate the incidence of dengue infection and disease in a cohort of subjects recruited from different geographic areas over time in Brazil. The study will also identify and train potential sites for the conduct of phase III studies in the future.

1.2. Rationale for the study

The estimation of dengue incidence is critical when designing an efficacy trial for a dengue vaccine. In a given city or region, dengue incidence is highly variable from one year to another (variations in geography, season, year-to-year variations in mosquito vector populations in different areas, etc.,) and cannot yet be predicted with sufficient accuracy in many locations being considered for clinical trials. In a dengue vaccine efficacy trial, volunteers are recruited from geographically defined sites. Some of these sites may experience high dengue transmission during the trial duration while others may have limited transmission. Therefore, making a valid assumption regarding the average incidence over time and across sites in order to assess the efficacy of an investigational vaccine is important.

In addition, a critical factor for the success of an efficacy trial is training staff at the various sites to perform study procedures such as febrile illness detection, dengue laboratory diagnosis and data collection in a cohort of subjects.

The proposed study will estimate the incidence of dengue infection and disease across geographically distinct locations in order to validate attack rate assumptions in future efficacy trials. We will enrol individuals from a random sample of households from selected communities of geographically different sites in Brazil and assess incidence of dengue infection and disease, by conducting scheduled serosurveys and an enhanced surveillance of febrile disease.

This study will also prepare potential sites for future clinical trials by setting up the logistics and training staff on site to enrol a cohort of subjects, perform dengue surveillance and other study procedures.

Finally, in line with the overarching strategy of building a strong partnership with the Ministry of Health (MoH), this study will serve as a useful comparison for evaluation of dengue surveillance systems

2. OBJECTIVES

2.1. Primary objective

- To estimate the incidence of laboratory-confirmed symptomatic dengue infection in the study population by year/season.

Refer to Section 8.1.1 for the primary endpoint.

2.2. Secondary objectives

- To estimate the serotype-specific incidence of virologically-confirmed symptomatic dengue infection in the study population overall and by season.
- To estimate the incidence of symptomatic dengue infection (including laboratory-confirmed and probable cases) in the study population by study site, gender, age-group, previous dengue exposure (primary or secondary), overall and by season.
- To estimate the prevalence of previous dengue infection (dengue seroprevalence) in the study population overall and by study site, gender and age-group at enrolment.
- To estimate the incidence of primary inapparent, dengue infection, in the study population overall and by study site, gender age-group and by season.
- To describe symptoms and spectrum of dengue disease in the study population.

Refer to Section 8.1.2 for the secondary endpoints.

2.3. Tertiary objectives (optional) (Amended 16 October 2017)

- To investigate potential risk factors for symptomatic dengue infection.
- To investigate the role of dengue neutralizing antibodies elicited through natural infection in protection against subsequent infection.
- To estimate the degree of underreporting of dengue cases using the passive national notification surveillance.
- ***To estimate the incidence of symptomatic Zika virus infection (including laboratory-confirmed and probable cases) in the study population by study site, gender, age-group, previous flavivirus exposure, overall and by season.***
- ***To estimate the incidence of symptomatic chikungunya virus infection (including laboratory-confirmed and probable cases) in the study population by study site, gender, age-group, overall, and by season.***
- To describe previous Yellow Fever (YF) antibody profile in selected dengue cases to investigate the role of previous YF immunity on the dengue neutralizing antibody profile following dengue infection.
- To describe the spatial and temporal distribution of dengue cases among cohort participants in the study areas.

- ***To describe the spatial and temporal distribution of Zika and chikungunya cases among cohort participants in the study areas.***
- ***To describe other infectious aetiologies related to differential diagnosis of dengue (chikungunya and Zika) in subjects with episodes of febrile illness referred to as "suspected dengue case".***
- ***To estimate the seroprevalence of antibodies against Zika and chikungunya virus at selected timepoints.***

Refer to Section 8.1.3 for tertiary endpoints

3. STUDY DESIGN OVERVIEW (AMENDED 16 OCTOBER 2017)

- **Type of design**: Prospective, multicenter, community-based, household-sampling, cohort study in Brazil.
- The study period, initially planned to be one year, has been extended by additional three years (overall four years) to cover three additional dengue seasons (Amendment 2).
- Initial cohort subjects were invited to extend their participation.
- (See glossary of terms for the definition of initial and expansion cohort subjects).
- In each cohort: replacement subjects may be enrolled in order to compensate for subjects who did not wish to extend their participation for three additional years (for subjects from the initial cohort – Amendment 2), subjects who prematurely terminate participation or subjects who are lost to follow-up.

  This will be done to maintain a cohort size of at least 500 subjects per site, in the Initial and Expansion cohorts, at the beginning of each additional study year/season.

  In last three months of the of penultimate year of the study, each site will review the number of active subjects and drop-out rate. If the risk of having fewer than the required number of active participants at the end of the study is high, recruitment for participants into the replacement cohort may be performed with sponsor approval. If the risk is high, recruitment into the replacement cohort should begin even if the number of active subjects is 500 or above 500.

  (See glossary of terms for the definition of replacement subjects, subjects lost to follow-up and subjects who prematurely terminate participation).
- Informed consent (and assent, if applicable) will be obtained:
  - From the subject (and parents/LAR of the subjects, if applicable) at the study start for the initial cohort.
  - From the subject (and parents/LAR of the subjects, if applicable) of the initial cohort prior to participation in the additional three years of the study.
  - From the subject (and parents/LAR of the subjects, if applicable) at the study start for the expansion cohort.

- From the subject (and parents/LAR of the subjects, if applicable) prior to enrolment in the study for the replacement subjects.

- **Study population:** Subjects six months of age and older at the time of enrolment, who either live in households in study areas with support from the Family Health Physician Program (FHP) or the Larval Index Rapid Assay (LIRA) or with field research experience in the community (preferred) or where a similar system of mapped communities with potential for surveillance exists.

- There will be 2 waves of enrolment for recruiting 3600 subjects:
  - Initial Cohort = about 1800 subjects – up to 6 visits (Amendment 2) – INITIAL sites
  - Expansion Cohort = about 1800 subjects – up to 4 visits – NEW sites

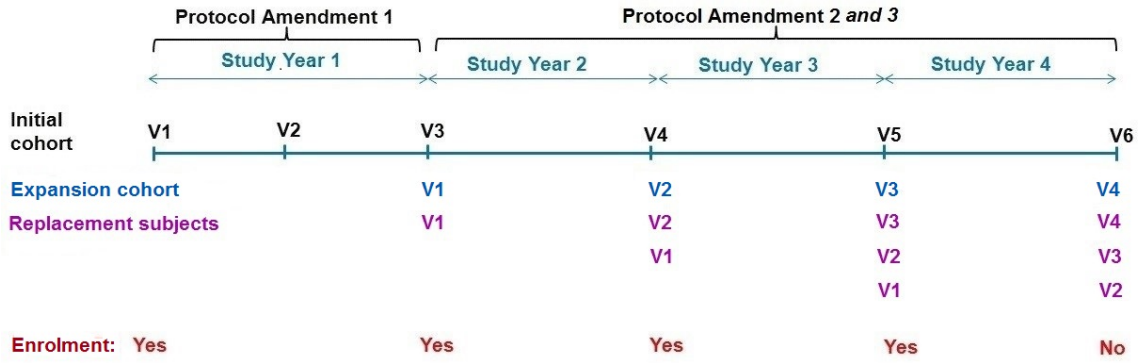
- **Study visits:** The visits will be as follows and further shown in Figure 1:
  - Initial cohort subjects consenting to extend participation for the three additional years will have:
    - three scheduled visits in the first year (Visit 1, Visit 2 and Visit 3) at approximately six months intervals ***(+/- 28 days)***,
    - and thereafter, one scheduled visit per year preferably during a period of low dengue transmission for the three additional years; i.e., one visit at study Year 2, Year 3 and Year 4 (Visit 4, Visit 5 and Visit 6 respectively).
  - Initial cohort subjects who do not consent to extend participation for three additional years will:
    - have three scheduled visits (Visit 1, Visit 2 and Visit 3) at approximately six months intervals ***(+/- 28 days)***.
    - conclude their participation in the study at Visit 3 or the last follow up visit if the subject is suspected of having a dengue case on-going at Visit 3.
  - Subjects from the expansion cohort will have only three years of follow-up, with at most 4 scheduled visits.
  - For replacement subjects, the number of scheduled visits will depend on the study year in which they are enrolled. Replacement subjects will not be enrolled until after the third visit of the first study year is concluded for the initial cohort. The number of scheduled visits will range between two and four.

    The yearly scheduled visits for the initial and expansion cohorts and replacement subjects will preferably occur during the period of usually low dengue transmission.

    In last three months of the of penultimate year of the study, each site will review the number of active subjects and drop-out rate. If the risk of having fewer than the required number of active participants at the end of the study is high, recruitment for participants into the replacement cohort may be performed with sponsor approval. If the risk is high, recruitment into the

replacement cohort should begin even if the number of active subjects is 500 or above 500.

Figure 1 Schematic representation of the scheduled visits



V=Visit
The scheduled visits for initial cohort subjects are shown in black.
New enrolments will be done yearly (as needed) till the end of study Year 3.
Enrollment of expansion cohort subjects will start preferably during study Year 2
For replacement subjects, the number of visits will depend on the year enrolled.

- **Type of study**: self-contained
- **Data collection**: Electronic Case Report Form (eCRF)
- **Duration of the study**: Four years for the overall study. For an individual subject, it will range between one and four years.
  - **Epoch 001:** prospective data collection starting at Visit 1, Day 0 and ending at the last subject last visit .

Table 1 Study groups and epochs foreseen in the study

Study Group	Number of subjects*	Age (Minimum)	Epoch
Prospective study cohort	Initial Cohort: 1800 at enrolment (first year) At least 1500 in each subsequent year	6 months and older	Epoch 001
	Expansion Cohort: 1800 at enrolment (first year). At least 1500 in each subsequent year		

*The final number of participants will take into account the active subjects, subjects who prematurely terminate participation and subjects lost to follow up.

3.1. Discussion of study design

3.1.1. Selection of study sites

Selection of study sites

At least six sites covering different regions of the country will be selected for this study. Selection criteria of the sites include support from the Family Health Physician Program (FHP), the Larval Index Rapid Assay (LIRA) or with field research experience in the community. The FHP is a Brazilian government public health strategy that consists of multidisciplinary teams of health care workers that cover geographically defined areas to provide basic/preventive care to community members. The teams include a doctor, a nurse, a nurse auxiliary, and community health care workers who pay regular visits to the household under its responsibility to deliver health promotion activities and facilitate triage and referral into the health unit, among other activities. Community health care workers are local residents who are trained for basic tasks.

LIRA is an alternative vector surveillance strategy that consists of random sampling of a number of dwellings in which surveillance of *Aedes*-positive breeding places is carried out, and has been widely implemented in Brazil (Pontes, 2000).

At least one community of households within each site will be selected.

3.1.2. Rationale for study design

A single cohort study was chosen to estimate the incidence of dengue in the community. Data will be collected from a population of individuals aged 6 months and older for maximum four dengue seasons. Inclusion of individuals of various ages will further allow for a better understanding of disease dynamics, clinical spectrum, and validate attack rates for potential subgroups to be included in future dengue vaccine trials.

This study will use cluster (household) sampling to take into account the correlation that may exist within households. Individuals within a household are expected to have a more similar risk of infection compared to individuals from different households. For example, individuals living in the same household are more likely to share similar risk of being exposed to DENV infected mosquitoes. The sample size has been adjusted to account for this between-cluster variability.

A possible bias ***may arise from the*** non-response rate. ***To reduce the possibility of this potential*** bias***,*** visits ***will be scheduled*** on weekends or at times ***more convenient to the subject.*** Where applicable, FHP community health care workers will facilitate communication between study staff and participants.

4. STUDY POPULATION

Subjects six months of age and older at the time of enrolment who live in the selected study sites in Brazil, covering different regions of the country.

4.1. Selection of communities

This study plan*s* to enrol a sample of subjects from randomly selected households from different communities within the selected sites. Community selection is based on the following characteristics:

- preferably areas where the Brazilian FHP or LIRA has been fully implemented or where access to the community is already established by a government program, e.g., registry of families by Secretary of Health, or other institutional program, such as university, in order to allow for random sampling of households;
- safe access by study personnel;
- high population density; and
- low migration rate.

4.2. Selection of households

Households will be randomly selected through LIRA strategy or from a list provided by the FHP or equivalent.

The FHP or LIRA will provide an exhaustive list of households to the study sites and will serve as the liaison between the study personnel and the household, and will not have any other role in this study.

Sites with access to LIRA will use LIRA's specific sampling strategy. LIRA's sampling strategy (preferred strategy) consists of dividing the administrative neighbourhoods of the city into blocks in which each building is enumerated by a unique code number. Within each block a corner is chosen and moving leftward, one in every four houses is systematically selected for vector inspection.

Where LIRA is not implemented but has FHP coverage, a random sample of households will be selected from the FHP database.

Where another type of household registry offers the only access to the community, a random sample will be drawn from the registry database.

Refer to Section 8.2 for a detailed description of the criteria used in the estimation of sample size.

4.3. Overview of the recruitment plan

At least 3600 subjects will be recruited in the study in 2 waves of enrolment. Each wave, initial and expansion cohorts, will enrol about 600 subjects/site in order to maintain at least 500 subjects/site at the beginning of each dengue season.

Subjects will be recruited from randomly selected households originating from pre-selected mapped communities. Preferably the recruitment period will occur outside of the peak dengue transmission season, and will continue until each site has reached its foreseen target. The expected period for recruiting the target sample size is approximately three months. Recruitment of replacement subjects will be done during the low dengue transmission and the recruitment period will depend on the number of subjects that need to be replaced.

Recruitment will be organized by study staff at participating sites according to the appropriate strategy for each site. Community agents or equivalent will serve as the liaison to schedule visits and may accompany study staff during the visits. The study will be explained to the individuals living in the household, and if any individual is interested in study participation, informed consent (and assent when applicable) will be obtained, eligibility criteria will be checked and subjects will be enrolled and interviewed for potential study participation.

If the target household is found empty or all members refuse to participate, the first household to the left will be approached. Those households refusing to participate will be recorded as such. A household refusal will be characterized when all individuals in the household refuse to participate in the study. Individual refusals in a given household will not preclude inclusion of other ***individuals living in the households***, and will be recorded as such.

In the subsequent study years/seasons, the cohort size will be maintained at ***a minimum of*** 500 subjects per site. Since subjects who prematurely terminate participation and/or lost to follow-up will be replaced, the final number of participants across the four study years may exceed 3600 subjects. Yearly, there will be an evaluation of active subjects. If the number of active subjects/site becomes < 500, there will be enrolment of replacement subjects to maintain a cohort size of at least 500 subjects/site. Enrolment will preferably take place during the low dengue transmission. Recruitment of replacement subjects could occur at the end of study year 1, 2 or 3. The recruitment approach will be the same as for the initial enrolment.

In order to better represent the Brazilian population distribution, subjects enrolled in the expansion cohort should be composed of at least 30% of subjects younger than 18 years old and should not exceed 20 % of adults 50 years or older. Subjects enrolled as replacement subjects for the initial and expansion cohort will be recruited to approach as much as possible a minimal representation of 30% of subjects younger than 18 years old and a maximal representation of 20% for adults 50 years or older.

Household visits may be scheduled to occur during weekends or at convenient times (like evenings) if deemed necessary.

4.4. Inclusion criteria for enrolment (Amended 16 October 2017)

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

- Written, signed or thumb-printed informed consent (and assent when applicable) must be obtained from the subject or subject's parent(s)/LAR(s). If the subject/subject's parent(s)/LAR(s) are illiterate the consent form will be countersigned by a witness.
- Male or female at least 6 months of age at the time of enrolment.
- Subject and/or subject's parent(s)/LAR(s) who the study staff believes can comply with the requirements of the protocol (e.g., willingness to go to the hospital/clinic for visit/s if dengue, ***Zika and/or chikungunya*** is suspected, able to observe the signs of dengue, ***Zika and/or chikungunya*** and to understand how to take and report body temperature, etc.).
- Subject who plans, at the time of enrolment, to remain at same residence/study area during their study participation period.

4.5. Exclusion criteria for enrolment

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. (see the glossary for full definition)
- Participation (current or planned) in another epidemiological study or in a clinical trial that would conflict with the current study, based on investigator's judgement.
- Terminal illness or severe mental incapacity.

5. STUDY CONDUCT

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

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP), any applicable local guidelines, including the Document of the Americas, 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 Harmonized 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 from the appropriate regulatory agency, in accordance with applicable regulatory requirements, 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)/legally acceptable representative (LAR[s]) informed consent and subject informed assent, as appropriate
- Investigator reporting requirements as stated in the protocol.

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

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

GSK Biologicals will prepare a model Informed Consent Form (ICF) which will embody the applicable ICH GCP 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 Harmonized Tripartite Guidelines for Good Clinical Practice, those subjects who can only be enrolled in the study with the consent of the subject's parent(s)/LAR(s) (e.g. minors), should be informed about the study to the extent

compatible with the subject's understanding and, if capable, the subject should sign and personally date a written informed assent. ***It*** is required ***to*** be signed by each subject, if capable, in addition to the informed consent that is to be signed by his/her parent or legal representative. ***Requirement of assent*** should be assessed ***based*** on the age of the study population and the local requirements.

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

If a subject is unable to read or if a legally acceptable representative is unable to read, the informed consent will be obtained in the presence of an impartial witness, according to the International Conference on Harmonization (ICH) guidelines. For all participants unable to read for whatever reason, the subject's fingerprint must be obtained on the ICF.

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

5.2. Subject identification

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

5.3. General study aspects

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

5.4. Outline of study procedures

Study procedures are outlined in Table 2 and Table 3 for initial and expansion cohorts, respectively in Table 4 for replacement subjects.

Table 2 List of study procedures for initial cohort subjects (Amended 16 October 2017)

	Surveillance											Suspected Dengue/*Zika*/*Chikungunya* Visits (at hospital/clinic) as applicable		
Procedure	**Visit 1 Day 0**	**Monthly contact between visits**	**Visit 2 Month 6**	**Monthly contact between visits**	**Visit 3 ~ Month 12 (Year 1)**	**Monthly contact between visits**	**Visit 4 ~ Month 24 (Year 2)**	**Monthly contact between visits**	**Visit 5 ~ Month 36 (Year 3)**	**Monthly contact between visits**	**Visit 6 ~ Month 48 (Year 4)**	**First visit Day 0**	**Return Visit [2]**	**Follow-up visit Day 21**
Informed consent (and assent if applicable)	●													
Re-consent (and assent if applicable)[8]			●		●		●		●		●	●	●	●
Subject number and ***initial*** household number attribution	●													
Check inclusion/ exclusion criteria	●													
Record socio-demographic information ***or updates (including household characteristics if applicable)***	●						●		●		●			
Medical history ***including*** YF ***and dengue*** vaccination history or updates	●		●		●		●		●		●			
Distribute subject ID card distribution and suspected dengue instruction kit	O													
Blood sample for serology (5 mL)	●		●		●		●		●		●			
Instruct/remind subjects/subject's parent(s)/LAR(s) on suspected dengue, ***chikungunya and Zika*** assessment procedures (where applicable)	O	O	O	O	O	O	O	O	O	O				

	Surveillance											Suspected Dengue/*Zika*/*Chikungunya* Visits (at hospital/clinic) as applicable		
Procedure	**Visit 1 Day 0**	**Monthly contact between visits**	**Visit 2 Month 6**	**Monthly contact between visits**	**Visit 3 ~ Month 12 (Year 1)**	**Monthly contact between visits**	**Visit 4 ~ Month 24 (Year 2)**	**Monthly contact between visits**	**Visit 5 ~ Month 36 (Year 3)**	**Monthly contact between visits**	**Visit 6 ~ Month 48 (Year 4)**	**First visit Day 0**	**Return Visit** [2]	**Follow-up visit Day 21**
Issue diary logs to parents/LARs to be filled out in the event of a suspected dengue, ***chikungunya and Zika*** case[1]	O		O		O		O		O			O		
Contact subject regarding any dengue, ***chikungunya and Zika*** symptoms and remind subject of procedures for suspected dengue, ***chikungunya and Zika***		O		O		O		O		O				
Physical examination/record current medical conditions(see Section 5.6)												●	●	●
Blood sample for dengue/***zika/chikungunya*** infection ***differential*** diagnosis[3]												●		●
Blood sample for CBC and hematocrit ***(mandated for subjects presenting within 14 days following symptoms[6] onset)***												●		
Record body temperature												●		
Report SAEs related to study procedures[4]	●		●		●		●		●		●	●	●	●
Collect or verify diary logs if applicable												O	O	O
Study conclusion[5]											●			●

● is used to indicate a study procedure that requires documentation in the individual eCRF.
○ is used to indicate a study procedure that does not require documentation in the individual eCRF.
[1] Diary logs given at scheduled visits are only to be filled out in the event that the subject has fever ***and/or rash according to the case definition for*** suspected dengue***/chikungunya/Zika***. See Section 5.5.2.5 for details with regard to the diary log.
[2] A return visit is a visit linked to suspected dengue***/chikungunya/Zika***, occurring if the evolution of the subject's physical condition necessitates medical evaluation, according to local medical practice. The investigator will determine whether this return visit is necessary and whether it is considered as part of the same episode. See Section 5.6 for details.
[3] ***Blood sampling for dengue diagnosis which includes*** testing for RT-qPCR, dengue IgG and IgM ELISA, NS1 ELISA, neutralizing antibodies to dengue and ***virological/humoral***, ***testing for Zika and chikungunya infection***.
[4] SAE = Serious adverse event. See Section 6.1.1 for definition.
[5] The study conclusion information in the eCRF is collected once for each subject at the last scheduled visit or at the last follow-up contact for a suspected dengue***/zika/chikungunya*** case, should this case occur during the last scheduled visit or earlier if the subject terminates study participation or is lost to follow-up.
[6] ***Symptoms are fever for dengue, Zika and chikungunya, and rash for Zika.***
[8] ***The field staff will re-consent at the first opportunity***

Table 3 List of study procedures for expansion cohort subjects (Amended 16 October 2017)

	Surveillance							Suspected Dengue/*Zika/Chikungunya* Visits (at hospital/clinic) as applicable		
Procedure	**Visit 1 # Day 0**	**Monthly contact between visits**	**Visit 2**	**Monthly contact between visits**	**Visit 3**	**Monthly contact between visits**	**Visit 4**	**First visit Day 0**	**Return visit [2]**	**Follow-up visit21**
Informed consent (and assent if applicable)	●									
Re-consent (and assent if applicable)@			●		●		●	●	●	●
Subject number and ***initial*** household number attribution	●									
Check inclusion/ exclusion criteria	●									
Record socio-demographic information ***or updates (including household characteristics if applicable)***	●		●		●		●			
Medical history, ***including*** YF and ***dengue*** vaccination history or updates	●		●		●		●			
Distribute subject ID card distribution and suspected dengue instruction kit	O									
Blood sample for serology (5 mL)	●		●		●		●			
Instruct/remind subjects/subject's parent(s)/LAR(s) on suspected dengue, ***chikungunya and Zika assessments*** procedures (where applicable)	O	O	O	O	O	O				
Issue diary logs to parents/LARs to be filled out in the event of a suspected dengue, ***chikungunya and Zika*** case [1]	O		O		O			O		
Contact subject regarding any dengue ***chikungunya and Zika*** symptoms and remind subject of procedures for suspected dengue, ***chikungunya and Zika***		O		O		O				
Physical examination/record current medical conditions (see Section 5.6)								●	●	●

	Surveillance							Suspected Dengue/*Zika/Chikungunya* Visits (at hospital/clinic) as applicable		
Procedure	**Visit 1 # Day 0**	**Monthly contact between visits**	**Visit 2**	**Monthly contact between visits**	**Visit 3**	**Monthly contact between visits**	**Visit 4**	**First visit Day 0**	**Return visit [2]**	**Follow-up visit21**
Blood sample for dengue, ***Zika/chikungunya*** infection ***differential*** diagnosis[3]								●		●
Blood sample for CBC and hematocrit ***(mandated for subjects presenting within 14 days following symptoms[6] onset)***								●		
Record body temperature								●		
Report SAEs related to study procedures [4]	●		●		●		●	●	●	●
Collect or verify diary logs if applicable								O	O	O
Study conclusion [5]							●			●

● 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.
Enrolment of expansion cohort subjects will start preferably during study Year 2.
[1] Diary logs given at scheduled visits are only to be filled out in the event that the subject has fever ***and/or rash according to the case definition for*** suspected dengue/***chikungunya/Zika***.
[2] A return visit is a visit linked to suspected dengue/***Zika/chikungunya***, occurring if the evolution of the subject's physical condition necessitates medical evaluation, according to local medical practice. The investigator will determine whether this return visit is necessary and whether it is considered as part of the same episode. See Section 5.6 for details.
[3] ***Blood sampling for dengue diagnosis which includes*** testing for RT-qPCR, dengue IgG and IgM ELISA, NS1 ELISA, neutralizing antibodies to dengue and ***virological/humoral***, ***testing for Zika and chikungunya infection***.
[4] SAE = Serious adverse event. See Section 6.1.1 for definition.
[5] The study conclusion information in the eCRF is collected once for each subject at the last scheduled visit or at the last follow-up contact for a suspected dengue/***Zika/chikungunya*** case, should this case occur during the last scheduled visit or earlier if the subject terminates study participation or is lost to follow-up.
[6] ***Symptoms are fever for dengue, Zika and chikungunya, and rash for Zika.***
@ ***The field staff will re-consent at the first opportunity***

Table 4 List of study procedures for replacement subjects (Amended 16 October 2017)

	Surveillance							Suspected Dengue/*Zika*/*Chikungunya* Visits (at hospital/clinic) as applicable		
Procedure	**Visit 1 Day 0**	**Monthly contact Year 2**	**Visit 2**	**Monthly contact between visits**	**Visit 3 as applicable**	**Monthly contact between visits**	**Visit 4 as applicable**	**First visit Day 0**	**Return visit[2]**	**Follow-up visit Day 21**
Informed consent (and assent if applicable)	●									
Re-consent (and assent if applicable)@			●		●		●	●	●	●
Subject number and ***initial*** household number attribution	●									
Check inclusion/ exclusion criteria	●									
Record/ update socio-demographic information ***or updates (including household characteristics if applicable)***	●		●		●		●			
Medical history, ***including*** YF and ***dengue*** vaccination history or updates	●		●		●		●			
Distribute subject ID card distribution and suspected dengue instruction kit	O		O		O					
Blood sample for serology (5 mL)	●		●		●		●			
Instruct/remind subjects/subject's parent(s)/LAR(s) on suspected dengue, ***chikungunya and Zika*** assessment procedures (where applicable)	O	O	O	O	O	O				
Issue diary logs to parents/LARs to be filled out in the event of a suspected dengue***, chikungunya and Zika*** case [1]	O		O		O			O		
Contact subject regarding any dengue, ***chikungunya and Zika*** symptoms and remind subject of procedures for suspected dengue***, chikungunya and Zika***		O		O		O				
Physical examination/record current medical conditions (see Section 5.6)								●	●	●

	Surveillance							Suspected Dengue/*Zika/Chikungunya* Visits (at hospital/clinic) as applicable		
Procedure	**Visit 1 Day 0**	**Monthly contact Year 2**	**Visit 2**	**Monthly contact between visits**	**Visit 3 as applicable**	**Monthly contact between visits**	**Visit 4 as applicable**	**First visit Day 0**	**Return visit[2]**	**Follow-up visit Day 21**
Blood sample for dengue, ***Zika/chikungunya*** infection ***differential*** diagnosis[3]								●		●
Blood sample for CBC and hematocrit ***(mandated for subjects presenting within 14 days following symptoms[6] onset)***								●		
Record body temperature								●		
Report SAEs related to study procedures [4]	●		●		●		●	●	●	●
Collect or verify diary logs if applicable								O	O	O
Study conclusion [5]							●			●

● 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.
[1] Diary logs given at scheduled visits are only to be filled out in the event that the subject has a fever ***and/or rash according to the case definition for*** suspected dengue, ***Zika/chikungunya*** symptoms. See Section 5.5.2.5 for details with regard to the diary log.
[2] A return visit is a visit linked to suspected dengue***/Zika/chikungunya***, occurring if the evolution of the subject's physical condition necessitates medical evaluation, according to local medical practice. The investigator will determine whether this return visit is necessary and whether it is considered as part of the same episode. See Section 5.6 for details.
[3] ***Blood sampling for dengue diagnosis which includes*** testing for RT-qPCR, dengue IgG and IgM ELISA, NS1 ELISA, neutralizing antibodies to dengue and ***virological/humoral***, ***testing for Zika and chikungunya infection***.
[4] SAE = Serious adverse event. See Section 6.1.1 for definition.
[5] The study conclusion information in the eCRF is collected once for each subject at the last scheduled visit or at the last follow-up contact for a suspected dengue***/Zika/chikungunya*** case, should this case occur during the last scheduled visit or earlier if the subject terminates study participation or is lost to follow-up.
[6] ***Symptoms are fever for dengue, Zika and chikungunya, and rash for Zika.***
@ ***The field staff will re-consent at the first opportunity***

Table 5 Intervals between study visits/contacts for INITIAL cohort

Study Year*	Visits Interval	Optimal length of interval *	Allowed interval**
Year 1	Visit 1 (Day 0) → Visit 2 (Month 6)	6 months	± 28 days
	Visit 2 (Month 6) → Visit 3 (Month 12)	6 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	
Year 2	Visit X → Visit X+1¶ Note: Visit X ***is*** visit 3 for subjects enrolled in study year 1. For subjects enrolled in study year 2, visit X would be visit 1.	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	
Year 3	Visit X+1 → Visit X+2	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	
Year 4	Visit X → Visit X+3	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	

*Whenever possible the investigator should arrange study visits/contacts within this interval
**Subjects might not be eligible for inclusion in the ATP cohort for analysis if they make the study visit/contact outside this interval

Table 6 Intervals between study visits/contacts for EXPANSION cohort (Amended 16 October 2017)

Interval	Optimal length of interval*	Allowed interval**
Visit 1 (Day 0) → Visit 2 (Year 1)	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	±28 days
Visit 2 (Year 1) → Visit 3 (Year 2)	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	±28 days
Visit 3 (Year 2) → Visit 4 (Year 3)	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	±28 days

*Whenever possible the investigator should arrange study visits/contacts within this interval
**Subjects might not be eligible for inclusion in the ATP cohort for analysis if they make the study visit/contact outside this interval

Table 7 Interval between visits for suspected dengue/*chikungunya/Zika* case (Amended 16 October 2017)

	Optimal length of time	Allowed interval
Suspected dengue/***chikungunya/Zika*** case: Interval between first and follow-up visit	21 days	+ 7 days

Note that this interval applies to all subjects enrolled regardless of the year of enrolment.

5.5. Detailed description of study procedures

5.5.1. Procedures prior to study participation

See Section 4.2 for household selection

5.5.1.1. Informed consent

Before performing any other study procedure, the signed/thumb printed informed consent of the subject or subject's parent(s)/LAR(s) must be obtained. The signed/thumb printed assent form from any subject who is below the age of consent but at the age for assent should also be obtained in addition to the signed informed consent by his/her parent(s)/LAR(s) according to local rules and regulations. Refer to Section 5.1 for the requirements on how to obtain informed consent and assent, as appropriate.

- Initial cohort subjects will be required to have a new consent/assent (where applicable) form signed for continuous participation in the study to allow coverage of three additional dengue seasons.
- Consent/assent (where applicable) will also be obtained prior to participation for replacement subjects.

5.5.1.2. Check inclusion and exclusion criteria

Check all applicable inclusion and exclusion criteria as described in Sections 4.4 and 4.5 before enrolment.

5.5.2. Procedures at scheduled Visit 1 (enrolment visit) (Amended 16 October 2017)

Ideally, Visit 1 (the enrolment visit) will be scheduled before the peak incidence of dengue based on previous years.

A subject number and ***initial*** household number will be attributed at Visit 1.

5.5.2.1. Collect socio-demographic data

Socio-demographic data such as age, gender and household conditions will be recorded in the subject's source document for subsequent recording in the eCRF. The geographical location of the household (block) will also be recorded in the eCRF.

5.5.2.2. Collect specific medical history data (Amended 16 October 2017)

A baseline medical history, including history of dengue infection, ***Zika and chikungunya infection, and YF and dengue vaccination history will be taken.*** Results will be recorded in the subject's source document for subsequent recording in the eCRF.

YF ***and dengue*** vaccination status will be recorded, along with the source of the information (either written or oral record). A vaccination record, if available, is preferable but self-reported history will be recorded if the vaccination record is not available.

Information on medical history (e.g., diabetes, cardiovascular diseases, asthma, cancers, hematologic diseases, genetic disorders) will be collected.

5.5.2.3. Collect blood sample

- A blood sample for serology will be collected from all enrolled subjects (see Section 5.8 for details regarding sample collection). For children <2 years of age, the total amount of blood collected will not exceed 5 mL at any visit.

5.5.2.4. Instruction on enhanced passive dengue, chikungunya and Zika surveillance by the subject or subject's parent(s)/LAR(s) (Amended 16 October 2017)

Study personnel will train subjects/subject's parent(s)/LAR(s) to recognize dengue, ***Zika and chikungunya*** symptoms and will instruct them to contact the study personnel or come to a designated study hospital/clinic for medical evaluation within 5 days of the occurrence of ***fever and/or rash*** that may be associated with suspected dengue, ***chikungunya or Zika*** (defined in Section 5.7.1, 5.7.2 and 5.7.3).

Each household will also be given at least one dengue kit, as applicable, which includes thermometers, study contact information (phone numbers) and an instruction card with information about dengue, ***Zika and chikungunya*** symptoms***. This card will also provide instructions on who to contact and what to do if any of these infections are suspected.***

Participants or LAR will be instructed on how to take body temperature and how to record the temperatures in a diary log.

See Section 5.6 for details regarding management of suspected dengue, ***chikungunya and Zika*** cases.

5.5.2.5. Diary log distribution (Amended 16 October 2017)

A diary log will be given to all subjects in the household to be used in the event of suspected dengue***, chikungunya and/or Zika illness***. Diary logs will be distributed at the first scheduled visit and if they are needed, at subsequent visits (if the subject no longer has one, e.g., the previous one was lost or used). A new diary log will be issued by the study staff whenever necessary.

The subject will be instructed to start recording any symptoms***, including rash*** and body temperature ***at*** any time dengue, ***chikungunya and/or Zika infections are*** suspected. Refer to Section 5.6.1 for details.

5.5.2.6. Recording of serious adverse events related to a study procedure

The subject/subject's parent(s)/LAR(s) will be instructed to contact the study staff should any serious adverse event related to a study procedure occur during the study.

Refer to Section 6.2 for procedures for the Investigator to record SAEs that are related to study participation and for guidelines on how to report these SAEs to GSK Biologicals.

5.5.3. Procedures at subsequent scheduled visits (2-6, as applicable) (Amended 16 October 2017)

- For initial cohort subjects, Visit 2 will occur approximately 6 months after Visit 1 and Visit 3 approximately 6 months after Visit 2. Initial cohort subjects willing to extend their participation to three additional years will be re-consented at Visit 3 (i.e. at the end of study Year 1).
- For expansion cohort subjects, scheduled visits should ideally be performed at yearly intervals, preferably during periods of usually low dengue transmission. Expansion cohort subjects will have at most 4 scheduled visits, depending on the time of enrolment.
- For replacement subjects enrolled in the subsequent study years, scheduled visits should ideally be performed at yearly intervals, preferably during periods of usually low dengue transmission.
- A blood (serum) sample will be collected for serology.

  Note: Indirect DENV IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for DENV IgG at the previous scheduled visit.

  Zika IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for Zika IgG at the previous scheduled visit.

  Chikungunya IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for chikungunya IgG at the previous scheduled visit.

  Even if the tests are not repeated, the volume of the blood sample remains unchanged.
- ***Socio-demographic and household information will be updated in eCRF (for e.g. change of home location).***
- Medical and vaccination histories will be updated in the eCRF.
- The investigator will remind the subjects about the procedures in case of signs and symptoms of dengue, ***Zika and chikungunya***.

- A new diary log will be distributed for continued dengue, ***chikungunya and Zika*** surveillance if needed, and any completed diary logs will be verified, as applicable. Subjects will be asked to contact the study staff in the event of the occurrence of a symptom that may be associated with suspected dengue, ***chikungunya or Zika*** (see Section 5.7.1.1, 5.7.2 and 5.7.3 for suspected dengue, ***chikungunya or Zika*** definition).

- The subject will be instructed to start recording any symptoms, ***including rash*** and body temperature any time dengue***, chikungunya and/or Zika*** is suspected. Refer to Section 5.6.1 for details.

- Any SAEs related to study procedures will be recorded.

5.5.4. Study conclusion (Amended 16 October 2017)

The Study Conclusion screen page in the eCRF will be completed at the last study contact. This last contact could occur at the last scheduled visit or follow up visit for a suspected dengue***, chikungunya or Zika*** case (if it is on-going at the last scheduled visit) or earlier if the subject terminates study participation or is lost-to-follow-up.

The study staff will review data collected to ensure accuracy and completeness and will complete the Study Conclusion screen page 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. If so, the investigator will ask each subject/subject's parent(s)/LAR(s) if he or she would be willing to participate (or let their child participate) in a long-term follow-up study and subject will be asked to sign a new consent form.

5.5.5. Dengue, *chikungunya and Zika* case detection (Amended 16 October 2017)

Suspected cases (defined in Section 5.7.1, 5.7.2 and 5.7.3) in the study cohort may arise from three sources: 1) referred by study personnel during scheduled home visits; 2) through enhanced passive surveillance; and 3) as a result of active surveillance between scheduled visits.

5.5.5.1. Case detection during scheduled home visits (Amended 16 October 2017)

If a subject is identified as suspected case ***of dengue, chikungunya and/or Zika*** during any home visit, he or she will be referred to the designated study hospital/clinic for medical evaluation (refer to Section 5.6).

5.5.5.2. Case detection through enhanced passive surveillance (Amended 16 October 2017)

The subject/subject's parent(s)/LAR(s) will be instructed to contact the study staff (the local study coordinator) at any time dengue ***or chikungunya*** is suspected (i.e., body temperature ≥38°C measured (by any route) for at least two consecutive days) ***or if Zika is suspected (fever [i.e., body temperature ≥38°C measured (by any route) for at least two consecutive days] and/or rash)***.

The subject/subject's parent(s)/LAR(s) will be instructed to contact the hospital or study clinic should the subject manifest any signs or symptoms they/the subject's parent(s)/LAR(s) perceive as an emergency or severe.

The phone contact will be preferably made during working hours/days for logistical reasons. A reasonable schedule for phone contacts to the local study coordinator is from Monday to Saturday, 7:00 AM to 8:00 PM. ***In the case of an emergency outside of the defined hours, the patient should contact or present to their local emergency room, and contact the study staff point of contact as soon as convenient to the subject/subject's parent(s)/LAR(s).***

When a subject/subject's parent(s)/LAR(s) contacts the study staff, the local study coordinator will then arrange for an appointment at the designated study hospital/clinic. The subject/subject's parent(s)/LAR(s) will be instructed to record ***any symptom including rash and*** body temperature on the diary log daily until the appointment.

The visit should be arranged as soon as possible, preferably during week days for logistical reasons. If the subject contacts the study staff during the weekend, the visit should be scheduled for the following Monday. All efforts should be made to guarantee that the subject be seen by the fifth day of disease onset, at latest.

During holidays, arrangements will be made with study physicians and the subjects should be seen at the designated study hospital/clinic.

Although subjects will be instructed to contact the study staff in the event of suspected dengue***, chikungunya and/or Zika***, there may be cases where the subject is taken directly to the hospital or clinic. If this occurs when the study physician is not available, the staff at the designated hospital should notify the local study coordinator and the study physician.

The study staff should ensure that:

- the acute sample will be properly collected,
- all clinical information will be retrievable,
- a return visit (if applicable) will be scheduled
- and a follow-up visit will be scheduled.

See Section 5.6 for cases that present to a non-study hospital/clinic.

5.5.5.3. Case detection through active surveillance (Amended 16 October 2017)

Telephone calls (or home visits when applicable, if a phone call is not feasible) will be conducted at least monthly and more frequently if needed. During the phone call or visit, a structured script will be used to inquire about dengue, ***chikungunya and Zika*** symptoms since the last contact.

An example of questions to be included in the structured script is provided in APPENDIX B.

If dengue, ***chikungunya and/or Zika*** is suspected during active surveillance, an appointment will be arranged at the designated study hospital/clinic, and at least one additional visit will be required for case follow-up (see Section 5.6).

Subjects whose status cannot be ascertained during active surveillance will be tracked by the study personnel through an active tracking algorithm.

Figure 2 Active tracking algorithm

5.6. Management of suspected dengue, *chikungunya* and *Zika* cases (Amended 16 October 2017)

All study subjects with suspected dengue***, chikungunya and/or Zika*** should be seen at a designated study hospital or clinic by the study physician.

The first visit to the study hospital/clinic will include a detailed clinical examination to assess the subject's general condition, body temperature, height and weight, cardiac and respiratory rates, blood pressure, dengue, ***chikungunya and Zika*** associated clinical signs/symptoms and relevant clinical signs/symptoms (e.g., fatigue, headache, pain behind the eyes, abdominal pain, nausea, vomiting, muscle ache, joint ache, diffuse rash, photophobia and pruritus).

A blood sample should be collected by the hospital/clinic for laboratory confirmation of dengue***, chikungunya, Zika and differential diagnosis***. This sample is referred to as the 'acute' serum sample. In addition, a blood sample for haematology (CBC and HCT) will be collected ***(mandated procedure for subjects presenting within the first 14 days***

following onset of fever and/or rash). ***If blood chemistry is conducted according to local clinical practice, these data may also be collected.***

If dengue***, chikungunya or Zika*** is still suspected after the first visit to the study hospital/clinic, a return visit for suspected dengue***, chikungunya and/or Zika*** may be needed and will be conducted as directed by National guidelines by the study physician.

A return visit is a visit linked to suspected dengue, ***chikungunya and/or Zika***, occurring if the evolution of 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. If a return visit occurs, the diary log will be verified and possibly used by the physician to complete the clinical symptoms in the eCRF.

Medical data related to any suspected dengue***, chikungunya and/or Zika*** case will be collected for hospitalized study subjects and for those followed in the outpatient setting at the return visit. Treatment will be given according to local standard routines, following national guidelines.

Approximately 21(+7) days after the first visit for suspected dengue***, chikungunya and/or Zika***, a follow-up (convalescent) visit will be required. A physical examination will be conducted and a 'convalescent' serum sample (i.e., a blood sample collected after the acute phase for dengue***, chikungunya and/or Zika***) should be collected at this follow-up visit. Ideally, this collection should be done at the study hospital/clinic but may be done at the subject's home if directed by the study physician or delegated study staff, if the subject is unable to come to the hospital/ clinic.

Subjects who, for any reason, seek medical assistance at any non-study health care facility**,** will be identified through active surveillance, and clinical data will be retrospectively collected on the eCRF. The study physician will be responsible for collecting the retrospective data and informing the local study coordinator for appropriate follow-up.

5.6.1. Diary log instructions for suspected dengue*, chikungunya and/or Zika* cases (Amended 16 October 2017)

The subject/subject's parent(s)/LAR(s) will be instructed to record body temperature ***and rash*** on the diary log daily until the appointment. See Section 5.6 for subjects who do not reach the study staff and go directly to the hospital/clinic. The subject will be instructed to start recording any symptoms, ***including rash*** and body temperature any time dengue***, chikungunya and/or Zika virus infection*** is suspected.

The information collected in the diary log will be provided to the study physician as a tool in the medical evaluation of the subject. If the physician considers that the subject meets the criteria for suspected dengue***, chikungunya and/or Zika***, he could use the information contained in the diary log to complete the description of first clinical symptoms in the eCRF.

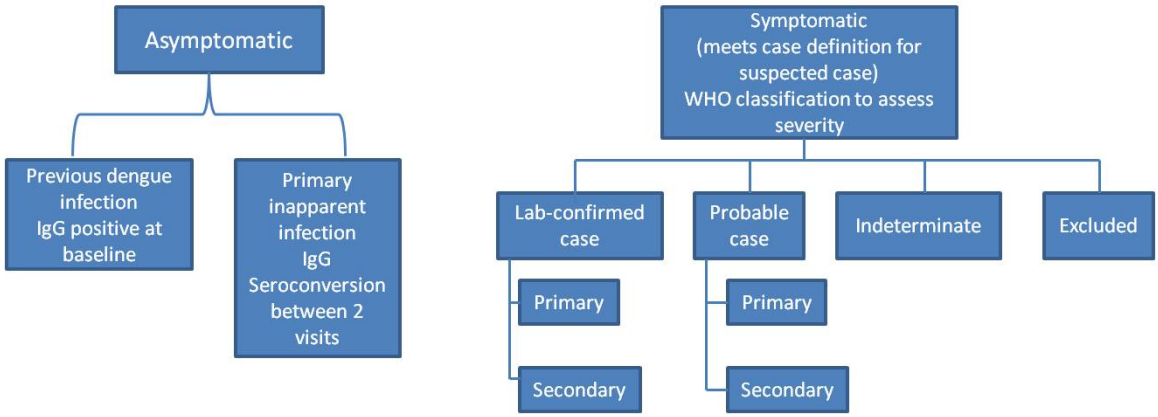
If dengue***, chikungunya and/or Zika*** is suspected, the diary log will be verified by the investigator or designee at the ***follow-up*** visit if the subject is not hospitalized. If the subject is hospitalized, information with regard to dengue, ***chikungunya and/or Zika*** infection will be recorded in the hospital record. Information from diary log and hospital records can be used to update clinical data in the eCRF.

5.7. Case definitions

5.7.1. Dengue

The following classification of dengue cases will be used in this study.

Figure 3 Classification of dengue cases



5.7.1.1. Suspected symptomatic dengue case (Amended 16 October 2017)

Febrile illness with body temperature ≥38°C measured (by any route) on at least two consecutive days and less than 14 days with or without the presence of other dengue symptoms or signs, without an obvious aetiology unrelated to dengue, based on investigator's judgement.

Although subjects are asked to come to a study hospital if fever (body temperature ≥ 38°C) ***is*** sustained for two consecutive days, a subject ***could*** present on the first day of fever. In this case the physician may still consider the subject as a suspected dengue case based on medical judgement and collect a blood sample for laboratory diagnosis.

Subjects should be examined at the health care facility during the 5 days following fever onset, as this is a requirement for RT-qPCR diagnosis of dengue infection and serotype identification.

However, subjects presenting until 30th (included) day of fever onset will be considered for investigation. After the 30th day, subjects will not be considered as suspected dengue cases.

A suspected dengue case presenting at the health care facility within 5 days following the onset of fever will be defined as an 'early presenter'.

Subjects presenting for care ***between sixth and 30th day*** of fever onset will be defined as 'late presenters'.

An example of other signs and symptoms of dengue, associated with fever, include but are not limited to: fatigue, headache, pain behind the eyes, abdominal pain, nausea, vomiting, muscle ache, joint ache, diffuse rash on the trunk, abnormal sensitivity to light (photophobia) and itching of skin (pruritus).

5.7.1.2. Laboratory-confirmed symptomatic dengue case (Amended 16 October 2017)

Early presenter:

At least one of the following findings must be met for a laboratory-confirmed dengue case:

- Dengue virus identification through RT-qPCR on the acute serum sample
- Dengue virus NS1 positive on acute serum sample through ELISA.
- Anti-dengue IgM seroconversion between acute and convalescent serum samples through ELISA.

Late presenter:

- ***Anti-dengue IgM seroconversion between acute and convalescent serum samples through ELISA.***

5.7.1.3. Virologically confirmed symptomatic dengue infection

A virologically confirmed symptomatic dengue infection is defined as a dengue case confirmed by RT-qPCR.

5.7.1.4. Moderate to severe dengue

A moderate to severe case of dengue is defined as follows (in accordance to the "dengue with warning signs" and "severe dengue" definitions in the 2009 WHO guidelines for dengue).

One or more of the WHO 2009 warning signs or one or more of the WHO 2009 criteria for severe dengue are met:

Warning signs

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargement >2 cm
- Laboratory: increase in hematocrit (HCT) concurrent with rapid decrease in platelet count

Criteria for severe dengue

- Severe plasma leakage leading to:
  - Shock (Dengue Shock Syndrome)
  - Fluid accumulation with respiratory distress
- Severe bleeding as evaluated by clinician
- Severe organ involvement
  - Liver: Alanine aminotransferase (ALT) or Aspartate aminotransferase (AST) ≥1000
  - Central Nervous System (CNS): Impaired consciousness
  - Heart and other organs

5.7.1.5. Previous dengue infection (Amended 16 October 2017)

A subject will be considered as having previous dengue infection at visit 1(baseline) if:

- Dengue IgG positive at visit 1 (baseline)

or

- Laboratory-confirmed symptomatic dengue case detected at visit 1 (baseline)

A subject will be considered as having previous dengue infection at any time after visit 1 (baseline), if:

- ***Dengue IgG positive at previous schedule visit(s)***

or

- ***Laboratory-confirmed symptomatic dengue case detected previously at study surveillance***

5.7.1.6. Primary symptomatic dengue case (Amended 16 October 2017)

A primary symptomatic dengue case is a subject with ***laboratory*** confirmed ***or probable*** symptomatic dengue infection without evidence of ***previous*** dengue ***infection*** (***absence*** of Ig G antibodies at the previous scheduled visit and ***absence of*** laboratory-confirmed symptomatic case ***detected previously at study surveillance***).

5.7.1.7. Secondary symptomatic dengue case (Amended 16 October 2017)

A secondary symptomatic dengue case is a subject with ***laboratory*** confirmed ***or probable*** symptomatic dengue infection with evidence of ***previous*** dengue infection (presence of Ig G antibodies at the previous scheduled visit(s) or laboratory-confirmed symptomatic case ***detected previously at study surveillance).***

5.7.1.8. Primary inapparent dengue infection (Amended 16 October 2017)

This condition is defined as a documented seroconversion (anti-dengue IgG antibodies) between two sequential sera samples obtained during the scheduled visits without clinical suspicion of dengue (identified during the time period in which seroconversion occurred).

5.7.1.9. Probable dengue case (Amended 16 October 2017)

- ***For early presenters, a probable case will be that case without laboratory confirmation, presenting IgG positive in the convalescent sample.***
- ***For late presenters, a probable case will be the case without seroconversion of IgM, presenting at least one IgG positive in one sample (acute or convalescent).***

5.7.1.10. Negative Dengue case

For early and late presenters, a negative dengue case is a participant with a negative result for all planned laboratory tests on both acute and convalescent samples.

5.7.1.11. Indeterminate dengue case (Amended 16 October 2017)

An indeterminate dengue case is a participant evaluated as an SDC (Section 5.7.1.1.) and not classified as laboratory confirmed case (Section 5.7.1.2), probably case (Section 5.7.1.9) or negative case (Section 5.7.1.10).

5.7.2. Chikungunya

5.7.2.1. Suspected symptomatic chikungunya case

Febrile illness with body temperature ≥ 38°C measured (by any route) on at least two consecutive days and less than 14 days with or without the presence of other chikungunya symptoms or signs, without an obvious aetiology unrelated to chikungunya, based on investigator's judgement.

Although subjects are asked to come to a study hospital if fever (body temperature ≥ 38°C) is sustained for two consecutive days, a subject could present on the first day of fever. In this case the physician may still consider the subject as a suspected chikungunya case based on medical judgement and collect a blood sample for laboratory diagnosis.

Subjects should be examined at the health care facility during the 5 days following fever onset, as this is a requirement for RT-qPCR diagnosis of dengue infection in the case of co-infection.

However, subjects presenting until 30th (included) day of fever onset will be considered for investigation. After the 30th day, subjects will not be considered as suspected chikungunya cases.

A suspected chikungunya case presenting at the health care facility within 5 days following the onset of fever will be defined as an 'early presenter'.

Subjects presenting for care between sixth and 30th day of fever onset will be defined as 'late presenters'.

An example of other signs and symptoms of chikungunya, associated with fever, include but are not limited to:

- ***Polyarthralgia is usually bilateral and symmetric, and can be debilitating***
- ***Other symptoms may include headache, myalgia, arthritis, conjunctivitis, nausea/vomiting, or maculopapular rash.***

Rare complications include uveitis, retinitis, myocarditis, hepatitis, nephritis, bullous skin lesions, hemorrhage, meningoencephalitis, myelitis, Guillain-Barré syndrome, and cranial nerve palsies.

5.7.2.2. Laboratory-confirmed symptomatic chikungunya case

Early presenter:

At least one of the following findings must be met for a laboratory-confirmed chikungunya case:

- ***Chikungunya virus identification through RT-qPCR on the acute serum sample***
- ***Anti-chikungunya IgM seroconversion between acute and convalescent serum samples through ELISA.***

Late presenters:

- ***Anti-chikungunya IgM seroconversion between acute and convalescent serum samples through ELISA.***

5.7.2.3. Virologically confirmed symptomatic chikungunya infection

A virologically confirmed symptomatic chikungunya infection is defined as a chikungunya case confirmed by RT-qPCR.

5.7.2.4. Previous chikungunya infection at baseline

A subject will be considered as having previous chikungunya infection at baseline (enrolment visit/Visit 1), if:

- ***Chikungunya IgG positive at baseline visit OR***
- ***Laboratory-confirmed symptomatic chikungunya case at baseline***

5.7.2.5. Inapparent chikungunya infection

This condition is defined as a documented seroconversion (anti-chikungunya IgG antibodies) between two sequential sera samples obtained during the scheduled visits without clinical suspicion of chikungunya (identified during the time period in which seroconversion occurred).

5.7.2.6. Probable chikungunya case

For early and late presenters, a probable chikungunya case will be that case without a lab-confirmed criteria, presenting at least one IgG positive in one sample (acute or convalescent).

5.7.2.7. Negative chikungunya case

For early and late presenters, a negative chikungunya case is a participant with a negative result for all planned laboratory tests on both acute and convalescent samples.

5.7.2.8. Indeterminate chikungunya case

An indeterminate chikungunya case is a participant evaluated as an suspected chikungunya case (Section 5.7.2.1.) and not classified as laboratory confirmed case (Section 5.7.2.2), probably case (Section 5.7.2.6) or negative case (Section 5.7.2.7).

5.7.3. Zika

5.7.3.1. Suspected symptomatic Zika case

Rash and/or febrile illness on at least two consecutive days and less than 14 days with or without the presence of other Zika symptoms or signs, without an obvious aetiology unrelated to Zika, based on investigator's judgement.

Although subjects are asked to come to a study hospital if fever and/or rash is sustained for two consecutive days, a subject could present on the first day of fever and/or rash. In this case the physician may still consider the subject as a suspected Zika case based on medical judgement and collect a blood sample for laboratory diagnosis.

Subjects should be examined at the health care facility during the 5 days following fever and/or rash onset, as this is optimal for RT-qPCR diagnosis of Zika infection.

However, subjects presenting until 30th (included) day of fever and/or rash onset will be considered for investigation. After the 30th day, subjects will not be considered as suspected Zika cases.

A suspected Zika case presenting at the health care facility within 5 days following the onset of fever and/or rash will be defined as an 'early presenter'. Subjects presenting for care between sixth and 30th day of fever and/or rash onset will be defined as 'late presenters'. At the clinical presentation of the first visit, fever will take priority in the classification of "early" or "late" presenter if the participant reported both fever and rash are present. If the participant reported the rash without fever, the date of onset of rash will determine classification.

An example of other signs and symptoms include arthralgia, arthritis, or conjunctivitis (non-purulent/hyperaemic).

5.7.3.2. Laboratory-confirmed symptomatic Zika case

Early presenters:

At least one of the following findings must be met for a laboratory-confirmed Zika case.

- ***Zika virus identification through RT-qPCR on the acute serum sample***
- ***Anti-Zika IgM seroconversion between acute and convalescent serum samples through ELISA and confirmed by Zika neutralization antibody testing.***

Late presenters:

- ***Anti-Zika IgM seroconversion between acute and convalescent serum samples through ELISA and confirmed by Zika neutralization antibody testing.***

5.7.3.3. Virologically confirmed symptomatic Zika infection

A virologically confirmed symptomatic Zika infection is defined as a Zika suspected case confirmed by RT-qPCR.

5.7.3.4. Previous Zika infection at baseline

A subject will be considered as having previous Zika infection at baseline (enrolment visit/Visit 1), if:

- ***Zika IgG positive at baseline visit as confirmed by neutralizing antibody tests OR***
- ***Laboratory-confirmed symptomatic Zika case at baseline***

5.7.3.5. Inapparent Zika infection

This condition is defined as a documented seroconversion and confirmed by Zika neutralising antibody testing between two sequential sera samples obtained during the scheduled visits without clinical suspicion of Zika (identified during the time period in which seroconversion occurred).

5.7.3.6. Probable Zika case

- ***For early and late presenters, a probable Zika case will be that case without a lab-confirmed criteria, presenting at least one IgM/IgG positive in one sample (acute or convalescent).***

5.7.3.7. Negative Zika Case

For early and late presenters, a negative Zika case is a participant with a negative result for all planned laboratory tests on both acute and convalescent samples.

5.7.3.8. Indeterminate Zika case

An indeterminate Zika case is a participant evaluated as an suspected Zika case (Section 5.7.3.1.) and not classified as laboratory confirmed case (Section 5.7.3.2), probably case (Section 5.7.3.6) or negative case (Section 5.7.3.7).

5.8. Biological sample handling and analysis

The biological samples collected in this study are whole blood.

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

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

Collected samples may be used for future testing, according to local regulations, in other assays, for test improvement or test development of analytical methods related to the pathogens under study. Under these circumstances, additional testing on the samples may be performed by GSK Biologicals or a GSK designated laboratory outside the scope of this protocol. Further research on the sample will only be conducted by GSK Biologicals after further assessment and approval(s) by Ethics Committee(s) and the National Commission of Ethics in Research (when applicable) for the test(s) to be done and also after the subjects' informed consent. Information on further investigations and their rationale can be obtained from GSK Biologicals.

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

Any human pharmacogenetic testing will require specific consent from the individual subjects/subject's parent(s)/LAR(s) and the ethics committee approval.

Refer also to the Investigator Agreement, the Investigator cannot perform any other biological assays except those described in the protocol or its amendment(s).

Collected samples will be stored for up to 10 years (counting from when the last subject performed the last study visit/contact), with the option to extend the retention time.

5.8.1. Use of specified study materials

When materials are provided by GSK Biologicals, that all samples (including serum samples) must be collected and stored exclusively using those materials in the appropriate manner; this is MANDATORY. The use of other materials could result in the exclusion of the subject from the ATP analysis (See Section 8.3 for the definition of study cohorts to be evaluated). 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 SPM.

5.8.2. Biological samples evaluation

A detailed description of the assays performed and laboratory addresses are provided in the appendices.

Assays will be performed using standardized and validated procedures.

5.8.2.1. Laboratory assays

Table 8 Humoral Immunity (Amended 16 October 2017)

System	Component	Method	Kit / Manufacturer	Laboratory
Serum	IgM to Dengue	ELISA	Commercial kit Panbio or equivalent	Local# or ***GSK designated laboratory***
	IgG to Dengue	ELISA	Indirect IgG Panbio or equivalent (for scheduled visits)	Local# or ***GSK designated laboratory***
			IgG Capture PanBio or equivalent (for ***unscheduled visits***)	Local# or ***GSK designated laboratory***
Serum	Dengue virus types 1-4 neutralizing antibodies	Dengue neutralization assay	In-house	Laboratório de Tecnologia Virológica, Bio-Manguinhos, Fiocruz Rio de Janeiro or GSK designated laboratory
Serum	***Antibodies against Zika and chikungunya***	***To be determined***	***To be determined***	***GSK designated laboratory***
Serum	YF virus neutralizing antibody*	YF neutralization assay	To be determined	GSK designated laboratory

*For subjects residing in a yellow fever endemic region or vaccinated against yellow fever
#***Testing performed according to local practices***

Table 9 Virology (Amended 16 October 2017)

System	Component	Method	Kit/Manufacturer	Laboratory
Serum	DENV RNA	RT-qPCR	In-house	Laboratório de Tecnologia Virológica, Bio-Manguinhos, Fiocruz Rio de Janeiro (GSK as back-up)
Serum	***Zika RNA***	***RT-PCR***	***To be determined***	***GSK designated laboratory***
Serum	***Chikungunya RNA***	***RT-PCR***	***To be determined***	***GSK designated laboratory***
Serum	NS1	ELISA	Commercial kit Biorad,Platelia	Local

Although not a specific study endpoint, viral isolation and sequencing may be performed if deemed necessary for future research (the aliquot reserved for RT-qPCR will be used in this case).

Table 10 Haematology

System	Component	Method	Kit/Manufacturer	Laboratory
Whole Blood	CBC	Per local standard practice	Per local standard practice	Local
	Hematocrit			Local

Assays performed on an as needed basis if dengue is suspected

The GSK Biologicals' and Fiocruz 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 should also have a Quality System established and supported by procedures.

Aliquots for PCR and the neutralization assay will be sent to Fiocruz with the possibility of sending reserve aliquots to GSK for retesting if necessary. Aliquots for re-testing will be sent from Fiocruz

5.8.2.2. Laboratory read-outs

In case of insufficient blood sample volume to perform all assays, the samples will be analyzed according to priority ranking provided in Table 11, Table 12 and Table 13.

Table 11 Laboratory read-outs at each time point, and priority ranking for scheduled visits (Amended 16 October 2017)

SCHEDULED VISITS								
Blood sampling time point		**No. Subjects (anticipated)**	**Subset***	**Possible assays**	**Serum aliquot volume**	***Aliquot* priority rank**	***Test priority rank***	**Aliquot number**
Type of contact/ Amount of whole blood	**Sampling time point**							
Scheduled Visit 1 whole blood 5 mL	(Day 0)	3600 (1800 from wave 1 & 2***)	-	Dengue IgG ELISA**	250 µL	1	***1***	1
			yes	Dengue virus 1-4 neutralizing antibodies	1 mL	2	***1***	2
				YF neutralization			***4***	
				Zika neutralization			***3***	
				Zika serology (IgG)#			***2***	
				Chikungunya serology (IgG)#				
			yes	***Chikungunya neutralization***	remaining volume	3	***1***	3
Scheduled Visit 2 5 mL whole blood	Visit 1+ 6 months for subject enrolled in Study Year 1; Visit 1+ 12 months for subject enrolled in beginning of Study Year 2, 3 or 4)	3190 (1660 from wave 1 and 1530 from wave 2)	yes	Dengue IgG ELISA** (only for subjects IgG negative at the previous visit)	250 µL	1	***1***	1
			yes	Dengue virus 1-4 neutralizing antibodies	1 mL	2	***1***	2
				YF neutralization			***4***	
				Zika neutralization			***3***	
				Zika serology (IgG)#			***2***	
				Chikungunya serology (IgG)#				
			yes	***Chikungunya neutralization***	remaining volume	3	***1***	3

SCHEDULED VISITS								
Blood sampling time point		**No. Subjects (anticipated)**	**Subset***	**Possible assays**	**Serum aliquot volume**	***Aliquot* priority rank**	***Test priority rank***	**Aliquot number**
Type of contact/ Amount of whole blood	**Sampling time point**							
Scheduled Visit 3 5 mL whole blood	(Visit 2+ 6 months for subject enrolled in Study Year 1; Visit 2+12 months for subject enrolled in beginning of Study Year 2 or 3)	3030 (1530 from wave 1 and 1500 from wave 2)	yes	Dengue IgG ELISA** (only for subjects IgG negative at the previous visit)	250 µL	1	***1***	1
			yes	Dengue virus 1-4 neutralizing antibodies	1 mL	2	***1***	2
				YF neutralization			***4***	
				Zika neutralization			***3***	
				Zika serology (IgG)#				
				Chikungunya serology (IgG)#			***2***	
			yes	***Chikungunya neutralization***	remaining volume	3	***1***	3
Subsequent Yearly Visit(s) as applicable 5 mL whole blood	Yearly	3000 for Visit 4 and 1500 for visit 5 and 6 which applies only to subjects of the wave 1	yes	Dengue IgG ELISA** (only for subjects IgG negative at the previous visit)	250 µL	1	***1***	1
			yes	Dengue virus 1-4 neutralizing antibodies	1 mL	2	***1***	2
				YF neutralization			***4***	
				Zika neutralization			***3***	
				Zika serology (IgG)#				
				Chikungunya serology (IgG)#			***2***	
			yes	***Chikungunya neutralisation***	remaining volume	3	***1***	3

*tertiary (exploratory) analysis, number of subjects in the subset will be determined during analysis

- not applicable,

** Dengue IgG indirect ELISA

*** Wave 1 enrolment = initial cohort / Wave 2 enrolment = Expansion cohort

 # Indirect DENV IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for DENV IgG at the previous scheduled visit. Zika IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for Zika IgG at the previous scheduled visit; chikungunya IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for chikungunya IgG at the previous scheduled visit

Table 12 Laboratory read-outs at each time point, and priority ranking for suspected dengue, chikungunya and/or Zika visits for EARLY presenters (Amended 16 October 2017)

SUSPECTED DENGUE, CHIKUNGUNYA AND/OR ZIKA VISITS – EARLY PRESENTERS								
Blood sampling time point		**No. Subjects**	**Subset**	**Component**	**Serum aliquot volume**	***Aliquot* priority rank**	***Test priority rank***	**Aliquot number**
Type of contact/ Amount of whole blood	**Sampling time point**							
First visit (amount of blood= 5 mL for < 2 years of age and 10 mL for all other subjects)	Sample collected ≤ 5 days of fever and/or rash	unknown	-	***Dengue*** RT-qPCR	750 µL	1	***1***	1
				Zika and chikungunya RT-qPCR			***2***	
				Dengue virus 1-4 neutralizing antibodies	750 µL	***3***	***1***	2
				Dengue IgM and NS1 ELISA	500 µL	2	***1***	3
				Zika neutralising antibodies	1 mL	4	***1***	4
				Chikungunya neutralizing antibodies and Serology for Zika (IgG/IgM) and chikungunya (IgG/IgM)	remaining volume	5	***1***	5
	Optional for severe cases			PCR	200 µL	-		
First visit (total amount of blood per local practice	***Sample collected before day 14 of onset of fever/rash***	unknown		CBC, HCT	No aliquot	-		

SUSPECTED DENGUE, CHIKUNGUNYA AND/OR ZIKA VISITS – EARLY PRESENTERS								
Blood sampling time point		**No. Subjects**	**Subset**	**Component**	**Serum aliquot volume**	***Aliquot* priority rank**	***Test priority rank***	**Aliquot number**
Type of contact/ Amount of whole blood	**Sampling time point**							
Suspected dengue follow-up visit (total amount of blood 5 mL)	Approximately 21 days after first visit	unknown	-	Dengue IgM and Dengue capture IgG ELISA	500 µL	1	**1**	1
				Dengue virus 1-4 neutralizing antibodies	1 mL	2	**1**	2
				Serology for Zika (IgM/IgG) and chikungunya (IgM/IgG)			**2**	
				Zika neutralizing antibodies			**3**	
				Chikungunya neutralizing antibodies	remaining volume	3	**1**	3

- not applicable

Table 13 Laboratory read-outs at each time point, and priority ranking for suspected dengue, chikungunya and/or Zika visits for LATE presenters

SUSPECTED DENGUE, CHIKUNGUNYA AND/OR ZIKA VISITS – LATE PRESENTERS								
Blood sampling time point		No. Subjects	Subset	Component	Serum aliquot volume	*Aliquot* priority rank	*Test priority rank*	Aliquot number
Type of contact/ Amount of whole blood	Sampling time point							
First visit (total amount of whole blood 5 mL)	Sample collected > 5 days or more after fever and/or rash	unknown	-	Dengue IgM and Dengue capture IgG ELISA	500 µL	1	***1***	1
				Dengue virus 1-4 neutralizing antibodies	1 mL	2	***1***	2
				Zika and chikungunya RT-qPCR			***2***	
				Serology for Zika (IgG/IgM) and chikungunya (IgG/IgM)	remaining volume	3	***1***	3
First visit (total amount of blood per local practice	***Sample collected before day 14 of onset of fever/rash***	unknown		CBC, HCT	No aliquot	-		
Suspected dengue, ***chikungunya and Zika*** follow-up visit (total amount of blood 5 mL)	Approximately 21 days after first visit	unknown	-	Dengue IgM and Dengue capture IgG ELISA	500 µL	1	***1***	1
				Dengue virus 1-4 neutralizing antibodies	1 mL	2	***1***	2
				Serology for Zika (IgG/IgM) and chikungunya (IgG/IgM)			***2***	
				Zika neutralizing antibodies			***3***	
				Chikungunya neutralizing antibodies	remaining volume	3	***1***	3

- not applicable

6. SERIOUS ADVERSE EVENTS

In this prospective cohort study no test product/vaccine will be given. However blood samples will be collected at scheduled visits and ***unscheduled visits*** for suspected dengue, ***chikungunya and/or Zika***.

In order to fulfil international reporting obligations, serious adverse events (SAEs) that are related to study procedures (blood collection) will be collected and recorded from the time the subject consents to participate in the study until the study conclusion for each subject.

The investigators or site staff 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 or 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.

6.1. Serious adverse events

6.1.1. Definition of a serious adverse event

A serious adverse event is any AE that:

a. Results in death

b. Is life-threatening

   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 hospitalization or prolongation of existing hospitalization,

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

   Hospitalization 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, OR

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

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

f. 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.

6.2. Detecting and recording serious adverse events

6.2.1. Evaluation of serious adverse events related to a study procedure

Any SAE either observed by the investigator or his/her staff or reported by the subject/subject's parents/LAR(s) related to study procedures will be evaluated by the investigator. The nature of each event, data and time (where appropriate) of onset, outcome, intensity and relationship to the study procedures should be established.

When an SAE related to a study procedure 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 on the eCRF or SAE Report screens as applicable. It is not acceptable for the investigator to send photocopies of the subject's medical records to GSK Biologicals instead of the appropriate completed SAE screens in the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK Biologicals. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to GSK Biologicals.

The investigator will attempt to establish a diagnosis pertaining to the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the SAE and not the individual signs/symptoms.

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

There may be situations when a SAE has occurred and the investigator has minimal information to include in the initial report to GSK Biologicals. However it is very important that the investigator always makes an assessment of causality for every event prior to submission of the SAE to GSK Biologicals. The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE information accordingly.

An SAE related to a study procedure will be examined by the investigator to the extent to enable determination of all contributing factors applicable to each SAE.

Possible contributing factors include:

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

Outcome of any reportable SAE during the entire study will be assessed as:

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

6.2.2. Prompt reporting of SAEs related to a study procedure

SAEs related to a study procedure that occurs at any time during the study will be reported promptly to GSK within the timeframes described in Table 14, once the investigator determines that the event meets the protocol definition of a SAE.

Table 14 Timeframes for submitting SAE reports to GSK

Type of Event	Initial Reports		Follow-up of Relevant Information on a Previous Report	
	Timeframe	Documents	Timeframe	Documents
SAEs related to a study procedure	24 hours*	SAE screen	24 hours*	SAE screen

* Timeframe allowed after receipt or awareness of the information

6.2.3. Contact information for reporting serious adverse events and other events to GSK Biologicals

Study Contact for Reportable SAEs
During office hours: Local Name: Dr. PPD GlaxoSmithKline Biologicals PPD, PPD, PPD, Brazil CEP 22783-110 Email: PPD Phone: PPD Cell: PPD Name: PPD Clinical Safety Analyst Pharmacovigilance Department GlaxoSmithKline Brazil PPD, PPD, PPD, PPD Brazil 22783-110 Email: PPD Phone: PPD
Back-up Study Contact for Reporting SAEs
24/24 hour and 7/7 day availability: GSK Biologicals Clinical Safety & Pharmacovigilance Fax: PPD or PPD

Once an investigator becomes aware that a study procedure related SAE has occurred in a study subject, the investigator (or designate) must complete the information in the SAE screens of the eCRF WITHIN 24 HOURS. The SAE screens will always be completed as thoroughly as possible with all available details of the event. Even if the investigator does not have all information regarding a SAE, the SAE screens should still be completed within 24 hours. Once additional information is received, the SAE screens in the eCRF should be updated WITHIN 24 HOURS.

The investigator will always provide an assessment of causality at the time of the initial report.

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

If the electronic SAE reporting system does not work, the investigator (or designate) must complete, then date and sign a SAE Report Form 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 SAE reporting system is not working and NOT if the system is slow. As soon as the electronic SAE reporting system is working again, the investigator (or designate) must complete the SAE screens in the eCRF within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic SAE reporting system.

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

When additional information is received on a SAE after freezing of the subject's eCRF, new or updated information is to be recorded on a SAE Report Form, with all changes signed and dated by the investigator. The updated form should be faxed to the GSK Biologicals Clinical Safety and Pharmacovigilance department or to the Study Contact for Reporting SAEs (refer to the Sponsor Information Sheet) WITHIN 24 HOURS of receipt of the follow-up information.

6.2.4. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to GSK in accordance with the procedures detailed in Section 6.2.2. Prompt notification of SAEs by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

6.3. Follow-up of SAEs related to study procedure

After the initial SAE report, the investigator is required to proactively follow each subject and provide further information on the subject's condition to GSK Biologicals.

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

Investigators will follow-up subjects with an SAE until the event has resolved, subsided, stabilized, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up.

If a subject dies during participation in the study or during a recognized follow-up period, GSK Biologicals will be provided with a copy of any available post-mortem findings, including histopathology.

6.4. Treatment of adverse events

Treatment of any SAE related to a study procedure is at the sole discretion of the investigator and according to current good medical practice. Any medication administered for the treatment of an SAE should be recorded in the subject's eCRF.

6.5. Subject Cards

Study subjects will be provided with the address and telephone number of the main contact for information about the epidemiological study.

Investigator/delegate should therefore provide a "subject card" to each subject. The aim of this card is to inform any physician having contact with a study subject, that the patient is participating in this epidemiology study.

Subjects must be instructed to keep these cards in their possession at all times.

7. SUBJECT COMPLETION AND WITHDRAWAL

7.1. 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.

7.2. Subject withdrawal

Subjects who are withdrawn because of an SAE must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn as result of a SAE until resolution of the event.

All data collected until the date of withdrawal/last contact of the subject will be used for the analysis. Withdrawals/drop-outs will be replaced (see Section 7.3 for further details).

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

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

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

- Serious adverse event.
- Non-serious adverse event.
- Protocol violation (specify).
- Consent withdrawal, not due to an adverse event*
- Moved from the study area.
- Lost to follow-up.
- Other (specify).

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

7.3. Subject replacement (Amended 16 October 2017)

Enrolment of new subjects to replace:

- subjects not wishing to continue after amendment 2 (initial cohort), or
- subjects who prematurely terminate participation (initial and expansion cohorts) or
- subjects lost to follow-up will be done (initial and expansion cohorts).

The aim is to maintain a critical cohort size of ≥ 500 subjects/site. This replacement will be done at the beginning of each study year: during the scheduled visits starting at the end of study Year 1 up till the end of study Year 3.

The number of subjects to be recruited as replacements will be defined based on the number of subjects still active in the study. If at the end of each study year we have ≥500 subjects/site, no replacements will be needed.

In last three months of the of penultimate year of the study, each site will review the number of active subjects and drop-out rate.. If the risk of having fewer than the required number of active participants at the end of the study is high, recruitment for participants into the replacement cohort may be performed with sponsor approval. If the risk is high, recruitment into the replacement cohort should begin even if the number of active subjects is 500 or above 500.

The time window for the recruitment of replacements will be during the low dengue transmission, ***preferably***.

8. DATA EVALUATION: CRITERIA FOR EVALUATION OF OBJECTIVES

8.1. Endpoints

8.1.1. Primary endpoint

- Laboratory-confirmed symptomatic dengue infection (all DENV types).

Symptomatic dengue infection is defined in Section 5.7.1.2

8.1.2. Secondary endpoints

- DENV-type specific primary laboratory-confirmed symptomatic dengue infection.
- DENV-type specific secondary laboratory-confirmed symptomatic dengue infection.
- Primary symptomatic dengue infection (including laboratory-confirmed and probable cases).

- Secondary symptomatic dengue infection (including laboratory-confirmed and probable cases).
- Previous dengue infection(s) (dengue seroprevalence) at baseline (defined in Section 5.7.1.5)
- Primary inapparent dengue infection..
- Severity of symptoms of symptomatic dengue (using the 2009 WHO guidelines).

8.1.3. Tertiary endpoints (Amended 16 October 2017)

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

- ***Symptomatic laboratory-confirmed Zika virus infection***
- ***Symptomatic Zika virus infection (including laboratory-confirmed and probable cases).***
- ***Symptomatic laboratory-confirmed chikungunya infection***
- ***Symptomatic chikungunya infection (including laboratory-confirmed and probable cases).***
- Risk factors for dengue infection and disease.
- Neutralizing antibodies titers against DENV 1-4.
- Neutralizing antibody titers against YF virus.
- Spatial and temporal distribution of dengue cases (confirmed and probable symptomatic cases and inapparent infections).
- ***Spatial and temporal distribution of laboratory confirmed and probable Zika virus cases***
- ***Spatial and temporal distribution of patients with laboratory confirmed and probable chikungunya virus infection***
- ***Occurrence of laboratory confirmed or probable Zika and/or chikungunya infection in suspected dengue cases (differential diagnosis of dengue), retrospectively***
- ***Antibody titre against Zika and chikungunya virus at the scheduled visits***

8.2. Sample size consideration

8.2.1. Assumptions:

A target to obtain at least 500 subjects/site still active at the end of the first year of the study ***is planned***. ***Since*** subjects might leave the study due to a variety of reasons, e.g. moving away geographically, sample size is increased to allow for this. On the basis of

an annual drop-out rate between 15 and 20%, approximately 600 subjects/site will be enrolled at the beginning of this study. The recruitment will be cluster sampling , i.e. the unit of sampling will be the households. Different areas where the FHP has been fully implemented or where access to the community is already established by a government program will be first selected, followed by a selection of households in the respective areas. All subjects in each household will be invited for recruitment to the study.

New sites will start enrolling subjects preferably during study Year 2. The target is to enrol approximately 600 subjects per additional site. Replacement subjects may be recruited in order to maintain at least 500 subjects/site still active at the beginning of the next dengue season.

In order to better represent the Brazilian population distribution, subjects enrolled in the expansion cohort should be composed of at least 30% of subjects younger than 18 years old and should not exceed 20 % of adults 50 years or older. Subjects enrolled as replacement subjects for the initial and expansion cohort will be recruited to approach as much as possible a minimal representation of 30% of subjects younger than 18 years old and a maximal representation of 20% for adults 50 years or older.

The total study duration per site will last approximately 4 years for the initial cohort and approximately 3 years for the expansion cohort, and new subjects will be enrolled if the number of subjects still active in the study is below 500/site after year 1 and at the beginning of each additional study year/season.

Between 2007 and 2010, the average incidence of reported dengue cases in the Brazilian population was about 0.3% per year (or 0.003 cases per person per year). Assuming an underreporting factor of 3 (Brazil MoH, 2011) the average incidence of dengue is expected to be about 1% per year (or 0.01 cases per person per year).

8.2.2. 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 1.8 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 3. Although in theory *rho* can have a value up to 1, in practice values higher than 0.4 are uncommon. We used a conservative estimate of 0.4 for this study (Bennett, 1991).The design effect is then estimated to 1.8.

8.2.3. Expected precision of incidence rate estimates

Table 15 shows the 95% CI for a range of expected incidences based on the Poisson distribution (exact method, normal approximation and the normal approximation accounting for a design effect of 1.8) in different scenarios. The scenarios were defined according to the following criteria:

- Number of subjects enrolled at the beginning of the study (for some sites it will be the second year). At season level and for all sites: 2000, 4000, 1800, 3600, 1500, 3000. For all seasons and for all sites: 4000, 3600 and 3000. At season level and site level: 600 and 500.
- Number of years of follow-up. At study level: 1 or 4 years, but half of the sites will be followed for 3 years and this is taken into account for the calculations. At site level: 1, 3 or 4 years.
- Overall incidences: 3, 6, 9, 12 or 15 events per 1000 person-years.

With an overall sample size of a minimum of about 3,600 subjects enrolled and a follow-up period of one year, the 95% confidence interval (CI) for an expected incidence of dengue of 9 cases per 1000 person-years using a cluster design is [4.7 ; 13.3]. With 3600 subjects enrolled, 4 years of follow-up and new enrolments to keep the number of subjects above 3000 at the beginning of each subsequent season, the 95% CI is [6.3 ; 11.7].

Table 15 Precision of the expected incidences of dengue for a single cohort of subjects in Brazil

	Cohort size	Number of years	Number of person-years	Number of cases	Incidence of dengue (per 1000 person-years)	95% CI of incidence rate (n / 1000 P-Y)						New enrolment		
						Exact (Poisson)		Normal approx		Normal approx with design effect		After Y1	After Y2	After Y3
						Lower	Upper	Lower	Upper	Lower	Upper			
Overall	2000	1	1845.9	6	3.0	1.0	6.7	0.5	5.5	-0.4	6.4			
	2000	1	1845.9	11	6.0	3.0	10.7	2.5	9.5	1.3	10.7			
	2000	1	1845.9	17	9.0	5.2	14.5	4.7	13.3	3.2	14.8			
	2000	1	1845.9	22	12.0	7.5	18.1	7.0	17.0	5.3	18.7			
	2000	1	1845.9	28	15.0	9.9	21.7	9.4	20.6	7.5	22.5			
	4000	1	3691.9	11	3.0	1.5	5.4	1.2	4.8	0.6	5.4			
	4000	1	3691.9	22	6.0	3.8	9.1	3.5	8.5	2.6	9.4			
	4000	1	3691.9	33	9.0	6.2	12.6	5.9	12.1	4.9	13.1			
	4000	1	3691.9	44	12.0	8.7	16.1	8.5	15.5	7.3	16.7			
	4000	1	3691.9	55	15.0	11.3	19.5	11.0	19.0	9.7	20.3			
	4000	4	9086.6	27	3.0	2.0	4.4	1.9	4.1	1.5	4.5			327
	4000	4	9086.6	55	6.0	4.5	7.8	4.4	7.6	3.9	8.1			327
	4000	4	9086.6	82	9.0	7.2	11.2	7.0	11.0	6.4	11.6			327
	4000	4	9086.6	109	12.0	9.9	14.5	9.7	14.3	9.0	15.0			327
	4000	4	9086.6	136	15.0	12.6	17.7	12.5	17.5	11.6	18.4			327
	1800	1	1661.3	5	3.0	0.9	7.2	0.4	5.6	-0.5	6.5			
	1800	1	1661.3	10	6.0	2.8	11.1	2.3	9.7	1.0	11.0			
	1800	1	1661.3	15	9.0	5.0	14.9	4.4	13.6	2.9	15.1			
	1800	1	1661.3	20	12.0	7.3	18.6	6.7	17.3	4.9	19.1			
	1800	1	1661.3	25	15.0	9.7	22.1	9.1	20.9	7.1	22.9			
	3600	1	3322.7	10	3.0	1.4	5.6	1.1	4.9	0.5	5.5			
	3600	1	3322.7	20	6.0	3.6	9.3	3.4	8.6	2.5	9.5			

	Cohort size	Number of years	Number of person-years	Number of cases	Incidence of dengue (per 1000 person-years)	95% CI of incidence rate (n / 1000 P-Y)						New enrolment		
						Exact (Poisson)		Normal approx		Normal approx with design effect		After Y1	After Y2	After Y3
						Lower	Upper	Lower	Upper	Lower	Upper			
	3600	1	3322.7	30	9.0	6.1	12.9	5.8	12.2	4.7	13.3			
	3600	1	3322.7	40	12.0	8.6	16.3	8.3	15.7	7.0	17.0			
	3600	1	3322.7	50	15.0	11.1	19.8	10.8	19.2	9.4	20.6			
	3600	4	8611.3	26	3.0	2.0	4.4	1.8	4.2	1.4	4.6		170	450
	3600	4	8611.3	52	6.0	4.5	7.9	4.4	7.6	3.8	8.2		170	450
	3600	4	8611.3	78	9.0	7.1	11.2	7.0	11.0	6.3	11.7		170	450
	3600	4	8611.3	103	12.0	9.8	14.5	9.7	14.3	8.9	15.1		170	450
	3600	4	8611.3	129	15.0	12.5	17.8	12.4	17.6	11.5	18.5		170	450
	1500	1	1384.5	4	3.0	0.8	7.6	0.1	5.9	-0.9	6.9			
	1500	1	1384.5	8	6.0	2.6	11.7	1.9	10.1	0.5	11.5			
	1500	1	1384.5	12	9.0	4.7	15.6	4.0	14.0	2.3	15.7			
	1500	1	1384.5	17	12.0	6.9	19.3	6.2	17.8	4.3	19.7			
	1500	1	1384.5	21	15.0	9.2	22.9	8.5	21.5	6.3	23.7			
	3000	1	2768.9	8	3.0	1.3	5.9	1.0	5.0	0.3	5.7			
	3000	1	2768.9	17	6.0	3.5	9.7	3.1	8.9	2.1	9.9			
	3000	1	2768.9	25	9.0	5.8	13.3	5.5	12.5	4.3	13.7			
	3000	1	2768.9	33	12.0	8.3	16.8	7.9	16.1	6.5	17.5			
	3000	1	2768.9	42	15.0	10.8	20.3	10.4	19.6	8.9	21.1			
	3000	4	8306.7	25	3.0	1.9	4.4	1.8	4.2	1.4	4.6	225	450	450
	3000	4	8306.7	50	6.0	4.4	7.9	4.3	7.7	3.8	8.2	225	450	450
	3000	4	8306.7	75	9.0	7.1	11.3	7.0	11.0	6.3	11.7	225	450	450
	3000	4	8306.7	100	12.0	9.8	14.6	9.6	14.4	8.8	15.2	225	450	450
	3000	4	8306.7	125	15.0	12.5	17.9	12.4	17.6	11.5	18.5	225	450	450

	Cohort size	Number of years	Number of person-years	Number of cases	Incidence of dengue (per 1000 person-years)	95% CI of incidence rate (n / 1000 P-Y)						New enrolment		
						Exact (Poisson)		Normal approx		Normal approx with design effect		After Y1	After Y2	After Y3
						Lower	Upper	Lower	Upper	Lower	Upper			
Site	600	1	553.8	2	3.0	0.2	12.3	-1.6	7.6	-3.1	9.1			
	600	1	553.8	3	6.0	1.2	17.0	-0.5	12.5	-2.7	14.7			
	600	1	553.8	5	9.0	2.7	21.4	1.1	16.9	-1.6	19.6			
	600	1	553.8	7	12.0	4.6	25.1	2.9	21.1	-0.2	24.2			
	600	1	553.8	8	15.0	6.5	29.2	4.8	25.2	1.3	28.7			
	600	3	1486.0	4	3.0	0.8	7.5	0.2	5.8	-0.7	6.7		67	
	600	3	1486.0	9	6.0	2.7	11.5	2.1	9.9	0.7	11.3		67	
	600	3	1486.0	13	9.0	4.8	15.3	4.2	13.8	2.5	15.5		67	
	600	3	1486.0	18	12.0	7.1	19.0	6.4	17.6	4.5	19.5		67	
	600	3	1486.0	22	15.0	9.4	22.6	8.8	21.2	6.6	23.4		67	
	600	4	1947.5	6	3.0	1.0	6.7	0.6	5.4	-0.3	6.3		67	75
	600	4	1947.5	12	6.0	3.1	10.6	2.6	9.4	1.4	10.6		67	75
	600	4	1947.5	18	9.0	5.3	14.3	4.8	13.2	3.3	14.7		67	75
	600	4	1947.5	23	12.0	7.6	17.9	7.1	16.9	5.5	18.5		67	75
	600	4	1947.5	29	15.0	10.1	21.5	9.6	20.4	7.7	22.3		67	75
	500	1	461.5	1	3.0	0.1	14.3	-2.0	8.0	-3.7	9.7			
	500	1	461.5	3	6.0	1.0	18.6	-1.1	13.1	-3.5	15.5			
	500	1	461.5	4	9.0	2.5	22.7	0.3	17.7	-2.6	20.6			
	500	1	461.5	6	12.0	4.2	26.8	2.0	22.0	-1.4	25.4			
	500	1	461.5	7	15.0	5.8	31.2	3.8	26.2	0.0	30.0			
	500	3	1384.5	4	3.0	0.8	7.6	0.1	5.9	-0.9	6.9	75	75	
	500	3	1384.5	8	6.0	2.6	11.7	1.9	10.1	0.5	11.5	75	75	
	500	3	1384.5	12	9.0	4.7	15.6	4.0	14.0	2.3	15.7	75	75	
	500	3	1384.5	17	12.0	6.9	19.3	6.2	17.8	4.3	19.7	75	75	

	Cohort size	Number of years	Number of person-years	Number of cases	Incidence of dengue (per 1000 person-years)	95% CI of incidence rate (n / 1000 P-Y)						New enrolment		
						Exact (Poisson)		Normal approx		Normal approx with design effect		**After Y1**	**After Y2**	**After Y3**
						Lower	Upper	Lower	Upper	Lower	Upper			
	500	3	1384.5	21	15.0	9.2	22.9	8.5	21.5	6.3	23.7	75	75	
	500	4	1845.9	6	3.0	1.0	6.7	0.5	5.5	-0.4	6.4	75	75	75
	500	4	1845.9	11	6.0	3.0	10.7	2.5	9.5	1.3	10.7	75	75	75
	500	4	1845.9	17	9.0	5.2	14.5	4.7	13.3	3.2	14.8	75	75	75
	500	4	1845.9	22	12.0	7.5	18.1	7.0	17.0	5.3	18.7	75	75	75
	500	4	1845.9	28	15.0	9.9	21.7	9.4	20.6	7.5	22.5	75	75	75

8.3. Study cohorts to be evaluated

8.3.1. Total cohort

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

8.3.2. According-To-Protocol cohort

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

8.4. Conduct of analyses

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

8.4.1. Sequence of analyses

An interim analysis will be performed when subjects from the initial cohort of first two sites have completed two years of surveillance (Visit 4) and the third site have completed at least one year of surveillance (Visit 3). All analyses of primary and secondary objectives will be performed on data as cleaned as possible.

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

8.5. Statistical methods

Any deviation or change from the original statistical plan will be described and justified in the final study report.

All the analyses of primary objectives will be performed on the ATP cohort. Analysis of demographics will be performed on the total cohort.

8.5.1. 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 summarized overall and by region, and at the beginning of each season using descriptive statistics.

8.5.2. Analysis of primary and secondary endpoints

The following analyses will be performed overall and by DENV type, study site, gender and age-group previous dengue exposure (Yes = DENV IgG antibodies at previous visit and No = no DENV IgG antibodies at previous visit):

- Incidence rate of laboratory-confirmed symptomatic dengue infection with 95% CI for each season separately: the numerator will be the number of subjects with lab-confirmed symptomatic dengue infection during the season (between the visits scheduled before and after the season). The denominator will be the total person-years at risk, i.e. from the visit scheduled before the season until the first RT-qPCR confirmed symptomatic dengue infection during the season, the next scheduled visit or the subject's withdrawal, whichever comes first.
- Incidence rate of laboratory-confirmed symptomatic dengue infection with 95% CI all seasons combined: the numerator will be the number of subjects with lab-confirmed symptomatic dengue infection during the study period. The denominator will be the total person-years at risk, i.e. from enrolment until the first RT-qPCR confirmed symptomatic dengue infection, the end of the study or the subject's withdrawal, whichever comes first.

The following analyses will be performed by study site, gender and age-group previous dengue exposure (primary or secondary):

- Incidence rate of laboratory confirmed or probable symptomatic dengue infections with 95% CI.for each season separately and overall. The numerator will be the number of subjects with symptomatic dengue infection (including laboratory-confirmed and probable cases) during the period. The denominator will be the total person-years at risk.
- Incidence rate of probable symptomatic dengue infection with 95% CI for each season separately and overall. The numerator will be the number of subjects with probable symptomatic dengue infection. The denominator will be the total person-years at risk.

The following analyses will be performed by study site, gender and age-group:

- The proportion of subjects with primary inapparent dengue infection at each subsequent scheduled visit (not at enrolment) with 95% CI will be calculated as the number of dengue IgG positive cases and at the visit divided by the total number of subjects IgG negative at the previous visit.
- The proportion of subjects with secondary symptomatic dengue infection (probable or confirmed) at each subsequent scheduled visit (not at enrolment) with 95% CI will be calculated as the number of subjects with secondary symptomatic infection since the previous visit divided by the total number of IgG positive subjects at the previous visit.

- The (crude) seroprevalence of dengue infection will be calculated at enrolment and at each scheduled visit as a proportion (i.e., the number of dengue IgG positive subjects tested positive at this visit or known to be positive from previous visits divided by the total number of subjects for whom dengue IgG serostatus is known).
- The clinical characteristics of symptomatic dengue infection (symptoms, hospitalizations, severity) will be presented as proportions of dengue cases overall and per sub-group (type (DENV 1-4), study site, gender and age group).

Endpoint numerators and denominators are described in Table 16 below:

Table 16 Numerators and Denominators within each strata (DENV type, study site, gender and age-group and previous dengue exposure (primary or secondary)

Analysis	Numerator	Denominator
Incidence rate of laboratory confirmed symptomatic infection by year	All confirmed acute cases during the season (between the visits scheduled before and after the season)	Total person-years at risk, i.e. from the visit scheduled before the season until the first RT-qPCR confirmed symptomatic dengue infection during the season, the next scheduled visit or withdrawal, whichever comes first.
Incidence rate of laboratory-confirmed symptomatic dengue infection for all seasons combined	All confirmed acute cases during the study period	Total person-years at risk, i.e. from enrolment until the first RT-qPCR confirmed symptomatic dengue infection, the end of the study or withdrawal, whichever comes first.
Incidence rate of laboratory confirmed or probable symptomatic dengue infections	All symptomatic dengue infections (including laboratory-confirmed and probable cases) during the period.	Total person-years at risk*.
Incidence rate of probable symptomatic dengue infection	All probable symptomatic dengue infection	Total person-years at risk*.
Proportion of subjects with primary inapparent dengue infection	All dengue IgG positive cases at the considered visit	Total number of IgG negative subjects at the previous visit
Proportion of subjects with secondary symptomatic dengue infection	All symptomatic infection since the previous visit	Total number of IgG positive subjects at the previous visit
Seroprevalence of dengue infection	All dengue IgG positive cases	Total number of subjects tested or previously positive

* Time at risk: In analyses by season the time at risk is calculated as the time from the visit scheduled before the season until the first event during the season, the next scheduled visit or the subject's withdrawal, whichever comes first.
In the analyses of all seasons combined the time at risk is calculated as the time from enrolment until the first event, the end of the study or the subject's withdrawal, whichever comes first.

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

8.5.3. Analysis of symptoms and spectrum of dengue disease

The clinical characteristics of symptomatic dengue infection (symptoms, hospitalizations, severity) will be presented.

8.5.4. Analysis of tertiary objectives (Amended 16 October 2017)

Analysis of tertiary objectives is optional and may or may not be performed. If these analyses are performed, they will be conducted by the Research and Development Department. Statistical methods for tertiary objectives will be described in the SAP.

The neutralization assay, if performed, will only be done on blood samples from a subset of subjects. YF virus antibody testing will be performed retrospectively, for subjects in YF endemic region and who was tested positive for dengue.

Analysis of risk factors for symptomatic dengue

Poisson regression models will be used for exploring the risk factors (e.g. region, age and gender, etc.) for symptomatic dengue. The analyses will account for clustering of observations within households for the analyses by centre and also the stratification by centres for the overall analyses.

Incidence rates of symptomatic Zika infections

The following analyses will be performed by study site, gender and age-group, previous dengue exposure and previous Zika infection:

- ***Incidence rate of laboratory-confirmed or probable symptomatic Zika infection with 95% CI for each season separately: the numerator will be the number of subjects with lab-confirmed or probable symptomatic Zika infection during the season (between the visits scheduled before and after the season). The denominator will be the total person-years at risk, i.e. from the visit scheduled before the season until the first lab-confirmed or probable symptomatic Zika infection during the season, the next scheduled visit or the subject's withdrawal, whichever comes first.***
- ***Incidence rate of laboratory-confirmed or probable symptomatic Zika infection with 95% CI all seasons combined: the numerator will be the number of subjects with lab-confirmed or probable symptomatic Zika infection during the study Zika follow-up period. The denominator will be the total person-years at risk, i.e. from start of the study Zika follow-up period until the first lab-confirmed or probable symptomatic Zika infection, the end of the study or the subject's withdrawal, whichever comes first.***

Incidence rates of symptomatic chikungunya infections

The following analyses will be performed by study site, gender and age-group, previous dengue exposure and previous chikungunya infection:

- ***Incidence rate of laboratory-confirmed or probable symptomatic chikungunya infection with 95% CI for each season separately: the numerator will be the number of subjects with lab-confirmed or probable symptomatic chikungunya infection during the season (between the visits scheduled before and after the season). The denominator will be the total person-years at risk, i.e. from the visit scheduled before the season until the first lab-confirmed or probable symptomatic chikungunya infection during the season, the next scheduled visit or the subject's withdrawal, whichever comes first.***
- ***Incidence rate of laboratory-confirmed or probable symptomatic chikungunya infection with 95% CI all seasons combined: the numerator will be the number of subjects with lab-confirmed or probable symptomatic chikungunya infection during the study chikungunya follow-up period. The denominator will be the total person-years at risk, i.e. from start of the study chikungunya follow-up period until the first lab-confirmed or probable symptomatic chikungunya infection, the end of the study or the subject's withdrawal, whichever comes first.***

Analysis for Zika and chikungunya viruses in suspected dengue cases

The proportions and associated exact 2-sided 95% confidence intervals (CI)of the Zika and chikungunya viruses in suspected dengue cases will be summarized.

Analysis for seroprevalence of antibody titers against Zika and chikungunya viruses at selected timepoints

Seroprevalence at each selected timepoint will be calculated as a proportion i.e. the number of seropositive samples at each selected timepoint over the total number of samples with antibody results. An exact 95% CI will be computed.

8.5.5. Statistical considerations for interim analyses

Since there is no hypothesis testing, no adjustment of type I error is needed for the interim analyses.

9. ADMINISTRATIVE MATTERS

To comply with ICH GCP or other applicable guidelines administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality and publications must be fulfilled. This study will be sponsored by GSK and co-funded by GSK and Fiocruz. As study sponsor, GSK will delegate some activities to Fiocruz, according to the provisions in their Cooperative Research and Development Agreement (CRADA).

9.1. Remote Data Entry instructions

Inform, a validated computer application, will be used as the method for data collection on electronic case report form (eCRF).

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 designate. In all cases, the investigator remains accountable for the study data.

The investigator will be provided with a CD-ROM of the final version of the data generated at the investigational site once the database is archived and the clinical study report is complete and approved by all parties.

9.2. Monitoring by GSK Biologicals

Monitoring visits by a GSK Site Monitor are for the purpose of confirming that GSK Biologicals' sponsored studies are being conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and that are consistent with Good Clinical practice (GCP) or other applicable guidelines and the applicable regulatory requirement(s) (verifying continuing compliance with the protocol, amendment(s), verifying that the site staff and facilities continue to be adequate to conduct the study).

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

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform an eCRF review and a Source Document Verification (SDV). By SDV we understand verifying eCRF entries by comparing them with the source data that will be made available by the investigator for this purpose.

The Source Documentation Agreement Form describes the source data for the different data in the eCRF. This document should be completed and signed by the site monitor and investigator and should be filed in the monitor's and investigator's study file. Any data item for which the eCRF will serve as the source must be identified, agreed and documented in the source documentation agreement form.

For eCRF, the monitor could validate the screens at each visit.

In accordance with applicable regulations, GCP or other applicable guidelines and GSK procedures, 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.

The study will be monitored to verify that, among 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 and any amendments, any other study agreements, GCP or other applicable guidelines and all applicable regulatory requirements.

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

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 or other applicable guidelines and GSK procedures.

9.3. Archiving of data at study sites

Following closure of the study, the investigator must maintain all site study records in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g. audit or inspection), and, whenever feasible, to allow any subsequent review of data 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 for studies with an eCRF); however, caution needs to be exercised before such action is taken. The investigator must assure 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 there is an acceptable back-up of these reproductions and that an acceptable quality control process exists 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 that site for the study, 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 ***25*** years.

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

9.4. Audits

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

9.5. Posting of information on public registers

Study information from this protocol will be posted on public registers (e.g. GSK Clinical Study Register, clinicaltrials.gov) before enrolment of subjects begins as applicable.

9.6. Ownership, confidentiality and publication

9.6.1. Ownership

All information provided by GSK and all data and information generated by the site as part of the study (other than a subject's medical records) are owned by GSK and Fiocruz who shall each be entitled to use the results according to the provisions of their Collaborative Research and Development Agreement (CRADA) on Dengue.

All rights, title, and interests in any inventions, know-how or other intellectual or industrial property rights which are conceived or reduced to practice by site staff during the course of or as a result of the study are the sole property of GSK, and are hereby assigned to GSK

9.6.2. Confidentiality

Documented evidence that a potential investigator is aware and agrees to the confidential nature of the information related to the study must be obtained by means of a confidentiality agreement.

All information provided by GSK, and all data and information generated by the site as part of the study (other than a subject's medical records) will be kept confidential by the investigator and other site staff. This information and data will not be used by the investigator or other site personnel for any purpose other than conducting the study. These restrictions do not apply to: (i) information which becomes publicly available through no fault of the investigator or site staff; (ii) information which is necessary to disclose in confidence to an IEC or IRB solely for the evaluation of the study; (iii) information which is necessary to disclose in order to provide appropriate medical care to

a study subject; or (iv) study results which may be published as described in the next paragraph.

If a written contract for the conduct of the study which includes confidentiality provisions inconsistent with this statement is executed, that contract's confidentiality provisions shall apply rather than this statement.

9.6.3. Publication

Prior to submitting for publication, presentation, use for instructional purposes, or otherwise disclosing the study results generated by the site (collectively, a 'Publication'), the investigator shall provide GSK and Fiocruz with a copy of the proposed Publication and allow GSK and Fiocruz a period to review the proposed Publication (at least twenty-one working days, or at least fifteen working days for abstracts/posters/presentations). Proposed Publications shall not include either GSK confidential information other than the study results or personal data on any subject, such as name or initials.

At GSK's request, the submission or other disclosure of a proposed Publication will be delayed a sufficient time to allow GSK to seek patent or similar protection of any inventions, know-how or other intellectual or industrial property rights disclosed in the proposed Publication.

If a written contract for the conduct of the study, which includes publication provisions inconsistent with this statement is executed, that contract's publication provisions shall apply rather than this statement.

9.6.4. Provision of study results to investigators and publication

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 or Fiocruz site or other mutually-agreeable location.

A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

10. COUNTRY SPECIFIC REQUIREMENTS

Not applicable

11. REFERENCES (AMENDED 16 OCTOBER 2017)

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APPENDIX A STUDY LABORATORIES (Amended 16 October 2017)

GSK Biologicals' laboratories (for back up samples)

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

Non GSK laboratories

Laboratory	Address
Local laboratories	**Details presented in a separated document**
Laboratório de Tecnologia Virológica – LATEV Bio-Manguinhos Fundação Oswaldo Cruz - Fiocruz	Av. Brasil, 4365, Manguinhos Pavilhão Rocha Lima, sala 403 Rio de Janeiro, RJ. CEP: 21.040-360 Brazil

APPENDIX B STRUCTURED SCRIPT

1. Have you/has your child been sick in the past month?

( ) Yes

( ) No

2. If yes, did you/your child have:

( ) Fever

( ) Headache

( ) retro-orbital pain

( ) rash

( ) muscle or joint pain

( ) bleeding

3. Have you sought medical care due to your/your child's symptoms?
4. If yes, what was the diagnosis and where did you seek medical care?
5. Are you aware of dengue, ***chikungunya or Zika*** cases in your neighborhood during the past month?

Any subject that meets the case definition for suspected dengue***, chikungunya or Zika*** the subject (and subject's parent(s)/LAR(s) as applicable) will be referred to the study hospital according to protocol

APPENDIX C AMENDMENTS AND ADMINISTRATIVE CHANGES TO THE PROTOCOL

GlaxoSmithKline Biologicals Clinical Research & Development **Protocol Amendment 1**	
eTrack study number and Abbreviated Title	116606 (EPI-DENGUE-006 BOD BR)
Amendment 1 version and date:	Final: 14 June 2013
Coordinating author:	PPD, Scientific Writer

Rationale/background for changes:
The Brazilian IRB requested that the protocol be amended to address the shared responsibilities of Fiocruz and GSK with regard to the following statement in the protocol *Section **9.6.1. Ownership.*** According to the Brazilian resolution CNS 292/99, "The onus and benefits came from the investigation process and research results must be distributed in a fair way between the involved parts, and must be described in the protocol."
After discussions between GSK and Fiocruz legal, it was decided that Fiocruz should also have the property of study data. GSK will continue to be the only sponsor but since the study will be funded by both institutions, both will have access to study data as in accordance with CRADA.
Additional changes related to this request, and minor unrelated changes have also been made. A detailed list of changes is provided below:

Section 9.6.1 Ownership:All information provided by GSK and all data and information generated by the site as part of the study (other than a subject's medical records) are ***owned by GSK and Fiocruz who shall each be entitled to use the results according to the provisions of their Collaborative Research and Development Agreement (CRADA) on Dengue.***
Section 9.6.3 Publication
Prior to submitting for publication, presentation, use for instructional purposes, or otherwise disclosing the study results generated by the site (collectively, a 'Publication'), the investigator shall provide GSK ***and Fiocruz*** with a copy of the proposed Publication and allow GSK ***and Fiocruz*** a period to review the proposed Publication (at least twenty-one working days, or at least fifteen working days for abstracts/posters/presentations).
Section 9.6.4 Provisions of study results to investigators and publication
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 ***or Fiocruz*** site or other mutually-agreeable location.

Other minor changes:
Title page:
Added line for Amendment 1 version and date
Changed 2012 to 2012-2013 in this line: Copyright ***2012-2013*** the GlaxoSmithKline group of companies
Signature pages:
Changed heading from Protocol Sponsor Signatory Approval to Protocol Amendment 1 Sponsor Signatory Approval and same change for Investigator Agreement
Page 3: Protocol Amendment Rationale added
List of Abbreviations: added CRADA and Fiocruz
Section 5.5.6.3 ***An example of questions to be included in the*** ~~The~~ telephone script is provided in …
Added Appendix C: Amendments and Administrative Changes to the protocol.

GlaxoSmithKline Biologicals
Clinical Research & Development
Protocol Amendment 2

eTrack study number and Abbreviated Title	116606 (EPI-DENGUE-006 BOD BR)
Amendment number:	Amendment 2
Amendment 2 Final Date:	Final: 23 July 2014
Coordinating author:	PPD, Scientific Writer, Out-contractor for GSK

Rationale/background for changes:
The protocol is amended to allow for extension of the study by three years to cover three additional dengue seasons. There will be three scheduled visits (Visit 1, 2 and 3) during the first study year and one scheduled visit per year during study Year 2, Year 3 and Year 4 (Visit 4, 5 and 6). The scheduled visits will preferably take place during the low transmission period. A blood sample will be collected at each scheduled visit. The objectives have been updated to reflect the study extension.

This protocol amendment will also allow enrolment of new subjects to replace the subjects not wishing to extend their participation for three additional years, subjects who prematurely terminate participation and subjects lost to follow-up in order to maintain a cohort size of at least 1500 subjects during each year/season. A section on how subjects will be replaced was added.

An interim analyses is also planned.

Additional minor corrections have been made.

Amendment 2 List of Changes: deleted text shown with strikethrough, new text shown with bold italics

Title page:
Added line for Amendment 2 version and date.
The word “multiyear” was added to the Detailed Title as follows: A prospective, multicentre, multiyear cohort study to determine dengue incidence in children and adults in communities from endemic regions in Brazil.

Co-ordinating and contributing authors for this amendment 2 were as follows:

Co-ordinating author: PPD, Scientific Writer / PPD ***, Scientific Writer, XPE working for GSK Biologicals***

GSK Contributing authors:

PPD	***Epidemiologist, Brazil***
PPD	Epidemiologist, Senior Manager
PPD	Epidemiologist
PPD	***Project Statistician***
PPD	Project Statistician
PPD	Senior Manager Lead statistician
PPD	Study Manager
PPD	***Study Manager***
PPD	***Study Delivery Lead***
PPD	Global Study Manager (advisory role)
PPD	***Global Vaccine Clinical Laboratories Manager***
PPD	***Local Delivery Lead***
PPD	***Clinical Laboratory Project Manager***
PPD	Senior Manager, Global Study Management
PPD	Clinical Operations Manager
PPD	Clinical Laboratory Project Manager
PPD	Global Vaccine Clinical Laboratories Manager
PPD	Clinical Safety representative
PPD	Study Data Manager
PPD	***Study Data Manager***
PPD	Medical Affairs Director, Brazil
PPD	***Medical Affairs Director, Brazil***
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PPD	Principal Investigator, Manaus, Fundação de Medicina Tropical Dr. Heitor Vieira Dourado
PPD	Principal Investigator, Salvador de Bahia, Fundação Oswaldo Cruz, Ministério da Saúde
PPD	***Principal Investigator, Rio de Janeiro, Fundação Oswaldo Cruz, Ministério da Saúde***

Changed 2012-2013 to 2012-2014 in this line: Copyright ***2012-2014*** the GlaxoSmithKline group of companies

Signature pages:

Changed heading from Protocol Amendment 1 to Protocol Amendment 2 for Sponsor Signatory Approval and same change for Investigator Agreement.

Page 4: Protocol Amendment 2 Rationale added

Additional minor corrections like bulleting and colouring the table headings grey were done.

GLOSSARY OF TERMS: added Block, Initial cohort subjects, Replacement subjects, Lost to follow-up, Prematurely terminate participation and Primary dengue infection.

Section 1.1 Background: The first and the last paragraphs were changed as follows:

Dengue, the most common arthropod-borne viral disease worldwide, is caused by four types of dengue viruses (DENV 1-4), ***transmitted*** primarily by *Aedes aegypti*, a mosquito that is highly adapted to urban environments. Dengue infection can cause a range of clinical illnesses, from inapparent to a life threatening hemorrhagic disease, often associated with pre-existing heterotypic dengue virus antibodies.

In this study, we will estimate the incidence of dengue infection and disease in a cohort of subjects recruited from different geographic areas ***over time*** in Brazil. The study will also identify and train potential sites for the conduct of phase III studies in the future.

Section 2 Objectives: The objectives were changed as follows with the same changes effected in the Synopsis objectives:

Section 2.1 Primary objective

- To estimate the incidence of laboratory-confirmed symptomatic dengue infection in the study population ***by year/season.***

Section 2.2 Secondary objectives

- ***To estimate the serotype-specific incidence of virologically-confirmed symptomatic dengue infection in the study population overall and by season.***
- To estimate the incidence of symptomatic dengue infection (including laboratory-confirmed and probable cases) in the study population by ~~DENV type~~ study site, gender, age-group, previous dengue exposure (primary or secondary)***, overall and by season***.
- To estimate the prevalence of previous dengue infection (dengue seroprevalence) in the study population overall and by study site, gender and age-group ***at enrolment***.
- To estimate the incidence of primary inapparent, dengue infection, in the study population overall and by study site, gender age-group ***and by season***.

Section 2.3 Tertiary objectives (optional): The following objective was added.

- ***To describe the spatial and temporal distribution of dengue cases among cohort participants in the study areas.***

Section 3 Study design overview
This section was changed as follows in the body and respective sections of the Synopsis :

- **Type of design**: Prospective, multicenter, community-based, household-sampling, cohort study ***in Brazil.***

- ***The study period, initially planned to be one year, is extended by three years to cover three additional dengue seasons.***

- ***Initial cohort subjects will be invited to extend their participation.***

  (See glossary of terms for the definition of initial cohort subjects).

- ***Replacement subjects may be enrolled in order to compensate for subjects who do not wish to extend their participation for three additional years, subjects who prematurely terminate participation or subjects who are lost to follow-up. This will be done to maintain a cohort size of at least 1500 subjects at the beginning of each additional study year/season.***

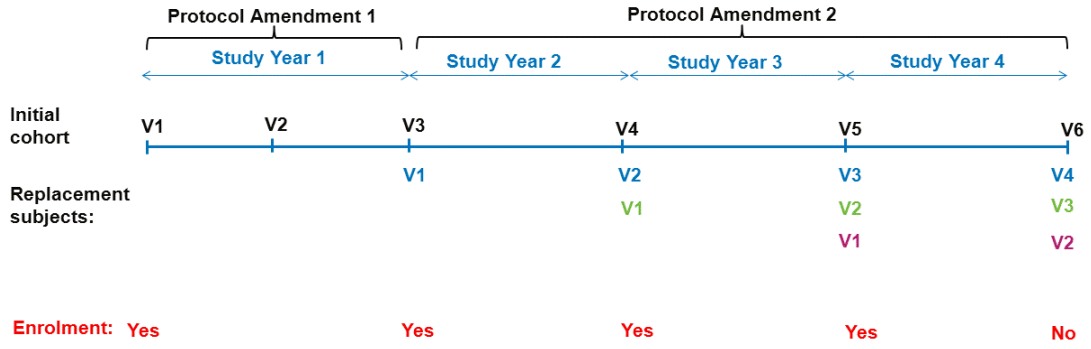
(See glossary of terms for the definition of replacement subjects, subjects lost to follow-up and subjects who prematurely terminate participation).

- ***Informed consent (and assent, if applicable) will be obtained:***
  - ***From the parents/LAR of the subjects (and from the subjects, if applicable) at the study start for the initial cohort.***
  - ***From the parents/LAR of the subjects (and from the subjects, if applicable) of the initial cohort prior to participation in the additional three years of the study.***
  - ***From the parents/LAR of the replacement subjects (and from the replacement subjects, if applicable) prior to enrolment in the study.***
- **Study visits:** ~~The study will consist of three scheduled household visits over a period of approximately one year, per subject. Preferably, the recruitment period will occur before the peak incidence of dengue based on previous years~~

  The visits will be as follows and further shown in Figure 1:
  - ***Initial cohort subjects consenting to extend participation for the three additional years will have:***
    - ***three scheduled visits in the first year (Visit 1, Visit 2 and Visit 3) at approximately six months intervals,***
    - ***and thereafter, one scheduled visit per year during a period of low dengue transmission for the three additional years; i.e; one visit at study Year 2, Year 3 and Year 4 (Visit 4, Visit 5 and Visit 6 respectively).***
  - ***Initial cohort subjects who do not consent to extend participation for three additional years will:***
    - ***Have three scheduled visits (Visit 1, Visit 2 and Visit 3) at approximately six months intervals.***
    - ***Conclude their participation in the study at Visit 3 or the last follow up visit if the subject is suspected of having a dengue case on-going at Visit 3.***
  - ***For replacement subjects, the number of scheduled visits will depend on the study year in which they are enrolled. Replacement subjects will not be enrolled until after the third visit of the first study year is concluded for the initial cohort. The number of scheduled visits will range between two and four.***

The yearly scheduled visits for the initial cohort and replacement subjects will preferably occur during the period of usually low dengue transmission.

(Synopsis) Figure 1 Schematic representation of the scheduled visits



V=Visit
The scheduled visits for initial cohort subjects are shown in black.
New enrolments will be done yearly (as needed) till the end of study Year 3.
For replacement subjects, the number of visits will depend on the year enrolled. These visits are shown in different colours for each year.

- **Duration of the study**: ~~approximately one year per subject from the time of consent~~ ***Four years for the overall study. For an individual subject, it will range between one and four years.***
  - **Epoch 001**: prospective data collection starting at Visit 1 Day 0 and ending at ~~Visit 3 Year 1~~ ***the last subject last visit***

(Synopsis)Table 2 Study groups and epochs foreseen in the study

Study Group	Number of subjects*	Age (Minimum)	Epoch
Prospective	1800 ~~(planned enrollment)~~ ***at enrolment (first year) At least 1500 in each subsequent year***	6 months	Epoch 001

****The final number of participants will take into account the active subjects, subjects who prematurely terminate participation and subjects lost to follow up.***

Section 3.1 Discussion of study design

3.1.1 Selection of study sites: Specific cities where the study will be conducted were deleted as follows:
At least three sites in Brazil covering at least 3 different regions of the country will be selected for this study~~, one each in Manaus, Salvador and Rio de Janeiro~~.

3.1.2 Rationale for study design: The second sentence in the first paragraph was modified as follows:
Data will be collected from a population of individuals aged 6 months and older for ~~at least one~~ ***maximum four*** dengue seasons.

Section 4 Study population: Specific cities where the study will be conducted were deleted as follows: This change was also implemented in the Synopsis.

Subjects six months of age and older at the time of enrolment who live in the selected study sites in Brazil, covering at least 3 different regions of the country~~.: North (Manaus), Northeast (Salvador) and Southeast (Rio de Janeiro).~~

4.2 Selection of households; Some specifics in the last paragraph were deleted and some words added as follows:

Approximately 1800 subjects~~, 600 per site~~ need to be recruited for this study in order to obtain an evaluable sample size of approximately 1500 subjects ***at the end of the first year of the study.***~~, 500 per site. Based on an average household size of 5 , approx. 125 households should be selected per site.~~

4.3 Overview of the recruitment plan; This section was changed as follows:

Subjects will be recruited from randomly selected households originating from pre-selected mapped communities. Preferably the recruitment period will occur outside of the peak dengue transmission season, ***and*** will continue until each site has reached its foreseen target. The expected period for recruiting the target sample size is approximately three months. ***Recruitment of replacement subjects will be done during the low dengue transmission and the recruitment period will depend on the number of subjects that need to be replaced.***

Recruitment will be organized by study staff at participating sites according to the appropriate strategy for each site. Community agents or equivalent will serve as the liaison to schedule ***visits*** and may accompany study staff during the visits. The study will be explained to the individuals living in the household, and if any individual is interested in study participation, informed consent (and assent when applicable) will be obtained, eligibility criteria will be checked and subjects will be enrolled and interviewed for potential study participation.

If the target household is found empty or all members refuse to participate, the first household to the left will be approached. Those households refusing to participate will be recorded as such. A household refusal will be characterized when all individuals in the household refuse to participate in the study. Individual refusals in a given household will not preclude inclusion of other housemates, and will be recorded as such.

In the subsequent study years/seasons, the cohort size will be maintained at, at least 1500 subjects. Since subjects who prematurely terminate participation and/or lost to follow-up will be replaced, the final number of participants across the four study years will exceed 1800. Yearly, there will be an evaluation of active subjects. If the number of active subjects becomes < 1500, there will be enrolment of replacement subjects to maintain a cohort size of 1500 subject. Enrolment will preferably take place during the low dengue transmission. Recruitment of replacement subjects could occur at the end of study year 1, 2 or 3. The recruitment approach will be the same as for the initial enrolment.

Household visits may be scheduled to occur during weekends ***or at convenient times (like evenings)*** if deemed necessary.

4.4 Inclusion criteria for enrolment; This section was changed as follows:

- Subject and/or subject's parent(s)/LAR(s) who the ~~investigator~~ ***study staff*** believes can comply with the requirements of the protocol (e.g., willingness to go to the hospital/clinic for visit/s if dengue is suspected, able to observe the signs of dengue and to understand how to take and report body temperature, etc.)
- Subject who plans, at the time of enrolment, to remain at same residence/study area during ***their*** study ***participation*** period.

Section 5 Study conduct

Section 5.4 Outline of study procedures; Table 2 was modified to procedures for initial cohort subjects including the three years extension and procedures for replacement subjects were included in a separate table, Table 3. Symbols for footnotes were changed to numbers.

Study procedures are outlined in Table 2 ***and Table 3 for initial cohort and replacement subjects respectively***

Table 2 List of study procedures for initial cohort subjects

	Surveillance											Suspected Dengue Visits (at hospital/clinic) as applicable*[3]		
Procedure	**Visit 1 Day 0**	**Monthly contact between visits**	**Visit 2 Month 6**	**Monthly contact between visits**	**Visit 3 ~Month 12 (Year 1)**	***Monthly contact between visits***	***Visit 4 ~Month 24 (Year 2)***	***Monthly contact between visits***	***Visit 5 ~Month 36 (Year 3)***	***Monthly contact between visits***	***Visit 6 ~Month 48 (Year 4)***	**First visit Day 0**	**Return visit **[2]**	**Follow-up visit Day 21**
Informed consent (and assent if applicable)	●													
Re-consent (and assent if applicable)					●bullet added									
Subject number and household number attribution	●													
Check inclusion/exclusion criteria	●													
Record socio-demographic information	●													
Medical history and YF vaccination history or updates	●		●		●		●		●		●			
Distribute subject ID card distribution and suspected dengue instruction kit	○													
Blood sample for serology 5 mL	●		●		●		●		●		●			
Instruct/ remind subjects/subject's parent(s)/LAR(s) on suspected dengue assessment procedures (***where applicable***)	○	○	○	○	○	○	○	○	○	○				
Issue diary logs to parents/LARs to be filled out in the event of a suspected dengue case‡[1]	○		○		○		○		○			○		
Contact subject regarding any dengue symptoms and remind subject of procedures for suspected dengue		○		○		○		○		○				
Physical examination/ record current medical conditions (see Section 5.6)												●	●	●

	Surveillance											Suspected Dengue Visits (at hospital/clinic) as applicable*[3]		
Procedure	**Visit 1 Day 0**	**Monthly contact between visits**	**Visit 2 Month 6**	**Monthly contact between visits**	**Visit 3 ~Month 12 (Year 1)**	***Monthly contact between visits***	***Visit 4 ~Month 24 (Year 2)***	***Monthly contact between visits***	***Visit 5 ~Month 36 (Year 3)***	***Monthly contact between visits***	***Visit 6 ~Month 48 (Year 4)***	**First visit Day 0**	**Return visit **[2]**	**Follow-up visit Day 21**
Blood sample for dengue infection diagnosis*[3]												●		●
Blood sample for CBC and hematocrit												●		
Record body temperature												●		
Report SAEs related to study procedures‡‡[4]	●		●		●		●		●		●	●	●	●
Collect or verify diary logs if applicable												○	○	○
Study conclusion§[5]											●			●

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

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

‡ [1]Diary logs given at scheduled visits are only to be filled out in the event that the subject has a fever or suspected dengue symptoms. See Section 5.5.2.5 for details with regard to the diary log.

** [2] A return visit is a visit linked to suspected dengue, occurring if the evolution of the subject's physical condition necessitates medical evaluation, according to local medical practice. The investigator will determine whether this return visit is necessary and whether it is considered as part of the same episode. See Section 5.6 for details.

* [3] May include testing for RT-qPCR, dengue IgG and IgM ELISA, NS1 ELISA, neutralizing antibodies to dengue and Yellow fever, as well as dengue virus isolation if deemed necessary.

‡‡[4]SAE = Serious adverse event- see Section 6.1.1 for definition.

§[5] The study conclusion information in the eCRF is collected once for each subject at the last scheduled visit or at the last follow-up contact for a suspected dengue case, should this case occur during the last scheduled visit or earlier if the subject terminates study participation or is lost to follow-up.

Table 3 List of study procedures for replacement subjects

	Surveillance							Suspected Dengue Visits (at hospital/clinic) as applicable[3]		
Procedure	Visit 1 Day 0	Monthly contact between visits	Visit 2*	Monthly contact between visits	Visit 3* as applicable	Monthly contact between visits	Visit 4 as applicable	First visit Day 0	Return visit[2]	Follow-up visit Day 21
Informed consent (and assent if applicable)	●									
Subject number and household number attribution	●									
Check inclusion/exclusion criteria	●									
Record/update socio-demographic information	●				●*					
Medical history and YF vaccination history or updates	●				●		●			
Distribute subject ID card distribution and suspected dengue instruction kit	O		O		O					
Blood sample for serology 5 mL	●		●		●		●			
Instruct/ remind subjects/subject's parent(s)/LAR(s) on suspected dengue assessment procedures (where applicable)	O	O	O	O	O	O	O			
Issue diary logs to parents/LARs to be filled out in the event of a suspected dengue case[1]	O		O		O		O	O		
Contact subject regarding any dengue symptoms and remind subject of procedures for suspected dengue		O		O		O				
Physical examination/ record current medical conditions (see Section 5.6)								●	●	●

	Surveillance							*Suspected Dengue Visits (at hospital/clinic) as applicable*[3]		
Procedure	***Visit 1 Day 0***	***Monthly contact between visits***	***Visit 2****	***Monthly contact between visits***	***Visit 3** as applicable***	***Monthly contact between visits***	***Visit 4 as applicable***	***First visit Day 0***	***Return visit*[2]**	***Follow-up visit Day 21***
***Blood sample for dengue infection diagnosis*[3]**								●		●
Blood sample for CBC and hematocrit								●		
Record body temperature								●		
***Report SAEs related to study procedures*[4]**	●		●		●		●	●	●	●
Collect or verify diary logs if applicable								O	O	O
***Study conclusion*[5]**							●			●

● 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.

**** This could be the first visit (enrolment) for some replacement subjects and the procedures for Visit 1 will be done as applicable.***

[1] Diary logs given at scheduled visits are only to be filled out in the event that the subject has a fever or suspected dengue symptoms. See Section 5.5.2.5 for details with regard to the diary log.

[2] A return visit is a visit linked to suspected dengue, occurring if the evolution of the subject's physical condition necessitates medical evaluation, according to local medical practice. The investigator will determine whether this return visit is necessary and whether it is considered as part of the same episode. See Section 5.6 for details.

[3] May include testing for RT-qPCR, dengue IgG and IgM ELISA, NS1 ELISA, neutralizing antibodies to dengue and Yellow fever, as well as dengue virus isolation if deemed necessary.

[4] SAE = Serious adverse event- see Section 6.1.1 for definition.

[5] *The study conclusion information in the eCRF is collected once for each subject at the last scheduled visit or at the last follow-up contact for the dengue case, should this case occur during the last scheduled visit or earlier if the subject prematurely terminates participation or is lost to follow-up.*

Table with intervals between visits was deleted and separate tables made for scheduled visits and suspected dengue visits as follows:

~~Interval~~	~~Optimal length of interval *~~	~~Allowed interval**~~
~~Visit 1 (Day 0) → Visit 2 (Month 6)~~	~~6 months~~	~~±28 days~~
~~Visit 2 (Month 6) → Visit 3 (Year 1)~~	~~6 months~~	~~±28 days~~
~~Interval between visit for a suspected dengue case and the final follow-up visit for a case~~	~~21 days~~	~~+7 days~~

~~*Whenever possible the investigator should arrange study visits/contacts within this interval~~
~~**Subjects will not be eligible for inclusion in the ATP cohort for analysis if they make the study visit/contact outside this interval~~

Table 4 Intervals between study visits

*Study Year**	*Visits Interval*	Optimal length of interval *	Allowed interval**
Year 1	Visit 1 (Day 0) → Visit 2 (Month 6)	6 months	±28 days
	Visit 2 (Month 6) → Visit 3 Month 12	***6 months*** ***The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission***	
Year 2	***Visit X → Visit X+1¶*** ***Note: Visit X may be visit 3 for subjects enrolled in study year 1. For subjects enrolled in study year 2, visit X would be visit 1.***	***12 months*** ***The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission***	
Year 3	***Visit X+1 → Visit X+2***	***12 months*** ***The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission***	
Year 4	***Visit X → Visit X+3***	***12 months*** ***The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission***	

*Whenever possible the investigator should arrange study visits/contacts within this interval
**Subjects might not be eligible for inclusion in the ATP cohort for analysis if they make the study visit/contact outside this interval

Table 5 Interval between visits for suspected dengue case

	Optimal length of time	Allowed interval
Suspected dengue case: Interval between first and follow-up visit	21 days	+7 days

Note that this interval applies to all subjects enrolled regardless of the year of enrolment.

Section 5.5.1.1 Informed consent; The following text was added:

- ***Initial cohort subjects will be required to have a new consent/assent (where applicable) form signed for continuous participation in the study to allow coverage of three additional dengue seasons.***

- ***Consent/assent (where applicable)will also be obtained prior to participation for replacement subjects.***

The sections below were deleted and merged into section 5.5.3 with the following additions:

~~5.5.3 Procedures at scheduled Visit 2~~

~~5.5.4 Procedures at scheduled Visit 3~~

- ~~Visit 3 will occur approximately 12 months after Visit 1.~~
- ~~A blood (serum) sample will be collected~~
- ~~Medical and vaccination histories will be updated in the eCRF~~
- ~~Any SAEs related to study procedures will be recorded.~~

Section 5.5.3 Procedures at subsequent scheduled visits (2-6, as applicable); following text was added:

- ***For initial cohort subjects***, Visit 2 will occur approximately 6 months after Visit 1 ***and Visit 3 approximately 6 months after Visit 2. Initial cohort subjects willing to extend their participation to three additional years will be re-consented at Visit 3 (ie at the end of study Year 1).***
- ***For replacement subjects enrolled in the subsequent study years, scheduled visits should ideally be performed at yearly intervals, preferably during periods of usually low dengue transmission***

Section 5.5.4 Study conclusion; The following text was added to the first paragraph:

The study conclusion screen page in the eCRF will be completed at the last study contact. This last contact could occur at ***the last scheduled visit or follow up visit for a suspected dengue case (if it is on-going at the last scheduled visit) or earlier if the subject terminates study participation or is lost to follow-up.***

Section 5.5.5.2 Case detection through enhanced passive surveillance

The last paragraph was bulleted as follows:

The study staff should ensure that:

- the acute sample will be properly collected,
- all clinical information will be retrievable,
- a return visit (if applicable) will be scheduled
- and a ***follow-up visit*** will be scheduled.

Section 5.5.5.3 Case detection through active surveillance

The second paragraph was changed as follows:

An example of questions to be included in the ~~telephone~~ ***structured*** script is provided in APPENDIX B

Section 5.6 Management of suspected dengue cases; The first and last paragraphs were changed as follows:

All study subjects with suspected dengue should be seen at a ***designated study hospital or clinic*** by the study physician.

Subjects who, for any reason, seek medical assistance at any ***non-study health care facility***, will be identified through active surveillance, and clinical data will be retrospectively collected on the eCRF. The study physician will be responsible for collecting the retrospective data and informing the local study coordinator for appropriate follow-up.

Section 5.7.1 Suspected symptomatic dengue case; The first paragraph was changed as follows:

Febrile illness with body temperature ≥38°C measured (by any route) on ***at least two consecutive days*** and less than 14 days with or without the presence of other dengue symptoms or signs, without an obvious etiology unrelated to dengue, based on investigator's judgement.

Section 5.7.2 Laboratory-confirmed symptomatic dengue case:

At least one of the following findings must be met for a laboratory-confirmed dengue case:

- Dengue virus identification through RT-qPCR on the acute serum sample
- Dengue virus NS1 positive on acute serum sample ***through ELISA*.**
- Anti-dengue IgM seroconversion between acute and convalescent serum samples ***through ELISA.***

Section 5.8.2.1 Laboratory assays; The footnote to Table 6 (Humoral immunity) was changed as follows:

*For subjects residing in ~~Manaus~~ ***a yellow fever endemic region or vaccinated against yellow fever***

Section 5.8.2.2 Laboratory read-outs; The initial Table 7: Laboratory read-outs at each time point, and priority ranking was split into scheduled visits, early presenters and late presenters as follows:

Table 7 Laboratory read-outs at each time point, and priority ranking

SCHEDULED VISITS						
Blood sampling time point		**No. Subjects (planned enrollment)**	**Subset***	**Component**	**Serum aliquot**	**Components priority rank**
Type of contact/ Amount of whole blood	**Sampling time point**					
Scheduled Visit 1 5 mL whole blood	(Day 0)	1800	-	Dengue IgG ELISA**	250 µL	1
			yes	Dengue virus 1-4 and YF neutralizing antibodies	2 X 1 mL	2
Scheduled Visit 2 5 mL whole blood	(Month 6)	1800	-	Dengue IgG ELISA**	250 µL	1
			yes	Dengue virus 1-4 and YF neutralizing antibodies	2 X 1 mL	2
Scheduled Visit 3 5 mL whole blood	(Month 12)	1800	-	Dengue IgG ELISA**	250 µL	1
			yes	Dengue virus 1-4 and YF neutralizing antibodies	2 X 1 mL	2
SUSPECTED DENGUE VISITS						
First visit- EARLY presenter (amount of blood= 5 mL for < 2 years of age and 10 mL for all other subjects)	Sample collected ≤ 5 days of fever and/or rash	unknown	-	RT-qPCR	750 µL	1
				RT-qPCR (reserve) Viral isolation and sequencing	750 µL	3
				Dengue IgM and NS1 ELISA	500 µL	2
				Dengue virus 1-4 neutralizing antibodies	2 X 1 mL or remaining volume	4
	Optional for severe cases***			PCR	200 µL	-
First visit- LATE presenter (total amount of whole blood 5 mL)	Sample collected > 5 days or more after fever/symptom/s	unknown	-	Dengue IgM and Dengue capture IgG ELISA NS1 ELISA	500 µL	1
				Dengue virus 1-4 neutralizing antibodies	2 x 1 mL or remaining volume	2
First visit (total amount of blood per local practice		unknown		CBC, HCT	No aliquot	-
Suspected dengue follow-up visit (total amount of blood 5 mL)	Approximately 21 days after first visit	unknown	-	Dengue IgM and Dengue capture IgG ELISA	500 µL	1
				Dengue virus 1-4 neutralizing antibodies	2 x 1 mL or remaining volume	2

*tertiary (exploratory) analysis, number of subjects in the subset will be determined during analysis
– not applicable
** Dengue IgG indirect ELISA
*** Sample taken per routine practice for standard patient care management

Table 9 Laboratory read-outs at each time point, and priority ranking for scheduled visits

SCHEDULED VISITS							
Blood sampling time point		**No. Subjects (anticipated)**	**Subset ***	**Possible assays**	**Serum aliquot *volume***	**Components priority rank**	***Aliquot number***
Type of contact/ Amount of whole blood	**Sampling time point**						
Scheduled Visit 1 5 mL whole blood	(Day 0)	1800	-	Dengue IgG ELISA**	250 µL	1	***1***
			yes	Dengue virus 1-4 and YF neutralizing antibodies	1 mL	2	***2***
			yes	***Dengue virus 1-4 and YF neutralizing antibodies***	***remaining volume***	***3***	***3***
Scheduled Visit 2 5 mL whole blood	***Visit 1+ 6 months for subject enrolled in Study Year 1; Visit 1+ 12 months for subject enrolled in beginning of Study Year 2, 3 or 4)***	1800	yes	Dengue IgG ELISA** ***(only for subjects IgG negative at the previous visit)***	250 µL	1	***1***
			yes	Dengue virus 1-4 and YF neutralizing antibodies	1 mL	2	***2***
			yes	***Dengue virus 1-4 and YF neutralizing antibodies***	***remaining volume***	***3***	***3***
Scheduled Visit 3 5 mL whole blood	***(Visit 2+ 6 months for subject enrolled in Study Year 1; Visit 2+12 months for subject enrolled in beginning of Study Year 2 or 3***)	***1500***	***yes***	Dengue IgG ELISA** ***(only for subjects IgG negative at the previous visit)***	250 µL	1	***1***
			yes	Dengue virus 1-4 and YF neutralizing antibodies	1 mL	2	***2***
			yes	***Dengue virus 1-4 and YF neutralizing antibodies***	***remaining volume***	***3***	***3***
Subsequent Yearly Visit(s) as applicable 5 mL whole blood	***Yearly***	***1500***	***yes***	Dengue IgG ELISA** ***(only for subjects IgG negative at the previous visit)***	***250 µL***	***1***	***1***
			yes	***Dengue virus 1-4 and YF neutralizing antibodies***	***1 mL***	***2***	***2***
			yes	***Dengue virus 1-4 and YF neutralizing antibodies***	***remaining volume***	***3***	***3***

*tertiary (exploratory) analysis, number of subjects in the subset will be determined during analysis
- not applicable,
** Dengue IgG indirect ELISA

Table 10 Laboratory read-outs at each time point, and priority ranking for suspected dengue visits for EARLY presenters

SUSPECTED DENGUE VISITS – EARLY PRESENTERS							
Blood sampling time point		**No. Subjects**	**Subset**	**Component**	**Serum aliquot volume**	**Components priority rank**	**Aliquot number**
Type of contact/ Amount of whole blood	**Sampling time point**						
First visit (amount of blood= 5 mL for < 2 years of age and 10 mL for all other subjects)	Sample collected ≤ 5 days of fever and/or rash	unknown	-	RT-qPCR	750 µL	1	1
				RT-qPCR (reserve) Viral isolation and sequencing	750 µL	3	2
				Dengue IgM and NS1 ELISA	500 µL	2	3
				Dengue virus 1-4 neutralizing antibodies	1 mL	4	4
				Dengue virus 1-4 neutralizing antibodies	***remaining volume***	***5***	***5***
	Optional for severe cases			PCR	200 µL	-	
First visit (total amount of blood per local practice		unknown		CBC, HCT	No aliquot	-	
Suspected dengue follow-up visit (total amount of blood 5 mL)	Approximately 21 days after first visit	unknown	-	Dengue IgM and Dengue capture IgG ELISA	500 µL	1	1
				Dengue virus 1-4 neutralizing antibodies	1 mL	4	4
				Dengue virus 1-4 neutralizing antibodies	***remaining volume***	***5***	***5***

- not applicable

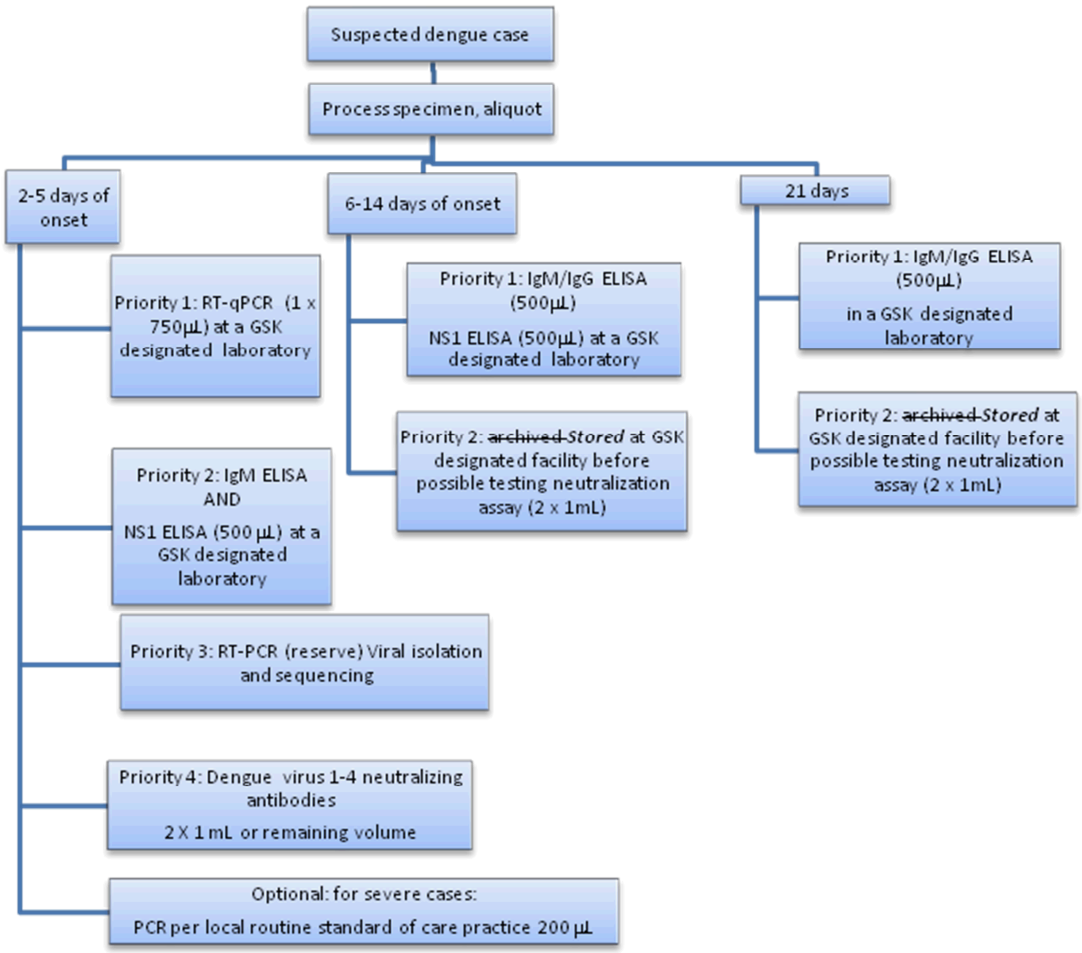
Table 11 Laboratory read-outs at each time point, and priority ranking for suspected dengue visits for LATE presenters

SUSPECTED DENGUE VISITS – LATE PRESENTERS							
Blood sampling time point		No. Subjects	Subset	Component	Serum aliquot volume	Components priority rank	Aliquot number
Type of contact/ Amount of whole blood	Sampling time point						
First visit (total amount of whole blood 5 mL)	Sample collected > 5 days or more after fever/symptom/s	unknown	-	Dengue IgM and Dengue capture IgG ELISA NS1 ELISA	500 µL	1	1
				Dengue virus 1-4 neutralizing antibodies	1 mL	2	2
				Dengue virus 1-4 neutralizing antibodies	***remaining volume***	***3***	***3***
First visit (total amount of blood per local practice		unknown		CBC, HCT	No aliquot	-	
Suspected dengue follow-up visit (total amount of blood 5 mL)	Approximately 21 days after first visit	unknown	-	Dengue IgM and Dengue capture IgG ELISA	500 µL	1	1
				Dengue virus 1-4 neutralizing antibodies	1 mL	2	2
				Dengue virus 1-4 neutralizing antibodies	***remaining volume***	***3***	***3***

- not applicable

In case of insufficient blood sample volume to perform ***all*** assays, the samples will be analyzed according to priority ranking provided in ***Table 9, Table 10 and Table 11***

Figure 4 Flow chart of serum aliquot testing for suspected dengue cases;



Section 6.2.3 Contact information for reporting serious adverse events and other events to GSK Biologicals

Study Contact for Reportable SAEs
During office hours: ***Local*** Name: Dr.-PPD PPD GlaxoSmithKline Biologicals PPD, PPD, PPD, Brazil CEP 22783-110 Email: PPD PPD Phone: PPD Cell: PPD PPD Fax: PPD Name: PPD -PPD GlaxoSmithKline Biologicals PPD PPD, PPD, PPD, Brazil 22783-110 Email: PPD PPD Phone: PPD Fax: PPD
Back-up Study Contact for Reporting SAEs
24/24 hour and 7/7 day availability: GSK Biologicals Clinical Safety & Pharmacovigilance Fax: PPD or PPD

Section 7.2 Subject withdrawal; The second paragraph was changed as follows:

All data collected until the date of withdrawal/last contact of the subject will be used for the analysis. Withdrawals/drop-outs will ~~not~~ be replaced (***see section 7.3 for further details***).

Section 7.3 on subject replacement was added as follows:

Section 7.3 Subject replacement

Enrolment of new subjects to replace:

- ***subjects not wishing to continue after this amendment, or***
- ***subjects who prematurely terminate participation or***
- ***subjects lost to follow-up will be done.***

The aim is to maintain a critical cohort size of ≥1500 subjects. This replacement will be done before each surveillance year during scheduled visits starting at the end of study Year 1 (Visit 3) up till the end of study Year 3 (Visit 5).

The number of subjects to be recruited as replacements will be defined based on the number of subjects still active in the study. If at the end of each study year we have

≥1500 subjects, no replacements will be needed. The time window for the recruitment of replacements will be during the low dengue transmission.

Section 8.1 Endpoints

Section 8.1.2 Secondary endpoints; The endpoints were updated as follows:

- DENV-type specific ~~by study site, gender, age-group, and previous dengue exposure (primary or secondary)~~ ***primary laboratory-confirmed symptomatic dengue infection***
- DENV-type specific ***secondary laboratory-confirmed symptomatic dengue infection***
- ***Primary symptomatic dengue infection (including laboratory-confirmed and probable cases)***
- ***Secondary symptomatic dengue infection (including laboratory-confirmed and probable cases)***

Section 8.1.3 Tertiary endpoints

The following endpoint was added:

- ***Spatial and temporal distribution of dengue cases (confirmed and probable symptomatic cases and inapparent infections).***

Section 8.2 Sample size consideration: This was re-written and formatted into sub sections with some rewording as follows:

~~Table 19 Precision for expected incidences of dengue for a single cohort of subjects in Brazil~~

-	-	-	-	95% CI of incidence rate (n / 100 P-Y)					
-	-	-	-	Poisson Exact CI		Normal approx.		Normal approx with design effect	
-	Number of person-years	Number of cases	Incidence of dengue (per 100 person-years)	lower	upper	lower	upper	lower	upper
Overall	2000	3	0.15	0.03	0.44	0.00	0.32	0.00	0.38
-	2000	6	0.30	0.11	0.65	0.06	0.54	0.00	0.62
-	2000	9	0.45	0.21	0.85	0.16	0.74	0.06	0.84
-	2000	12	0.60	0.31	1.05	0.26	0.94	0.14	1.06
-	2000	15	0.75	0.42	1.24	0.37	1.13	0.24	1.26
-	2000	18	0.90	0.53	1.42	0.48	1.32	0.34	1.46
-	2000	21	1.05	0.65	1.61	0.60	1.50	0.45	1.65
-	1800	3	0.17	0.03	0.49	0.00	0.36	0.00	0.42
-	1800	6	0.33	0.12	0.73	0.07	0.60	0.00	0.69
-	1800	9	0.50	0.23	0.95	0.17	0.83	0.06	0.94
-	1800	12	0.67	0.34	1.16	0.29	1.04	0.16	1.17
-	1800	15	0.83	0.47	1.37	0.41	1.26	0.27	1.40

-	-	-	-	95% CI of incidence rate (n / 100 P-Y)					
-	-	-	-	Poisson Exact CI		Normal approx.		Normal approx with design effect	
-	Number of person-years	Number of cases	Incidence of dengue (per 100 person-years)	lower	upper	lower	upper	lower	upper
-	1800	18	1.00	0.59	1.58	0.54	1.46	0.38	1.62
-	1800	21	1.17	0.72	1.78	0.67	1.67	0.50	1.84
-	1500	3	0.20	0.04	0.58	0.00	0.43	0.00	0.50
-	1500	6	0.40	0.15	0.87	0.08	0.72	0.00	0.83
-	1500	9	0.60	0.27	1.14	0.21	0.99	0.07	1.13
-	1500	12	0.80	0.41	1.40	0.35	1.25	0.19	1.41
-	1500	15	1.00	0.56	1.65	0.49	1.51	0.32	1.68
-	1500	18	1.20	0.71	1.90	0.65	1.75	0.46	1.94
-	1000	3	0.30	0.06	0.88	0.00	0.64	0.00	0.76
-	1000	6	0.60	0.22	1.31	0.12	1.08	0.00	1.24
-	1000	9	0.90	0.41	1.71	0.31	1.49	0.11	1.69
-	1000	12	1.20	0.62	2.10	0.52	1.88	0.29	2.11
-	1000	15	1.50	0.84	2.47	0.74	2.26	0.48	2.52
-	1000	18	1.80	1.07	2.84	0.97	2.63	0.68	2.92
site	500	3	0.60	0.12	1.75	0.00	1.28	0.00	1.51
-	500	6	1.20	0.44	2.61	0.24	2.16	0.00	2.49
-	500	9	1.80	0.82	3.42	0.62	2.98	0.22	3.38
-	500	12	2.40	1.24	4.19	1.04	3.76	0.58	4.22
-	500	15	3.00	1.68	4.95	1.48	4.52	0.96	5.04
-	500	18	3.60	2.13	5.69	1.94	5.26	1.37	5.83

*If three study sites and same number of subjects enrolled per region.

Section 8.2.1 Assumptions:

It is planned to obtain approximately 1500 subjects still active at the end of the first year of the study. Because subjects might leave the study due to a variety of reasons, e.g. moving away geographically, sample size is increased to allow for this., If we assume a ***an annual*** drop-out rate of about ***between*** 15 and 20% during the one-year follow-up period then we will need to recruit a total of about 1800 subjects to this study will be considered***.*** About ***Therefore, approximately*** 1800 subjects will be selected in ***enrolled at the beginning of*** this study using cluster sampling ***, i.e. the unit of sampling will be the households***. ***At least*** T three areas where the FHP has been fully implemented or where access to the community is already established by a government program will be first selected, followed by a selection of households in the respective areas. All subjects in each household will be recruited ***invited for recruitment*** to the study. ***The study will last approximately 4 years, and new subjects will be enrolled between seasons if the number of subjects still active in the study is below 1500.***

Between 2007 and 2010, the average incidence of reported dengue cases in the Brazilian population was about 0.3% per year (or 0.003 cases per person per year). Assuming an underreporting factor of 3 (Brazil MoH, 2011) the average incidence of dengue is expected to be about 1% per year (or 0.01 cases per person per year).

Section 8.2.2 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 1.8 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 3. Although in theory *rho* can have a value up to 1, in practice values higher than 0.4 are uncommon. We used a conservative estimate of 0.4 for this study (Bennett, 1991).The design effect is then estimated to 1.8.

Section 8.2.3 Expected precision of incidence rate estimates

Table 13 shows the 95% CI for a range of expected incidences based on the Poisson distribution (exact method, normal approximation and the normal approximation accounting for a design effect of 1.8). With an overall sample size of a minimum of about 1,800 subjects enrolled and a follow-up period of one year, the 95% confidence interval (CI) for an expected incidence of dengue of 9 cases per 1000 person-years using a cluster design is [2.9 ; 15.1]. ***With 4 years of follow-up and new enrolments to keep the number of subjects above 1500 at the beginning of each subsequent season, the 95% CI is [5.7 ; 12.3].***

Table 13 Precision for expected incidences of dengue for a single cohort of subjects in Brazil

	Cohort size	Number of years	Number of person-years	Number of cases	Incidence of dengue (per 1000 person-years)	95% CI of incidence rate (n / 1000 P-Y)					
						Exact (Poisson)		Normal approx		Normal approx with design effect	
						Lower	Upper	Lower	Upper	Lower	Upper
Overall	2000	1	1845.9	6	3.0	1.0	6.7	0.5	5.5	-0.4	6.4
	2000	1	1845.9	11	6.0	3.0	10.7	2.5	9.5	1.3	10.7
	2000	1	1845.9	17	9.0	5.2	14.5	4.7	13.3	3.2	14.8
	2000	1	1845.9	22	12.0	7.5	18.1	7.0	17.0	5.3	18.7
	2000	1	1845.9	28	15.0	9.9	21.7	9.4	20.6	7.5	22.5
	2000	4	6183.9	19	3.0	1.8	4.7	1.6	4.4	1.2	4.8
	2000	4	6183.9	37	6.0	4.2	8.3	4.1	7.9	3.4	8.6
	2000	4	6183.9	56	9.0	6.8	11.7	6.6	11.4	5.8	12.2
	2000	4	6183.9	74	12.0	9.4	15.0	9.3	14.7	8.3	15.7
	2000	4	6183.9	93	15.0	12.1	18.4	11.9	18.1	10.9	19.1
	1800	1	1661.3	5	3.0	0.9	7.2	0.4	5.6	-0.5	6.5
	1800	1	1661.3	10	6.0	2.8	11.1	2.3	9.7	1.0	11.0
	1800	1	1661.3	15	9.0	5.0	14.9	4.4	13.6	2.9	15.1
	1800	1	1661.3	20	12.0	7.3	18.6	6.7	17.3	4.9	19.1
	1800	1	1661.3	25	15.0	9.7	22.1	9.1	20.9	7.1	22.9
	1800	4	5842.4	18	3.0	1.8	4.8	1.6	4.4	1.1	4.9
	1800	4	5842.4	35	6.0	4.2	8.3	4.0	8.0	3.3	8.7
	1800	4	5842.4	53	9.0	6.7	11.8	6.6	11.4	5.7	12.3
	1800	4	5842.4	70	12.0	9.4	15.1	9.2	14.8	8.2	15.8
	1800	4	5842.4	88	15.0	12.0	18.5	11.9	18.1	10.8	19.2
	1500	1	1384.5	4	3.0	0.8	7.6	0.1	5.9	-0.9	6.9
	1500	1	1384.5	8	6.0	2.6	11.7	1.9	10.1	0.5	11.5
	1500	1	1384.5	12	9.0	4.7	15.6	4.0	14.0	2.3	15.7
	1500	1	1384.5	17	12.0	6.9	19.3	6.2	17.8	4.3	19.7
	1500	1	1384.5	21	15.0	9.2	22.9	8.5	21.5	6.3	23.7
	1500	4	5537.8	17	3.0	1.7	4.8	1.6	4.4	1.1	4.9
	1500	4	5537.8	33	6.0	4.1	8.4	4.0	8.0	3.3	8.7
	1500	4	5537.8	50	9.0	6.7	11.9	6.5	11.5	5.6	12.4
	1500	4	5537.8	66	12.0	9.3	15.2	9.1	14.9	8.1	15.9
	1500	4	5537.8	83	15.0	12.0	18.6	11.8	18.2	10.7	19.3

	Cohort size	Number of years	Number of person-years	Number of cases	Incidence of dengue (per 1000 person-years)	95% CI of incidence rate (n / 1000 P-Y)					
						Exact (Poisson)		Normal approx		Normal approx with design effect	
						Lower	Upper	Lower	Upper	Lower	Upper
Site	600	1	553.8	2	3.0	0.2	12.3	-1.6	7.6	-3.1	9.1
	600	1	553.8	3	6.0	1.2	17.0	-0.5	12.5	-2.7	14.7
	600	1	553.8	5	9.0	2.7	21.4	1.1	16.9	-1.6	19.6
	600	1	553.8	7	12.0	4.6	25.1	2.9	21.1	-0.2	24.2
	600	1	553.8	8	15.0	6.5	29.2	4.8	25.2	1.3	28.7
	600	4	1947.5	6	3.0	1.0	6.7	0.6	5.4	-0.3	6.3
	600	4	1947.5	12	6.0	3.1	10.6	2.6	9.4	1.4	10.6
	600	4	1947.5	18	9.0	5.3	14.3	4.8	13.2	3.3	14.7
	600	4	1947.5	23	12.0	7.6	17.9	7.1	16.9	5.5	18.5
	600	4	1947.5	29	15.0	10.1	21.5	9.6	20.4	7.7	22.3
	500	1	461.5	1	3.0	0.1	14.3	-2.0	8.0	-3.7	9.7
	500	1	461.5	3	6.0	1.0	18.6	-1.1	13.1	-3.5	15.5
	500	1	461.5	4	9.0	2.5	22.7	0.3	17.7	-2.6	20.6
	500	1	461.5	6	12.0	4.2	26.8	2.0	22.0	-1.4	25.4
	500	1	461.5	7	15.0	5.8	31.2	3.8	26.2	0.0	30.0
	500	4	1845.9	6	3.0	1.0	6.7	0.5	5.5	-0.4	6.4
	500	4	1845.9	11	6.0	3.0	10.7	2.5	9.5	1.3	10.7
	500	4	1845.9	17	9.0	5.2	14.5	4.7	13.3	3.2	14.8
	500	4	1845.9	22	12.0	7.5	18.1	7.0	17.0	5.3	18.7
	500	4	1845.9	28	15.0	9.9	21.7	9.4	20.6	7.5	22.5

Section 8.4.1 Sequence of analysis; The following text was added:

An interim analysis will be performed when all active subjects have achieved the visit 4. All analyses of primary and secondary objectives will be performed on data as cleaned as possible.

The final analysis will be performed when all prospective data have been collected and cleaned. ~~An interim analysis will not be performed.~~

Section 8.5.1 Analysis of demographics/baseline characteristics; The following words were added:

Socio-demographic and patient characteristics (e.g., age at study ***enrol~~l~~ment***, gender, household conditions, medical history and vaccination history) will be summarized overall and by region***, and at the beginning of each season*** using descriptive statistics.

Section 8.5.2 Analysis of primary and secondary endpoints

The following analyses will be performed ***overall and by DENV type, study site, gender and age-group and previous dengue exposure (Yes = DENV IgG antibodies at previous visit and No = no DENV IgG antibodies at previous visit)***:

- Incidence rate of laboratory-confirmed symptomatic dengue infection with 95% CI ***for each season separately***: the numerator will be the number of subjects with lab-confirmed symptomatic dengue infection during the ~~study period~~ ***season (between the visits scheduled before and after the season)***. The denominator will be the total person-years at risk***, i.e. from the visit scheduled before the season until the first RT-qPCR confirmed symptomatic dengue infection during the season, the next scheduled visit or the subject's withdrawal, whichever comes first.***
- ***Incidence rate of laboratory-confirmed symptomatic dengue infection with 95% CI all seasons combined: the numerator will be the number of subjects with lab-confirmed symptomatic dengue infection during the study period. The denominator will be the total person-years at risk, i.e. from enrolment until the first RT-qPCR confirmed symptomatic dengue infection, the end of the study or the subject's withdrawal, whichever comes first.***

The following analyses will be performed by study site, gender and age-group and previous dengue exposure (primary or secondary) :

- Incidence rate of laboratory confirmed or probable symptomatic dengue infections ***with 95% CI*** ~~by type (DENV 1-4), study site, gender and age group and previous dengue exposure (primary or secondary).~~***for each season separately and overall.*** The numerator will be the number of subjects with symptomatic dengue infection (including laboratory-confirmed and probable cases) ***during the period.*** The denominator will be the total person-years at risk.
- Incidence rate of probable symptomatic dengue infection ***with 95% CI for each season separately and overall***. The numerator will be the number of subjects with ***probable symptomatic dengue infection***. The denominator will be the total person-years at risk.

The following analyses will be performed by study site, gender and age-group:

- The proportion of subjects with primary inapparent dengue infection at at ~~6 and 12 months~~ ***each subsequent scheduled visit (not at enrolment)*** with 95% CI will be calculated as the number of dengue IgG positive cases and ~~acute cases at enrollment~~ ***at the visit*** divided by the total number of subjects ~~enrolled at baseline~~ ***IgG negative at the previous visit.***
- ~~Incidence rate of primary inapparent dengue infection with 95% CI : the numerator will be the number of cases of inapparent dengue infection and the denominator will be the total person-years at risk.~~
- The proportion of subjects with secondary symptomatic dengue infection ***(probable or confirmed) at each subsequent scheduled visit (not at enrolment) with 95% CI*** will be calculated as the number of subjects with secondary symptomatic infection ***since the previous visit*** divided by the total number of ***IgG positive*** subjects ~~with confirmed symptomatic dengue infection and IgG positive at visit 1 or baseline), with 95% CI.~~***at the previous visit.***
- The (crude) seroprevalence of dengue infection will be calculated at ***enrolment and at each scheduled visit*** as a proportion (i.e., the number of dengue IgG positive ~~cases and acute cases at enrollment divided by the total number of subjects enrolled~~ ***subjects tested positive at this visit or known to be positive from previous visits divided by the total number of subjects for whom dengue IgG serostatus is known)***.
- The clinical characteristics of symptomatic dengue infection (symptoms, hospitalizations, severity) will be presented as proportions of ~~mild, moderate and severe~~ dengue cases overall and per sub-group (type (DENV 1-4), study site, gender and age group).

~~Table 10 Endpoint Numerators and Denominators~~

~~Endpoint~~	~~Numerator~~	~~Denominator~~
~~Prevalence of past dengue infection at enrollment~~	~~Dengue IgG positive + acute cases at enrolment~~	~~All individuals enrolled~~
~~Incidence rate of laboratory confirmed symptomatic infection~~	~~All confirmed acute cases after enrollment~~	~~Total person-years at risk~~
~~Incidence rate of primary inapparent infection~~	~~Dengue IgG positive at 6 months or 12 months without history of fever~~	~~Total person-years at risk*~~
~~Cumulative incidence of primary inapparent infection over 6 months~~	~~Dengue IgG positive at 6 months and IgG negative at baseline (enrollment), without history of fever~~	~~Number of subjects IgG negative at baseline~~
~~Cumulative incidence of primary inapparent infection over 12 months~~	~~Dengue IgG positive at 6 months or at 12 months, and IgG negative at baseline without history of fever~~	~~Number of subjects IgG/IgM negative at baseline~~
~~Cumulative incidence of secondary symptomatic infection~~	~~All secondary symptomatic cases~~	~~Individuals with dengue IgG at baseline~~

~~* Time at risk is calculated as the time during which a subject is in the study population and remains dengue IgG negative at baseline (Visit 1) and/or at 6 months, and therefore at risk of seroconversion in 6 months (Visit 2) or 12 months (Visit 3), respectively.~~

Table 14 Numerators and Denominators within each strata (DENV type, study site, gender and age-group and previous dengue exposure (primary or secondary)

Analysis	Numerator	Denominator
Incidence rate of laboratory confirmed symptomatic infection by year	*All confirmed acute cases during the season (between the visits scheduled before and after the season)*	*Total person-years at risk, i.e. from the visit scheduled before the season until the first RT-qPCR confirmed symptomatic dengue infection during the season, the next scheduled visit or withdrawal, whichever comes first.*
Incidence rate of laboratory-confirmed symptomatic dengue infection for all seasons combined	*All confirmed acute cases during the study period*	*Total person-years at risk, i.e. from enrolment until the first RT-qPCR confirmed symptomatic dengue infection, the end of the study or withdrawal, whichever comes first.*
Incidence rate of laboratory confirmed or probable symptomatic dengue infections	*All symptomatic dengue infections (including laboratory-confirmed and probable cases) during the period.*	*Total person-years at risk*.*
Incidence rate of probable symptomatic dengue infection	*All probable symptomatic dengue infection*	*Total person-years at risk*.*
Proportion of subjects with primary inapparent dengue infection	*All dengue IgG positive cases at the considered visit*	*Total number of IgG negative subjects at the previous visit*
Proportion of subjects with secondary symptomatic dengue infection	*All symptomatic infection since the previous visit*	*Total number of IgG positive subjects at the previous visit*
Seroprevalence of dengue infection	*All dengue IgG positive cases*	*Total number of subjects tested or previously positive*

** Time at risk: In analyses by season the time at risk is calculated as the time from the visit scheduled before the season until the first event during the season, the next scheduled visit or the subject's withdrawal, whichever comes first.*
In the analyses of all seasons combined the time at risk is calculated as the time from enrolment until the first event, the end of the study or the subject's withdrawal, whichever comes first.

CIs ***for incidence rates and proportions*** will account for clustering of observations within households ~~and will be calculated using generalized estimating equations (GEE) assuming an exchangeable correlation matrix and robust variance estimation.~~ ***for the analyses by centre and also the stratification by centres for the overall analyses. Details of the methods will be described in the SAP.***

Section 8.5.4 Analysis of tertiary objectives

Analysis of tertiary objectives is optional and may or may not be performed. If these analyses are performed, they will be conducted by the Research and Development Department. ***Statistical methods for tertiary objectives will be described in the SAP.***

The neutralization assay, if performed, will only be done on blood samples from a subset of subjects. YF virus antibody testing will be performed retrospectively, for subjects in ~~Manaus~~ ***YF endemic region and*** who test***ed*** positive for dengue.

Analysis of risk factors for symptomatic dengue

~~In this study, we will use Generalized Estimating Equations (GEE) to obtain population-averaged estimates for clustered data (so-called marginal model). The GEE estimates the within-cluster similarity of the residuals, and then uses this estimated correlation to re-estimate the regression parameters and to calculate robust standard errors. To use GEE, we need to specify how we think observations in our data are correlated with each other. Here, we assume an *exchangeable* correlation structure/matrix also known as *compound symmetry*. This means that within a cluster (house-hold) any two observations are equally correlated, but that there is no correlation between observations from different clusters (house-holds). This structure is fully characterized by one correlation parameter which is the intra-class correlation coefficient.~~

~~A~~ Poisson regression model***s*** ~~using the GEE approach (using the GENMOD procedure in SAS)~~ will be used for exploring the risk factors (e.g. region, age and gender, etc.) for symptomatic dengue. ~~The saturated model (including all potential risk factors) and the model obtained after backward selection at P<0.05 level will be presented. Analyses will be performed using SAS 9.2~~. ***The analyses will account for clustering of observations within households for the analyses by centre and also the stratification by centres for the overall analyses.***

Section 8.5.5 Statistical considerations for interim analyses

~~No interim analysis is planned.~~ ***Since there is no hypothesis testing, no adjustment of type I error is needed for the interim analyses.***

Section 9.1 Remote Data Entry instructions; The first paragraph was changed as follows:

~~Remote Data Entry (RDE)~~***Inform*** (***electronic case report form ,eCRF),*** a validated computer application, will be used as the method for data collection.

Section 9.2 Monitoring by GSK Biologicals: RDE was replaced with eCRF including other changes as follows:

Monitoring visits by a GSK Site Monitor are for the purpose of confirming that GSK Biologicals' sponsored studies are being conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and that are consistent with Good Clinical practice (GCP) or other applicable guidelines and the applicable regulatory requirement(s) (verifying continuing compliance with the protocol, amendment(s), verifying that the site staff and facilities continue to be adequate to conduct the study).

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

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform an ~~RDE~~ ***eCRF*** review and a Source Document Verification (SDV). By SDV we understand verifying ~~RDE~~ ***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 ~~RDE~~ ***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 ~~RDE~~ ***eCRF*** will serve as the source must be identified, agreed and documented in the source documentation agreement form.

For ~~RDE~~ ***eCRF,*** the monitor ***could validate the screens*** at each visit.

In accordance with applicable regulations, GCP or other applicable guidelines and GSK procedures, 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.

The study ***will be monitored*** to verify that, among others, the:

APPENDIX A **STUDY LABORATORIES**: Non GSK laboratories were updated as follows:

Non GSK laboratories

Laboratory	Address
Fundação de Medicina Tropical ***Dr. Heitor Vieira Dourado - FMT Gerência de Virologia***	~~Dr. Heitor Vieira Dourado - FMT~~ ~~Gerência de Virologia~~ Av. Pedro Teixeira, 25, Dom Pedro Manaus, AM. CEP: 69.040-000 Brazil
Centro de Pesquisas Gonçalo Moniz - CPqGM Fundação Oswaldo Cruz - FiocruzLaboratório de Patologia e Biologia Molecular - ***LPBM***	Rua Waldemar Falcão, 121, Candeal Salvador, BA. CEP: 40.296-710 Brazil
I***nstituto de Pesquisa Clínica Evandro Chagas - IPEC, Fundação Oswaldo Cruz - Fiocruz*** ***Laboratório de Imunodiagnóstico*** ~~Bio-Manguinhos~~ ~~LATEV~~	Av. Brasil, 4365, Manguinhos ~~Pavilhão Rocha Lima sala 403~~ Rio de Janeiro, ~~21040-360~~ ***RJ. CEP: 21.040-900*** Brazil
Laboratório de Tecnologia Virológica – LATEV Bio-Manguinhos ***Fundação Oswaldo Cruz*** - Fiocruz	Av. Brasil, 4365, Manguinhos Pavilhão Rocha Lima, sala 403 Rio de Janeiro, RJ. CEP: 21.040-360 Brazil

APPENDIX B ~~TELEPHONE~~ ***STRUCTURED*** SCRIPT

GlaxoSmithKline Biologicals

Clinical Research & Development

Protocol Amendment 3

eTrack study number and Abbreviated Title	116606 (EPI-DENG-006 BOD BR)
Amendment 3 version and date:	Final: 09 January 2015

Rationale/background for changes:

- The protocol is amended to increase the size of the study population and expand the number of sites. The target number of subjects to be recruited in the new expansion sites is 1800: per site, it is set at 600 subjects at initiation and should be maintained to at least 500 subjects at the beginning of each subsequent study year. This improved regional coverage should allow to better capture the diversity of dengue epidemiology in the different regions of Brazil and strengthen the operational preparation of sites for potential future dengue vaccine efficacy trials.
- In order to better represent the Brazilian population distribution, a criteria for the age stratification of the population has been defined. In the first two sites (initial cohort) having completed enrolment, it has been observed that children were slightly less represented than in the Brazilian population (19% in the sites versus 33% in the country) and that older adults over 50 years of age were slightly over-represented (28.8% in the sites versus 20% in the Brazilian population). Thus, we proposed that subjects enrolled in the expansion cohort should be composed of at least 30% of children and should not exceed 20 % of adults 50 years or older. Subjects enrolled as replacement subjects for the initial and expansion cohort will be recruited to approach as much as possible a minimal representation of 30% of children and a maximal representation of 20% for adults 50 years or older.
- Clarification: the determination of whether a subject with dengue suspicion is either an early or a late presenter is based on the time of onset of fever.
- It is highlighted within the document that the indirect DENV IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for DENV IgG at the previous scheduled visit. A footnote was added in the study procedures tables and a note in the Procedures at subsequent scheduled visits section.
- The criteria to determine the trigger of the interim analysis have been further defined. The differences in the study start in the initial sites have led to the necessity of updating the scope of the interim analysis.
- The local laboratories details from APPENDIX A were deleted since new laboratories will be identified for testing study samples in the additional sites. The information with the details for all the local laboratories will be submitted in a separated document.

Coordinating author: PPD, Scientific Writer, XPE Pharma & Science for GSK Biologicals

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

Cover page

GSK Contributing authors:	
PPD	***Global Vaccine Clinical Laboratories Manager***
PPD	***Global Vaccine Clinical Project Manager***
PPD	***Study Data Manager***
Non-GSK Contributing authors	
PPD	***Bio-Manguinhos, Fiocruz***

Synopsis

Study design

- ***2 waves of enrolment for recruiting 3600 subjects:***
  - ***Initial Cohort = about 1800 subjects – up to 6 visits (Amendment 2) – INITIAL sites***
  - ***Expansion Cohort = about 1800 subjects – up to 4 visits (Amendment 3) – NEW sites***
- The study period, initially planned to be one year, ***has been***~~is~~ extended by three years to cover three additional dengue seasons ***(Amendment 2)***.
- Initial cohort subjects ~~will be~~***were*** invited to extend their participation.

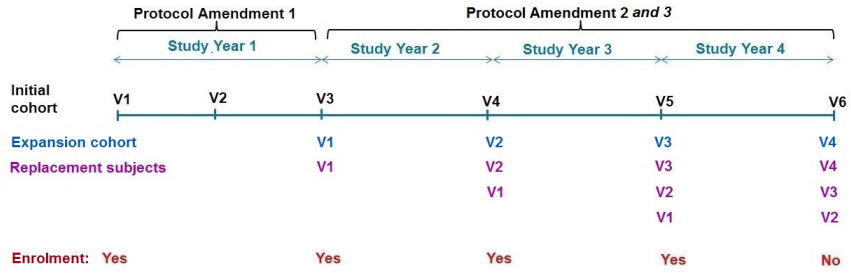
  (See glossary of terms for the definition of initial ***and expansion*** cohort subjects)
- ***In each cohort: r***~~R~~eplacement subjects may be enrolled in order to compensate for subjects who d***id***~~o~~ not wish to extend their participation for three additional years ***(for subjects from the initial cohort – Amendment 2)***, subjects who prematurely terminate participation or subjects who are lost to follow-up.

  This will be done to maintain a cohort size of at least ***500 subjects per site, in the Initial and Expansion cohorts,***~~1500 subjects~~ at the beginning of each additional study year/season.
- Informed consent (and assent, if applicable) will be obtained:
  - ***From the parents/LAR of the subjects (and from the subjects, if applicable) at the study start for the expansion cohort.***
  - ***Subjects from the expansion cohort will have only three years follow-up, with at most 4 scheduled visits.***
  - For replacement subjects, the number of scheduled visits will depend on the study year in which they are enrolled. Replacement subjects will not be enrolled until after the third

visit of the first study year is concluded for the initial cohort. The number of visits for the replacement subjects will range between two and four.

The yearly scheduled visits for the initial ***and expansion*** cohort***s*** and replacement subjects will preferably occur during the period of usually low dengue transmission.

Synopsis Figure 1 Schematic representation of the scheduled visits



V= Visit
The scheduled visits for initial cohort subjects are shown in black.
New enrolments will be done yearly (as needed) till the end of Study Year 3.
Enrollment of expansion subjects will start preferably during study Year 2.
For replacement subjects, the number of visits will depend on the year enrolled. ~~These visits are shown in different colours for each year.~~

Synopsis Table 1 Study group and epoch foreseen in the study

Study Group	Number of subjects*	Age (Min/Max)	Epoch
Prospective study cohort	***Initial cohort:*** 1800 at enrolment (first year) At least 1500 in each subsequent year	6 months of age and older	Epoch 001
	Expansion Cohort: 1800 at enrolment (first year). At least 1500 in each subsequent year		

* The final number of participants will take into account the active subjects, subjects who prematurely terminate participation and subjects lost to follow-up.

Discussion of study design

Selection of study sites

At least ~~three~~ ***six*** sites in Brazil will be selected for this study. Selection criteria include support from the Family Health Physician Program (FHP), the Larval Index Rapid Assay (LIRA) or with field research experience in the community. The FHP is a Brazilian government public health strategy that consists of multidisciplinary teams of health care workers that cover geographically defined areas to provide basic/preventive care to community members. The teams include a doctor, a nurse, a nurse auxiliary, other health professionals and community health care workers who pay regular visits to the household

under its responsibility to deliver health promotion activities and facilitate triage and referral into the health unit, among other activities. Community health care workers are local residents who are trained for basic tasks.

Number of subjects

Approximately ~~1800~~ ***3600*** subjects will be ~~initially~~ recruited in the study ***in 2 waves of enrolment. Each wave, initial and expansion cohorts, will enrol about 1800 subjects***. Replacement subjects may be recruited afterwards to compensate for subjects not willing to extend participation for the three additional years, subjects who prematurely terminate participation and subjects lost to follow-up. The recruitment of replacement subject aims at maintaining the number of subjects at a minimum of ~~1~~500 ***subjects/site*** at the beginning of study Year 2, 3 and 4. The total number of subjects enrolled in the study ~~will~~ ***may*** exceed ~~1800~~***3600***.

In order to better represent the Brazilian population distribution, subjects enrolled in the expansion cohort should be composed of at least 30% of subjects younger than 18 years old and should not exceed 20 % of adults 50 years or older. Subjects enrolled as replacement subjects for the initial and expansion cohort will be recruited to approach as much as possible a minimal representation of 30% of subjects younger than 18 years old and a maximal representation of 20% for adults 50 years or older.

GLOSSARY OF TERMS

Early presenter	***A suspected dengue case presenting at the health care facility within 5 days following the onset of fever.***
Expansion cohort subjects	***Subjects recruited in the additional sites for study expansion***
Late presenter	***A suspected dengue case presenting at the health care facility 6 days or more after the onset of fever***
Replacement subjects	These are subjects that will be enrolled to compensate for subjects: • ***from initial cohort:*** who will not want to extend participation for three additional years or • ***from initial and expansion cohorts:*** ~~subjects~~ who prematurely terminate participation or subjects lost to follow-up.

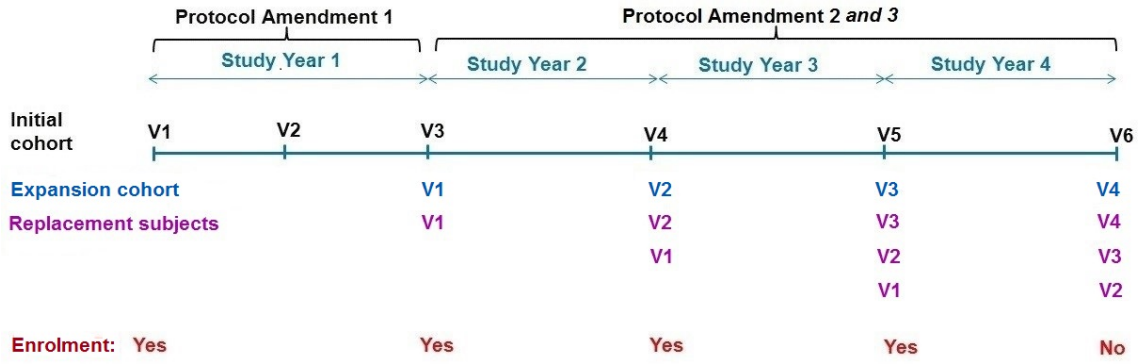
Section 3. STUDY DESIGN OVERVIEW

- The study period, initially planned to be one year, ***has been*~~is~~** extended by three years to cover three additional dengue seasons **(Amendment 2)**.
- Initial cohort subjects ~~will be~~***were*** invited to extend their participation.
- (See glossary of terms for the definition of initial ***and expansion*** cohort subjects).
- ***In each cohort: r*~~R~~**eplacement subjects may be enrolled in order to compensate for subjects who ~~do~~ ***did*** not wish to extend their participation for three additional years ***(for subjects from the initial cohort – Amendment 2)***, subjects who prematurely terminate participation or subjects who are lost to follow-up.

  This will be done to maintain a cohort size of at least ***500 subjects per site, in the Initial and Expansion cohorts,*** ~~1500 subjects~~ at the beginning of each additional study year/season.
- Informed consent (and assent, if applicable) will be obtained:
  - ***From the parents/LAR of the subjects (and from the subjects, if applicable) at the study start for the expansion cohort.***
  - From the parents/LAR of the replacement subjects (and from the replacement subjects, if applicable) prior to enrolment in the study.
- ***2 waves of enrolment for recruiting 3600 subjects:***
  - ***Initial Cohort = about 1800 subjects – up to 6 visits (Amendment 2) – INITIAL sites***
  - ***Expansion Cohort = about 1800 subjects – up to 4 visits (Amendment 3) – NEW sites***
- **Study visits:** The visits will be as follows and further shown in Figure 1:
  - ***Subjects from the expansion cohort will have only three years follow-up, with at most 4 scheduled visits.***
  - For replacement subjects, the number of scheduled visits will depend on the study year in which they are enrolled. Replacement subjects will not be enrolled until after the third visit of the first study year is concluded for the initial cohort. The number of scheduled visits will range between two and four.

    The yearly scheduled visits for the initial ***and expansion*** cohort***s*** and replacement subjects will preferably occur during the period of usually low dengue transmission.

Figure 1 Schematic representation of the scheduled visits



V=Visit
The scheduled visits for initial cohort subjects are shown in black.
New enrolments will be done yearly (as needed) till the end of study Year 3.
Enrollment of expansion cohort subjects will start preferably during study Year 2.
For replacement subjects, the number of visits will depend on the year enrolled. ~~These visits are shown in different colours for each year.~~

Table 1 Study groups and epochs foreseen in the study

Study Group	Number of subjects*	Age (Minimum)	Epoch
Prospective study cohort	***Initial cohort:*** 1800 at enrolment (first year) At least 1500 in each subsequent year	6 months and older	Epoch 001
	Expansion Cohort: 1800 at enrolment (first year). At least 1500 in each subsequent year		

*The final number of participants will take into account the active subjects, subjects who prematurely terminate participation and subjects lost to follow up.

Section 3.1.1. Selection of study sites

At least ~~three~~ ***six*** sites covering ~~at least 3~~ different regions of the country will be selected for this study. Selection criteria of the sites include support from the Family Health Physician Program (FHP), the Larval Index Rapid Assay (LIRA) or with field research experience in the community. The FHP is a Brazilian government public health strategy that consists of multidisciplinary teams of health care workers that cover geographically defined areas to provide basic/preventive care to community members. The teams include a doctor, a nurse, a nurse auxiliary, and community health care workers who pay regular visits to the household under its responsibility to deliver health promotion activities and facilitate triage and referral into the health unit, among other activities. Community health care workers are local residents who are trained for basic tasks.

Section 4. STUDY POPULATION

Subjects six months of age and older at the time of enrolment who live in the selected study sites in Brazil, covering ~~at least 3~~ different regions of the country.

Section 4.2. Selection of households

~~Approximately 1800 subjects need to be recruited for this study in order to obtain an evaluable sample size of approximately 1500 subjects at the end of the first year of the study.~~

Section 4.3. Overview of the recruitment plan

At least 3600 subjects will be recruited in the study in 2 waves of enrolment. Each wave, initial and expansion cohorts, will enrol about 600 subjects/site in order to maintain at least 500 subjects/site at the beginning of each additional study year/season.

In the subsequent study years/seasons, the cohort size will be maintained at~~,~~ at least ~~1~~500 subjects ***per site***. Since subjects who prematurely terminate participation and/or lost to follow-up will be replaced, the final number of participants across the four study years ~~will~~ ***may*** exceed ~~1800~~***3600 subjects***. Yearly, there will be an evaluation of active subjects. If the number of active subjects***/site*** becomes < ~~1~~500, there will be enrolment of replacement subjects to maintain a cohort size of ***at least*** ~~1~~500 subject***s/site***. Enrolment will preferably take place during the low dengue transmission. Recruitment of replacement subjects could occur at the end of study year 1, 2 or 3. The recruitment approach will be the same as for the initial enrolment.

In order to better represent the Brazilian population distribution, subjects enrolled in the expansion cohort should be composed of at least 30% of subjects younger than 18 years old and should not exceed 20 % of adults 50 years or older. Subjects enrolled as replacement subjects for the initial and expansion cohort will be recruited to approach as much as possible a minimal representation of 30% of subjects younger than 18 years old and a maximal representation of 20% for adults 50 years or older.

Section 5.4. Outline of study procedures

Study procedures are outlined in Table 2 and Table 3 for initial ***and expansion*** cohort***s,*** respectively in Table 4 for replacement subjects.

Table 2 List of study procedures for initial cohort subjects

	Surveillance											Suspected Dengue Visits (at hospital/clinic) as applicable[3]		
Procedure	**Visit 1 Day 0**	**Monthly contact between visits**	**Visit 2 Month 6**	**Monthly contact between visits**	**Visit 3 ~Month 12 (Year 1)**	**Monthly contact between visits**	**Visit 4 ~ Month 24 (Year 2)**	**Monthly contact between visits**	**Visit 5 ~ Month 36 (Year 3)**	**Monthly contact between visits**	**Visit 6 ~ Month 48 (Year 4)**	**First visit Day 0**	**Return visit [2]**	**Follow-up visit Day 21**
Blood sample for serology (5 mL)[6]	●		●		●		●		●		●			

[6] Indirect DENV IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for DENV IgG at the previous scheduled visit.

Table 3 List of study procedures for expansion cohort subjects

	Surveillance							*Suspected Dengue Visits (at hospital/clinic) as applicable[3]*		
Procedure	***Visit 1* Day 0***	***Monthly contact between visits***	***Visit 2***	***Monthly contact between visits***	***Visit 3***	***Monthly contact between visits***	***Visit 4***	***First visit Day 0***	***Return visit[2]***	***Follow-up visit Day 21***
Informed consent (and assent if applicable)	●									
Subject number and household number attribution	●									
Check inclusion/ exclusion criteria	●									
Record/ update socio-demographic information	●				●*					
Medical history and YF vaccination history or updates	●				●		●			
Distribute subject ID card distribution and suspected dengue instruction kit	*O*		*O*		*O*					
Blood sample for serology (5 mL)[6]	●		●		●		●			

	Surveillance							Suspected Dengue Visits (at hospital/clinic) as applicable[3]		
Procedure	Visit 1* Day 0	Monthly contact between visits	Visit 2	Monthly contact between visits	Visit 3	Monthly contact between visits	Visit 4	First visit Day 0	Return visit[2]	Follow-up visit Day 21
Instruct/remind subjects/subject's parent(s)/LAR(s) on suspected dengue assessment procedures (where applicable)	O	O	O	O	O	O	O			
Issue diary logs to parents/LARs to be filled out in the event of a suspected dengue case [1]	O		O		O		O	O		
Contact subject regarding any dengue symptoms and remind subject of procedures for suspected dengue		O		O		O				
Physical examination/record current medical conditions (see Section 5.6)								●	●	●
Blood sample for dengue infection diagnosis[3,]								●		●
Blood sample for CBC and hematocrit								●		
Record body temperature								●		
Report SAEs related to study procedures [4]	●		●		●		●	●	●	●
Collect or verify diary logs if applicable								O	O	O
Study conclusion [5]							●			●

● 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.

** Enrollment of expansion cohort subjects will start during Year 2, at the earliest.*

[1] Diary logs given at scheduled visits are only to be filled out in the event that the subject has a fever or suspected dengue symptoms. See Section 5.5.2.5 for details with regard to the diary log.

[2] A return visit is a visit linked to suspected dengue, occurring if the evolution of the subject's physical condition necessitates medical evaluation, according to local medical practice. The investigator will determine whether this return visit is necessary and whether it is considered as part of the same episode. See Section 5.6 for details.

[3] May include testing for RT-qPCR, dengue IgG and IgM ELISA, NS1 ELISA, neutralizing antibodies to dengue and Yellow fever, as well as dengue virus isolation if deemed necessary.

[4] SAE = Serious adverse event. See Section 6.1.1 for definition.

[5] The study conclusion information in the eCRF is collected once for each subject at the last scheduled visit or at the last follow-up contact for the dengue case, should this case occur during the last scheduled visit or earlier if the subject prematurely terminates participation or is lost to follow-up.

[6] Indirect DENV IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for DENV IgG at the previous scheduled visit.

Table 4 List of study procedures for replacement subjects

	Surveillance							Suspected Dengue Visits (at hospital/clinic) as applicable[3]		
Procedure	**Visit 1 Day 0**	**Monthly contact between visits**	**Visit 2 ***	**Monthly contact between visits**	**Visit 3* as applicable**	**Monthly contact between visits**	**Visit 4 as applicable**	**First visit Day 0**	**Return visit[2]**	**Follow-up visit Day 21**
Blood sample for serology (5 mL) [6]	●		●		●		●			

[6] Indirect DENV IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for DENV IgG at the previous scheduled visit.

Table 5 Intervals between study visits/contacts *for INITIAL cohort*

Study Year*	Visits Interval	Optimal length of interval *	Allowed interval**
Year 1	Visit 1 (Day 0) → Visit 2 (Month 6)	6 months	± -28 days
	Visit 2 (Month 6) → Visit 3 (Month 12)	6 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	
Year 2	Visit X → Visit X+1¶ Note: Visit X may be visit 3 for subjects enrolled in study year 1. For subjects enrolled in study year 2, visit X would be visit 1.	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	
Year 3	Visit X+1 → Visit X+2	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	
Year 4	Visit X → Visit X+3	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	

*Whenever possible the investigator should arrange study visits/contacts within this interval
**Subjects might not be eligible for inclusion in the ATP cohort for analysis if they make the study visit/contact outside this interval

Table 6 Intervals between study visits/contacts for EXPANSION cohort

Interval	*Optimal length of interval**	*Allowed interval***
Visit 1 (Day 0) → Visit 2 (Year 1)	***12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission***	***±28 days***
Visit 2 (Year 1) → Visit 3 (Year 2)	***12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission***	***±28 days***
Visit 3 (Year 2) → Visit 4 (Year 3)	***12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission***	***±28 days***
Interval between visit for a suspected dengue case and the final follow-up visit for a case	***21 days***	***+7 days***

Whenever possible the investigator should arrange study visits/contacts within this interval
****Subjects might not be eligible for inclusion in the ATP cohort for analysis if they make the study visit/contact outside this interval***

Section 5.5.3. Procedures at subsequent scheduled visits (2-6, as applicable)

- ***For expansion cohort subjects, scheduled visits should ideally be performed at yearly intervals, preferably during periods of usually low dengue transmission. Expansion cohort subjects will have at most 4 scheduled visits.***
- A blood (serum) sample will be collected for serology

  Note: Indirect DENV IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for DENV IgG at the previous scheduled visit. Even if the test is not repeated, the volume of the blood sample remains unchanged.

Section 5.7.1. Suspected symptomatic dengue case

A suspected dengue case presenting at the health care facility within 5 days following the onset of fever and/or rash will be defined as an 'early presenter'.

Subjects presenting for care after the fifth day of ***fever and/or rash*** onset will be defined as 'late presenters'.

An example of other signs and symptoms of dengue, ***associated with fever,*** include but are not limited to: fatigue, headache, pain behind the eyes, abdominal pain, nausea, vomiting, muscle ache, joint ache, diffuse rash on the trunk, abnormal sensitivity to light (photophobia) and itching of skin (pruritus).

Section 5.8.2.2. Laboratory read-outs

Table 11 Laboratory read-outs at each time point, and priority ranking for scheduled visits

SCHEDULED VISITS							
Blood sampling time point		**No. Subjects (anticipated)**	**Subset ***	**Possible assays**	**Serum aliquot volume**	**Components priority rank**	**Aliquot number**
Type of contact/ Amount of whole blood	**Sampling time point**						
Scheduled Visit 1 5 mL whole blood	(Day 0)	***3600 (1800 from wave 1 & 2***)*** ~~1800~~	-	Dengue IgG ELISA**	250 µL	1	1
			yes	Dengue virus 1-4 and YF neutralizing antibodies	1 mL	2	2
			yes	Dengue virus 1-4 and YF neutralizing antibodies	remaining volume	3	3
Scheduled Visit 2 5 mL whole blood	Visit 1+ 6 months for subject enrolled in Study Year 1; Visit 1+ 12 months for subject enrolled in beginning of Study Year 2, 3 or 4)	***3190 (1660 from wave 1 and 1530 from wave 2)*** ~~1800~~	yes	Dengue IgG ELISA** (only for subjects IgG negative at the previous visit)	250 µL	1	1
			yes	Dengue virus 1-4 and YF neutralizing antibodies	1 mL	2	2
			yes	Dengue virus 1-4 and YF neutralizing antibodies	remaining volume	3	3
Scheduled Visit 3 5 mL whole blood	(Visit 2+ 6 months for subject enrolled in Study Year 1; Visit 2+12 months for subject enrolled in beginning of Study Year 2 or 3)	***3030 (1530 from wave 1 and 1500 from wave 2)*** ~~1500~~	yes	Dengue IgG ELISA** (only for subjects IgG negative at the previous visit)	250 µL	1	1
			yes	Dengue virus 1-4 and YF neutralizing antibodies	1 mL	2	2
			yes	Dengue virus 1-4 and YF neutralizing antibodies	remaining volume	3	3
Subsequent Yearly Visit(s) as applicable 5 mL whole blood	Yearly	***3000 for Visit 4 and 1500 for visit 5 and 6 which applies only to subjects of the wave 1*** ~~1500~~	yes	Dengue IgG ELISA** (only for subjects IgG negative at the previous visit)	250 µL	1	1
			yes	Dengue virus 1-4 and YF neutralizing antibodies	1 mL	2	2
			yes	Dengue virus 1-4 and YF neutralizing antibodies	remaining volume	3	3

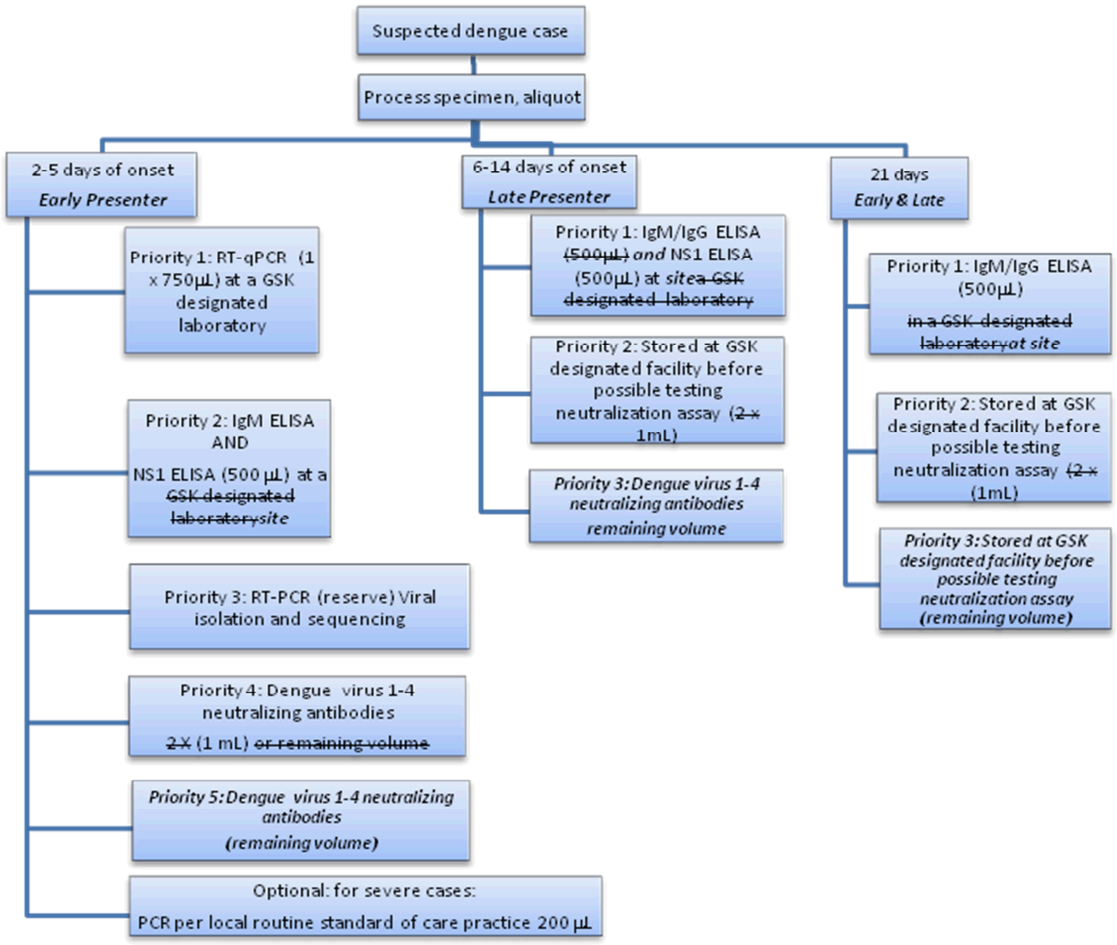
*tertiary (exploratory) analysis, number of subjects in the subset will be determined during analysis
- not applicable,
** Dengue IgG indirect ELISA
*** ***Wave 1 enrolment = initial cohort / Wave 2 enrolment = Expansion cohort***

Table 12 Laboratory read-outs at each time point, and priority ranking for suspected dengue visits for EARLY presenters

SUSPECTED DENGUE VISITS – EARLY PRESENTERS							
Blood sampling time point		No. Subjects	Subset	Component	Serum aliquot volume	Components priority rank	Aliquot number
Type of contact/ Amount of whole blood	Sampling time point						
Suspected dengue follow-up visit (total amount of blood 5 mL)	Approximately 21 days after first visit	unknown	-	Dengue IgM and Dengue capture IgG ELISA	500 µL	1	1
				Dengue virus 1-4 neutralizing antibodies	1 mL	~~4~~ ***2***	~~4~~ ***2***
				Dengue virus 1-4 neutralizing antibodies	remaining volume	~~5~~ ***3***	~~5~~ ***3***

- not applicable

Figure 4: Flow chart of serum aliquot testing for suspected dengue cases



Section 7.3. Subject replacement

Enrolment of new subjects to replace:

- subjects not wishing to continue after ~~this~~ amendment ***2 (initial cohort)***, or
- subjects who prematurely terminate participation ***(initial and expansion cohorts)*** or
- subjects lost to follow-up will be done ***(initial and expansion cohorts)***.

The aim is to maintain a critical cohort size of ≥ ~~1~~500 subjects***/site***. This replacement will be done ***at the beginning of*** ~~before~~ each ***study*** ~~surveillance~~ year: during ***the*** scheduled visits starting at the end of study Year 1 ~~(Visit 3)~~ up till the end of study Year 3 ~~(Visit 5)~~.

The number of subjects to be recruited as replacements will be defined based on the number of subjects still active in the study. If at the end of each study year we have ≥~~1~~500 subjects***/site***, no replacements will be needed. The time window for the recruitment of replacements will be during the low dengue transmission.

Section 8.2.1. Assumptions:

It is planned to obtain ~~approximately~~ ***at least*** ~~1~~500 subjects***/site*** still active at the end of the first year of the study. Because subjects might leave the study due to a variety of reasons, e.g. moving away geographically, sample size is increased to allow for this.~~,~~ ***On the basis of*** an annual drop-out rate between 15 and 20%, ~~will be considered. Therefore,~~approximately ~~1800~~ ***600*** subjects***/site*** will be enrolled at the beginning of this study***. The recruitment will be*** ~~using~~ cluster sampling , i.e. the unit of sampling will be the households. ~~At least three~~***Different*** areas where the FHP has been fully implemented or where access to the community is already established by a government program will be first selected, followed by a selection of households in the respective areas. All subjects in each household will be invited for recruitment to the study.

New sites will start enrolling subjects preferably during study Year 2. The target is to enrol approximately 600 subjects per additional site. Replacement subjects may be recruited in order to maintain at least 500 subjects/site still active at the beginning of the next dengue season.

In order to better represent the Brazilian population distribution, subjects enrolled in the expansion cohort should be composed of at least 30% of subjects younger than 18 years old and should not exceed 20 % of adults 50 years or older. Subjects enrolled as replacement subjects for the initial and expansion cohort will be recruited to approach as much as possible a minimal representation of 30% of subjects younger than 18 years old and a maximal representation of 20% for adults 50 years or older.

The ***total*** study ***duration per site*** will last approximately 4 years ***for the initial cohort and approximately 3 years for the expansion cohort***, and new subjects will be enrolled ~~between seasons~~ if the number of subjects still active in the study is below ~~1~~500***/site after year 1 and at the beginning of each additional study year/season***.

Section 8.2.3. Expected precision of incidence rate estimates

Table 13 shows the 95% CI for a range of expected incidences based on the Poisson distribution (exact method, normal approximation and the normal approximation accounting for a design effect of 1.8) ***in different scenarios. The scenarios were defined according to the following criteria:***

- ***Number of subjects enrolled at the beginning of the study (for some sites it will be the second year). At season level and for all sites: 2000, 4000, 1800, 3600, 1500, 3000. For all seasons and for all sites: 4000, 3600 and 3000. At season level and site level: 600 and 500.***
- ***Number of years of follow-up. At study level: 1 or 4 years, but half of the sites will be followed for 3 years and this is taken into account for the calculations. At site level: 1, 3 or 4 years.***
- ***Overall incidences: 3, 6, 9, 12 or 15 events per 1000 person-years.***

With an overall sample size of a minimum of about ~~1,800~~***3,600*** subjects enrolled and a follow-up period of one year, the 95% confidence interval (CI) for an expected incidence of dengue of 9 cases per 1000 person-years using a cluster design is [~~2.9~~***4.7*** ; ~~15.1~~***13.3***]. With ***3600 subjects enrolled,*** 4 years of follow-up and new enrolments to keep the number of subjects above ~~1500~~ ***3000*** at the beginning of each subsequent season, the 95% CI is [~~5.7~~***6.3*** ; ~~12.3~~***11.7***].

Table 15 Precision ~~for~~*of the* expected incidences of dengue for a single cohort of subjects in Brazil

	Cohort size	Number of years	Number of person-years	Number of cases	Incidence of dengue (per 1000 person-years)	95% CI of incidence rate (n / 1000 P-Y)						***New enrolment***		
						Exact (Poisson)		Normal approx		Normal approx with design effect		***After Y1***	***After Y2***	***After Y3***
						Lower	Upper	Lower	Upper	Lower	Upper			
Overall	2000	1	1845.9	6	3.0	1.0	6.7	0.5	5.5	-0.4	6.4			
	2000	1	1845.9	11	6.0	3.0	10.7	2.5	9.5	1.3	10.7			
	2000	1	1845.9	17	9.0	5.2	14.5	4.7	13.3	3.2	14.8			
	2000	1	1845.9	22	12.0	7.5	18.1	7.0	17.0	5.3	18.7			
	2000	1	1845.9	28	15.0	9.9	21.7	9.4	20.6	7.5	22.5			
	4000	1	3691.9	11	3.0	1.5	5.4	1.2	4.8	0.6	5.4			
	4000	1	3691.9	22	6.0	3.8	9.1	3.5	8.5	2.6	9.4			
	4000	1	3691.9	33	9.0	6.2	12.6	5.9	12.1	4.9	13.1			
	4000	1	3691.9	44	12.0	8.7	16.1	8.5	15.5	7.3	16.7			
	4000	1	3691.9	55	15.0	11.3	19.5	11.0	19.0	9.7	20.3			
	4000	4	9086.6	27	3.0	2.0	4.4	1.9	4.1	1.5	4.5			***327***
	4000	4	9086.6	55	6.0	4.5	7.8	4.4	7.6	3.9	8.1			***327***
	4000	4	9086.6	82	9.0	7.2	11.2	7.0	11.0	6.4	11.6			***327***
	4000	4	9086.6	109	12.0	9.9	14.5	9.7	14.3	9.0	15.0			***327***
	4000	4	9086.6	136	15.0	12.6	17.7	12.5	17.5	11.6	18.4			***327***
	1800	1	1661.3	5	3.0	0.9	7.2	0.4	5.6	-0.5	6.5			
	1800	1	1661.3	10	6.0	2.8	11.1	2.3	9.7	1.0	11.0			
	1800	1	1661.3	15	9.0	5.0	14.9	4.4	13.6	2.9	15.1			
	1800	1	1661.3	20	12.0	7.3	18.6	6.7	17.3	4.9	19.1			
	1800	1	1661.3	25	15.0	9.7	22.1	9.1	20.9	7.1	22.9			
	3600	1	3322.7	10	3.0	1.4	5.6	1.1	4.9	0.5	5.5			
	3600	1	3322.7	20	6.0	3.6	9.3	3.4	8.6	2.5	9.5			
	3600	1	3322.7	30	9.0	6.1	12.9	5.8	12.2	4.7	13.3			

	Cohort size	Number of years	Number of person-years	Number of cases	Incidence of dengue (per 1000 person-years)	95% CI of incidence rate (n / 1000 P-Y)						***New enrolment***		
						Exact (Poisson)		Normal approx		Normal approx with design effect		***After Y1***	***After Y2***	***After Y3***
						Lower	Upper	Lower	Upper	Lower	Upper			
	3600	1	3322.7	40	12.0	8.6	16.3	8.3	15.7	7.0	17.0			
	3600	1	3322.7	50	15.0	11.1	19.8	10.8	19.2	9.4	20.6			
	3600	4	8611.3	26	3.0	2.0	4.4	1.8	4.2	1.4	4.6		***170***	***450***
	3600	4	8611.3	52	6.0	4.5	7.9	4.4	7.6	3.8	8.2		***170***	***450***
	3600	4	8611.3	78	9.0	7.1	11.2	7.0	11.0	6.3	11.7		***170***	***450***
	3600	4	8611.3	103	12.0	9.8	14.5	9.7	14.3	8.9	15.1		***170***	***450***
	3600	4	8611.3	129	15.0	12.5	17.8	12.4	17.6	11.5	18.5		***170***	***450***
	1500	1	1384.5	4	3.0	0.8	7.6	0.1	5.9	-0.9	6.9			
	1500	1	1384.5	8	6.0	2.6	11.7	1.9	10.1	0.5	11.5			
	1500	1	1384.5	12	9.0	4.7	15.6	4.0	14.0	2.3	15.7			
	1500	1	1384.5	17	12.0	6.9	19.3	6.2	17.8	4.3	19.7			
	1500	1	1384.5	21	15.0	9.2	22.9	8.5	21.5	6.3	23.7			
	3000	1	2768.9	8	3.0	1.3	5.9	1.0	5.0	0.3	5.7			
	3000	1	2768.9	17	6.0	3.5	9.7	3.1	8.9	2.1	9.9			
	3000	1	2768.9	25	9.0	5.8	13.3	5.5	12.5	4.3	13.7			
	3000	1	2768.9	33	12.0	8.3	16.8	7.9	16.1	6.5	17.5			
	3000	1	2768.9	42	15.0	10.8	20.3	10.4	19.6	8.9	21.1			
	3000	4	8306.7	25	3.0	1.9	4.4	1.8	4.2	1.4	4.6	***225***	***450***	***450***
	3000	4	8306.7	50	6.0	4.4	7.9	4.3	7.7	3.8	8.2	***225***	***450***	***450***
	3000	4	8306.7	75	9.0	7.1	11.3	7.0	11.0	6.3	11.7	***225***	***450***	***450***
	3000	4	8306.7	100	12.0	9.8	14.6	9.6	14.4	8.8	15.2	***225***	***450***	***450***
	3000	4	8306.7	125	15.0	12.5	17.9	12.4	17.6	11.5	18.5	***225***	***450***	***450***

	Cohort size	Number of years	Number of person-years	Number of cases	Incidence of dengue (per 1000 person-years)	95% CI of incidence rate (n / 1000 P-Y)						***New enrolment***		
						Exact (Poisson)		Normal approx		Normal approx with design effect		***After Y1***	***After Y2***	***After Y3***
						Lower	Upper	Lower	Upper	Lower	Upper			
Site	600	1	553.8	2	3.0	0.2	12.3	-1.6	7.6	-3.1	9.1			
	600	1	553.8	3	6.0	1.2	17.0	-0.5	12.5	-2.7	14.7			
	600	1	553.8	5	9.0	2.7	21.4	1.1	16.9	-1.6	19.6			
	600	1	553.8	7	12.0	4.6	25.1	2.9	21.1	-0.2	24.2			
	600	1	553.8	8	15.0	6.5	29.2	4.8	25.2	1.3	28.7			
	600	3	1486.0	4	3.0	0.8	7.5	0.2	5.8	-0.7	6.7		***67***	
	600	3	1486.0	9	6.0	2.7	11.5	2.1	9.9	0.7	11.3		***67***	
	600	3	1486.0	13	9.0	4.8	15.3	4.2	13.8	2.5	15.5		***67***	
	600	3	1486.0	18	12.0	7.1	19.0	6.4	17.6	4.5	19.5		***67***	
	600	3	1486.0	22	15.0	9.4	22.6	8.8	21.2	6.6	23.4		***67***	
	600	4	1947.5	6	3.0	1.0	6.7	0.6	5.4	-0.3	6.3		***67***	***75***
	600	4	1947.5	12	6.0	3.1	10.6	2.6	9.4	1.4	10.6		***67***	***75***
	600	4	1947.5	18	9.0	5.3	14.3	4.8	13.2	3.3	14.7		***67***	***75***
	600	4	1947.5	23	12.0	7.6	17.9	7.1	16.9	5.5	18.5		***67***	***75***
	600	4	1947.5	29	15.0	10.1	21.5	9.6	20.4	7.7	22.3		***67***	***75***
	500	1	461.5	1	3.0	0.1	14.3	-2.0	8.0	-3.7	9.7			
	500	1	461.5	3	6.0	1.0	18.6	-1.1	13.1	-3.5	15.5			
	500	1	461.5	4	9.0	2.5	22.7	0.3	17.7	-2.6	20.6			
	500	1	461.5	6	12.0	4.2	26.8	2.0	22.0	-1.4	25.4			
	500	1	461.5	7	15.0	5.8	31.2	3.8	26.2	0.0	30.0			
	500	3	1384.5	4	3.0	0.8	7.6	0.1	5.9	-0.9	6.9	***75***	***75***	
	500	3	1384.5	8	6.0	2.6	11.7	1.9	10.1	0.5	11.5	***75***	***75***	
	500	3	1384.5	12	9.0	4.7	15.6	4.0	14.0	2.3	15.7	***75***	***75***	
	500	3	1384.5	17	12.0	6.9	19.3	6.2	17.8	4.3	19.7	***75***	***75***	

	Cohort size	Number of years	Number of person-years	Number of cases	Incidence of dengue (per 1000 person-years)	95% CI of incidence rate (n / 1000 P-Y)						***New enrolment***		
						Exact (Poisson)		Normal approx		Normal approx with design effect		***After Y1***	***After Y2***	***After Y3***
						Lower	Upper	Lower	Upper	Lower	Upper			
	500	3	1384.5	21	15.0	9.2	22.9	8.5	21.5	6.3	23.7	***75***	***75***	
	500	4	1845.9	6	3.0	1.0	6.7	0.5	5.5	-0.4	6.4	***75***	***75***	***75***
	500	4	1845.9	11	6.0	3.0	10.7	2.5	9.5	1.3	10.7	***75***	***75***	***75***
	500	4	1845.9	17	9.0	5.2	14.5	4.7	13.3	3.2	14.8	***75***	***75***	***75***
	500	4	1845.9	22	12.0	7.5	18.1	7.0	17.0	5.3	18.7	***75***	***75***	***75***
	500	4	1845.9	28	15.0	9.9	21.7	9.4	20.6	7.5	22.5	***75***	***75***	***75***

Section 8.4.1. Sequence of analyses

An interim analysis will be performed when ~~all active~~ subjects ***from the initial cohort of first two sites*** have ***completed two years of surveillance*** *(V*~~achieved the v~~isit 4) ***and the third site have completed at least one year of surveillance (Visit 3).*** All analyses of primary and secondary objectives will be performed on data as cleaned as possible.

APPENDIX A

Non GSK laboratories

Laboratory	Address
~~Fundação de Medicina Tropical ***Dr.*** Heitor Vieira Dourado - FMT Gerência de Virologia~~	~~Av. Pedro Teixeira, 25, Dom Pedro Manaus, AM. CEP: 69.040-000 Brazil~~
~~Centro de Pesquisas Gonçalo Moniz - CPqGM Fundação Oswaldo Cruz - FiocruzLaboratório de Patologia e Biologia Molecular - LPBM~~	~~Rua Waldemar Falcão, 121, Candeal Salvador, BA. CEP: 40.296-710 Brazil~~
~~Instituto de Pesquisa Clínica Evandro Chagas - IPEC, Fundação Oswaldo Cruz - Fiocruz Laboratório de Imunodiagnóstico~~	~~Av. Brasil, 4365, Manguinhos Rio de Janeiro, RJ. CEP: 21.040-900 Brazil~~
Local laboratories	***Details presented in a separated document***
Laboratório de Tecnologia Virológica – LATEV Bio-Manguinhos Fundação Oswaldo Cruz - Fiocruz	Av. Brasil, 4365, Manguinhos Pavilhão Rocha Lima, sala 403 Rio de Janeiro, RJ. CEP: 21.040-360 Brazil

GlaxoSmithKline Biologicals
Clinical Research & Development
Protocol Amendment 4

eTrack study number and Abbreviated Title	116606 (EPI-DENGUE-006 BOD BR)
Amendment 4 version and date:	Final: 16 October 2017
Coordinating author:	PPD, Scientific Writer

Rationale/background for changes:
Additional exploratory objectives have been added in this protocol amendment to explore the seroprevalence of antibodies to Zika and chinkungunya viruses in the study population, as well as the potential aetiological role of these viruses in episodes of febrile illness detected during follow-up .

Zika is a *flavivirus* and chikungunya virus is a member of the *alphavirus* genus, and *Togaviridae* family. These cause emerging mosquito-borne viral diseases in Latin America especially in Brazil. Zika virus infections have been laboratory confirmed in 19 states in Brazil between April and December 2015 [Boletim Epidemiológico n5, 2016]. In 2016, until epidemiological week 47, 196.976 cases were reported and 101,851 were confirmed [Boletim epidemiológico n 33, 2016].

In 2015, 20,661 autochthonous suspected chikungunya cases were reported in Brazil [Boletim Epidemiológico n5, 2016]. In 2016, 216,102 reported cases and 102,638 were confirmed up to epidemiological week 47 [Boletim epidemiológico n33, 2016]. Zika and chikungunya viruses cause symptoms often similar to dengue during the acute phase of illness.

The interpretation of the serological tests used to determine the seroprevalence of dengue antibodies or for diagnostic of acute dengue infection should take into account the possibility of cross-reaction with antibodies elicited by exposure to the Zika virus. The public health importance of Zika and chikungunya virus emergence, as well as the potential impact of these viruses on the interpretation of the laboratory assays, supports the inclusion of the exploratory objectives related to Zika and chikungunya infection.

Fever is the triggering event for a subject to be evaluated as a suspect dengue case in the study. While fever is common in suspected chikungunya cases, fever does not always present in suspected Zika cases. For this reason, rash is being added as a triggering event, in addition to fever, for Zika virus disease evaluation. A prospective surveillance will be maintained for febrile cases and added for rash cases. . Also, considering the epidemiological situation in Brazil for both Zika and chikungunya infection, a retrospective testing for these infections in subjects considered as suspect dengue case since the beginning of the study will also be done.

The current case definition for suspected dengue does not include a criteria specifying the maximum interval between fever onset and the initial medical visit. This is

specified in section 5.7.1.1.

Currently, haematology testing (HCT, CBC) is planned to be performed for all suspected dengue cases at the initial visit, regardless of the time between fever onset and the visit. However, for patients presenting more than 14 days after fever and/or rash onset, the relevance of these tests is low. Therefore, a maximum interval will be defined in this amendment.

The geographical distribution of the participants may impact the risk of infection. Therefore, updating information on the household of the study participants and their location at yearly visits is important during the follow-up period (which may extend to 4 years). Hence, socio-demographic and household information will be collected at yearly visits.

Some sites may have more than 500 active participants enrolled in the penultimate year of the study. However, their site may have experience with high drop-out rate and can expect to drop below 500 participants during the last year of the study. To address this challenge, criteria to allow for replacement cohort recruitment was clarified.

Also, the NS1 test has been removed for the late presenters since this test does not have clinical relevance in the case of late presenters.

Collection of dengue vaccination history has been added since a competitor dengue vaccine is currently available in the Brazilian market.

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

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Synopsis:

Tertiary (optional) objectives

- To investigate potential risk factors for symptomatic dengue infection.
- To investigate the role of dengue neutralizing antibodies elicited through natural infection in protection against subsequent infection.
- To estimate the degree of underreporting of dengue cases using the passive national notification surveillance.
- ***To estimate the incidence of symptomatic Zika virus infection (including laboratory-confirmed and probable cases) in the study population by study site, gender, age-group, previous flavivirus exposure, overall and by season.***
- ***To estimate the incidence of symptomatic chikungunya virus infection (including laboratory-confirmed and probable cases) in the study population by study site, gender, age-group, overall, and by season.***
- To describe previous Yellow Fever (YF) antibody profile in selected dengue cases to investigate the role of previous YF immunity on the dengue neutralizing antibody profile following dengue infection.
- To describe the spatial and temporal distribution of dengue cases among cohort participants in the study areas.
- ***To describe the spatial and temporal distribution of Zika and chikungunya cases among cohort participants in the study areas.***
- ***To describe other infectious aetiologies related to differential diagnosis of dengue (chikungunya and Zika) in subjects with episodes of febrile illness referred to as "suspected dengue case".***

- ***To estimate the seroprevalence of antibodies against Zika and chikungunya virus at selected timepoints.***

Study design

- **Type of design:** Prospective, multicenter, community-based, household-sampling, cohort study in Brazil.
- **Study population:** Subjects six months of age and older at the time of enrolment who live in the selected study sites in Brazil, covering different regions of the country.
- There will be 2 waves of enrolment for recruiting 3600 subjects:
  - Initial Cohort = about 1800 subjects – up to 6 visits (Amendment 2) – INITIAL sites
  - Expansion Cohort = about 1800 subjects – up to 4 visits – NEW sites
- The study period, initially planned to be one year, has been extended by additional three years (overall 4 years) to cover three additional dengue seasons (Amendment 2).
- Initial cohort subjects were invited to extend their participation.

  (See glossary of terms for the definition of initial and expansion cohort subjects)

  In each cohort: replacement subjects may be enrolled in order to compensate for subjects who did not wish to extend their participation for three additional years (for subjects from the initial cohort – Amendment 2), subjects who prematurely terminate participation or subjects who are lost to follow-up. This will be done to maintain a cohort size of at least 500 subjects per site, in the Initial and Expansion cohorts, at the beginning of each additional study year/season.

  At the end of the penultimate year of the study, the cohort size will be reviewed to address the potential need for replacement cohort recruitment.

  (See glossary of terms for the definition of replacement subjects, subjects who prematurely terminate participation and subjects lost to follow-up)
- Informed consent (and assent, if applicable) will be obtained:
  - From the subject (and parents/LAR of the subjects, if applicable) at the study start for the initial cohort.
  - From the subject (and parents/LAR of the subjects, if applicable) of the initial cohort prior to participation in the additional three years of the study.

- From the subject (and parents/LAR of the subjects, if applicable) at the study start for the expansion cohort.

From the subject (and parents/LAR of the subjects, if applicable) prior to enrolment in the study for the replacement subjects.

- **Study visits:** The visits will be as follows and shown in Synopsis Figure 1:
  - Initial cohort subjects consenting to continue participation for the three additional years will have:
    - three scheduled visits in the first year (Visit 1, Visit 2 and Visit 3) at six months intervals ***(+/- 28 days)***,
    - and thereafter, one scheduled visit per year during a period of low dengue transmission for the three additional years; i.e; one visit at study Year 2, Year 3 and Year 4 (Visit 4, Visit 5 and Visit 6 respectively).
  - Initial cohort subjects who do not consent to extend participation for three additional years will:
    - have three scheduled visits (Visit 1, Visit 2 and Visit 3) at six months interval ***(+/- 28 days)***.
    - conclude their participation in the study at Visit 3 or the last follow up visit if the subject is suspected of having a dengue case on-going at Visit 3.
  - Subjects from the expansion cohort will have only three years of follow-up, with at most 4 scheduled visits.
  - For replacement subjects, the number of scheduled visits will depend on the study year in which they are enrolled. Replacement subjects will not be enrolled until after the third visit of the first study year is concluded for the initial cohort. The number of visits for the replacement subjects will range between two and four.
- The yearly scheduled visits for the initial and expansion cohorts and replacement subjects will preferably occur during the period of usually low dengue transmission.
- **Suspected dengue, *chikungunya and/or Zika* case detection (Amended 16 October 2017):** Suspected dengue, ***Zika and/or chikungunya*** cases will be detected using three sources:
  1) referred by study personnel during scheduled home visits;
  2) through enhanced passive surveillance; and
  3) as a result of active surveillance between scheduled visits; (see Section ***5.5.3*** for details).
- If dengue, ***Zika and/or chikungunya*** is/***are*** suspected, a medical appointment with a physician at a designated study

hospital/clinic will be arranged (see Section 5.6 for management of suspected dengue, ***Zika and chikungunya*** cases).

- If the suspicion is confirmed during the visit:

iv. A blood sample for dengue, ***Zika and/or chikungunya*** diagnosis and for haematology (CBC and HCT) ***(mandated procedure for subjects presenting within the first 14 days following onset of fever and/or rash)*** will be collected at the first visit for suspected dengue, ***Zika and/or chikungunya*** (acute blood sample) and

v. a second blood sample (convalescent blood sample) will be collected approximately 21 (maximum 28 days) days later.

vi. In between, a return visit may be scheduled as needed.

- **Data collection**: eCRF
- **Type of study**: self-contained
- **Duration of the study:** Four years for the overall study. For an individual subject, it will range between one and four years.
- **Epoch 001:** prospective data collection starting at Visit 1 Day 0 and ending at the last subject last visit.

Endpoints: **Tertiary (optional)**

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

- ***Symptomatic laboratory-confirmed Zika virus infection***
- ***Symptomatic Zika virus infection (including laboratory-confirmed and probable cases).***
- ***Symptomatic laboratory-confirmed chikungunya infection***
- ***Symptomatic chikungunya infection (including laboratory-confirmed and probable cases).***
- Risk factors for dengue infection and disease
- Neutralizing antibodies titers against DENV 1-4
- Neutralizing antibody titers against YF virus
- Spatial and temporal distribution of dengue cases (confirmed and probable symptomatic cases and inapparent infections)
- ***Spatial and temporal distribution of laboratory confirmed and probable Zika virus cases***

- ***Spatial and temporal distribution of patients with laboratory confirmed and probable chikungunya virus infection***
- ***Occurrence of laboratory confirmed or probable Zika and/or chikungunya infection in suspected dengue cases (differential diagnosis of dengue), retrospectively***
- ***Antibody titre against Zika and chikungunya virus at the scheduled visits***

Glossary of terms

Diary log:	A diary log will be used in this study. The diary log is given to subjects to record body temperatures and symptoms in the event that a suspected dengue, ***Zika and/or chikungunya*** symptom occurs (see Section 5.7.1.1). The information collected in the diary log will be provided to the study physician as a tool in the medical evaluation of the subject. If the physician considers that the subject meets the criteria for suspected dengue, he/she will use the information contained in the diary log to complete the description of first clinical symptoms in the eCRF.
Early presenter	A suspected dengue, ***Zika and/or chikungunya*** case presenting at the health care facility within 5 days following the onset of fever ***and/or rash for Zika virus***.
Late presenter	A suspected dengue, ***Zika and/or chikungunya*** case presenting at the health care facility 6 to 30 days ~~or more~~ after the onset of fever ***and/or rash for Zika virus.***

List of abbreviations:

~~**DHF**~~	~~Dengue Hemorrhagic Fever~~
~~**ICU:**~~	~~Intensive Care Unit~~
~~**SST**~~	~~Serum Separator Tube~~

Glossary of terms

Diary log:	A diary log will be used in this study. The diary log is given to subjects to record body temperatures and symptoms in the event that a suspected dengue, ***Zika and/or chikungunya*** symptom occurs (see Section 5.7.1.1). The information collected in the diary log will be provided to the study physician as a tool in the medical evaluation of the subject. If the physician considers that the subject meets the criteria for suspected dengue, ***Zika and/or chikungunya***, he/she will use the information contained in the diary log to complete the description of first clinical symptoms in the eCRF.
Early presenter	A suspected dengue, ***Zika and/or chikungunya*** case presenting at the health care facility within 5 days following the onset of fever ***and/or rash for Zika virus***.
Late presenter	A suspected dengue, ***Zika and/or chikungunya*** case presenting at the health care facility 6 to 30 days after the onset of fever ***and/or rash for Zika virus.***
Seroconversion:	***Documented positive antibody test with previous documented negative antibody test.***

Section 1.1 Background

Dengue, the most common arthropod-borne viral disease worldwide, is caused by four types of dengue viruses (DENV 1-4), transmitted primarily by *Aedes aegypti*, a mosquito that is highly adapted to urban environments. Dengue infection can cause a range of clinical illnesses, from inapparent to a life threatening hemorrhagic disease, often associated with pre-existing heterotypic dengue virus antibodies.

An estimated 2.5 billion people are at risk of infection in tropical and subtropical countries worldwide (WHO, 2009) where major urban epidemics are responsible for substantial social and economic burden. Dengue has drastically increased in the last two decades in Central and South America with Brazil being one of the most affected countries (Siqueira-Junior, 2008). In Central Brazil, an almost 20-fold increase in reported cases of dengue have been reported from 1990 (1,660 cases) to 2000 (20,552 cases) (Siqueira, 2004). In all of Brazil, the number of yearly reported cases increased from 124,827 in 1995 to more than 1 million in 2010 (PAHO, 2011)

Currently, strategies to reduce disease burden rely mainly on mosquito control and human preventive behaviour modification, as treatment is limited to supportive care. The World Health Organization (WHO) has, therefore, considered the development of a vaccine a priority research area (Brandt, 1990), and a number of vaccine candidates are under development or being tested in clinical trials (Thomas, 2011).

In preparation for phase III trials, the WHO recommends that the primary efficacy endpoint be the presence of DENV in a patient with signs and/or symptoms of dengue disease (WHO, 2009; Edelman, 2008). However, mild cases of dengue are largely underreported, as shown in dengue cohort studies and capture-recapture studies (Wichmann, 2011; Vong, 2011; Suaya, 2007). For example, in Nicaragua an active dengue surveillance system in children was able to detect approximately 14 to 28 (average 21.3) times more dengue cases each year per 100,000 persons than passive surveillance among similar paediatric populations (Standish, 2010).

Zika is a flavivirus and chikungunya virus is a member of the alphavirus genus, and Togaviridae family. These are emerging mosquito-borne viral diseases in Latin America. Zika virus infections have been laboratory confirmed in 19 states in Brazil between April and December 2016 [Boletim Epidemiológico, 2017]. In 2016, until epidemiological week 52, 215,319 cases were reported and 130,701 were confirmed [Boletim Epidemiológico, 2017].

In 2015, 20,661 autochthonous suspected chikungunya cases were reported in Brazil [Boletim Epidemiológico, 2017]. In 2016, 271,824 reported cases and 151,318 were confirmed up to epidemiological week 47 [Boletim Epidemiológico, 2017]. Zika and chikungunya viruses cause symptoms often similar to dengue during the acute phase of illness.

Another challenge when conducting vaccine trials is to select geographic settings where the incidence of dengue would allow for a reliable assessment of vaccine efficacy. ***Endemicity for more than one dengue virus type is also*** highly desirable. Thus, selection of geographically diverse sites can potentially maximize the likelihood of having a sufficient number of exposed individuals for the conduct of a vaccine trial. Finally, ***identifying*** all other coexisting flaviviruses circulating at potential study sites ***is important***, as infection by such viruses might, in theory, modulate immune response and clinical course of dengue infection.

In this study, we will estimate the incidence of dengue infection and disease in a cohort of subjects recruited from different geographic areas over time in Brazil. The study will also identify and train potential sites for the conduct of phase III studies in the future.

Section 2.3 Tertiary Objectives

- To investigate potential risk factors for symptomatic dengue infection.
- To investigate the role of dengue neutralizing antibodies elicited through natural infection in protection against subsequent infection.
- To estimate the degree of underreporting of dengue cases using the passive national notification surveillance.
- ***To estimate the incidence of symptomatic Zika virus infection (including laboratory-confirmed and probable cases) in the study population by study site, gender, age-group, previous flavivirus exposure, overall and by season.***

- ***To estimate the incidence of symptomatic chikungunya virus infection (including laboratory-confirmed and probable cases) in the study population by study site, gender, age-group, overall, and by season.***
- To describe previous Yellow Fever (YF) antibody profile in selected dengue cases to investigate the role of previous YF immunity on the dengue neutralizing antibody profile following dengue infection.
- To describe the spatial and temporal distribution of dengue cases among cohort participants in the study areas.
- ***To describe the spatial and temporal distribution of Zika and chikungunya cases among cohort participants in the study areas.***
- ***To describe other infectious aetiologies related to differential diagnosis of dengue (chikungunya and Zika) in subjects with episodes of febrile illness referred to as "suspected dengue case".***
- ***To estimate the seroprevalence of antibodies against Zika and chikungunya virus at selected timepoints.***

Section 3 Study design overview

- **Type of design**: Prospective, multicenter, community-based, household-sampling, cohort study in Brazil.
- The study period, initially planned to be one year, has been extended by additional three years (overall four years) to cover three additional dengue seasons (Amendment 2).
- Initial cohort subjects were invited to extend their participation.
- (See glossary of terms for the definition of initial and expansion cohort subjects).
- In each cohort: replacement subjects may be enrolled in order to compensate for subjects who did not wish to extend their participation for three additional years (for subjects from the initial cohort – Amendment 2), subjects who prematurely terminate participation or subjects who are lost to follow-up.

  This will be done to maintain a cohort size of at least 500 subjects per site, in the Initial and Expansion cohorts, at the beginning of each additional study year/season.

  In last three months of the of penultimate year of the study, each site will review the number of active subjects and drop-out rate. If the risk of having fewer than the required number of active participants at the end of the study is high, recruitment for participants into the replacement cohort may be performed with sponsor approval. If the risk is high, recruitment into the replacement cohort should begin even if the number of active subjects is 500 or above 500.

  (See glossary of terms for the definition of replacement subjects, subjects lost to follow-up and subjects who prematurely terminate participation).
- Informed consent (and assent, if applicable) will be obtained:

  - From the subject (and parents/LAR of the subjects, if applicable) at the study start for the initial cohort.
  - From the subject (and parents/LAR of the subjects, if applicable) of the initial cohort prior to participation in the additional three years of the study.
  - From the subject (and parents/LAR of the subjects, if applicable) at the study start for the expansion cohort.
  - From the subject (and parents/LAR of the subjects, if applicable) prior to enrolment in the study for the replacement subjects.

- **Study population:** Subjects six months of age and older at the time of enrolment, who either live in households in study areas with support from the Family Health Physician Program (FHP) or the Larval Index Rapid Assay (LIRA) or with field research experience in the community (preferred) or where a similar system of mapped communities with potential for surveillance exists.

- There will be 2 waves of enrolment for recruiting 3600 subjects:
  - Initial Cohort = about 1800 subjects – up to 6 visits (Amendment 2) – INITIAL sites
  - Expansion Cohort = about 1800 subjects – up to 4 visits – NEW sites

- **Study visits:** The visits will be as follows and further shown in Figure 1:
  - Initial cohort subjects consenting to extend participation for the three additional years will have:
    - three scheduled visits in the first year (Visit 1, Visit 2 and Visit 3) at approximately six months intervals ***(+/- 28 days)***,
    - and thereafter, one scheduled visit per year preferably during a period of low dengue transmission for the three additional years; i.e., one visit at study Year 2, Year 3 and Year 4 (Visit 4, Visit 5 and Visit 6 respectively).
  - Initial cohort subjects who do not consent to extend participation for three additional years will:
    - have three scheduled visits (Visit 1, Visit 2 and Visit 3) at approximately six months intervals ***(+/- 28 days)***.
    - conclude their participation in the study at Visit 3 or the last follow up visit if the subject is suspected of having a dengue case on-going at Visit 3.
  - Subjects from the expansion cohort will have only three years of follow-up, with at most 4 scheduled visits.
  - For replacement subjects, the number of scheduled visits will depend on the study year in which they are enrolled. Replacement subjects will not be enrolled until after the third visit of the first study year is concluded for the initial cohort. The number of scheduled visits will range between two and four.

The yearly scheduled visits for the initial and expansion cohorts and replacement subjects will preferably occur during the period of usually low dengue transmission.

In last three months of the of penultimate year of the study, each site will review the number of active subjects and drop-out rate. If the risk of having fewer than the required number of active participants at the end of the study is high, recruitment for participants into the replacement cohort may be performed with sponsor approval. If the risk is high, recruitment into the replacement cohort should begin even if the number of active subjects is 500 or above 500.

Section 3.2.1 Rationale for study design:

A single cohort study was chosen to estimate the incidence of dengue in the community. Data will be collected from a population of individuals aged 6 months and older for maximum four dengue seasons. Inclusion of individuals of various ages will further allow for a better understanding of disease dynamics, clinical spectrum, and validate attack rates for potential subgroups to be included in future dengue vaccine trials.

This study will use cluster (household) sampling to take into account the correlation that may exist within households. Individuals within a household are expected to have a more similar risk of infection compared to individuals from different households. For example, individuals living in the same household are more likely to share similar risk of being exposed to DENV infected mosquitoes. The sample size has been adjusted to account for this between-cluster variability.

A possible bias ***may arise from the*** non-response rate. ***To reduce the possibility of this potential*** bias***,*** visits ***will be scheduled*** on weekends or at times ***more convenient to the subject.*** Where applicable, FHP community health care workers will facilitate communication between study staff and participants.

Section 4.3 Overview of the recruitment plan:

At least 3600 subjects will be recruited in the study in 2 waves of enrolment. Each wave, initial and expansion cohorts, will enrol about 600 subjects/site in order to maintain at least 500 subjects/site at the beginning of each dengue season.

Subjects will be recruited from randomly selected households originating from pre-selected mapped communities. Preferably the recruitment period will occur outside of the peak dengue transmission season, and will continue until each site has reached its foreseen target. The expected period for recruiting the target sample size is approximately three months. Recruitment of replacement subjects will be done during the low dengue transmission and the recruitment period will depend on the number of subjects that need to be replaced.

Recruitment will be organized by study staff at participating sites according to the appropriate strategy for each site. Community agents or equivalent will serve as the liaison to schedule visits and may accompany study staff during the visits. The study will be explained to the individuals living in the household, and if any individual is interested

in study participation, informed consent (and assent when applicable) will be obtained, eligibility criteria will be checked and subjects will be enrolled and interviewed for potential study participation.

If the target household is found empty or all members refuse to participate, the first household to the left will be approached. Those households refusing to participate will be recorded as such. A household refusal will be characterized when all individuals in the household refuse to participate in the study. Individual refusals in a given household will not preclude inclusion of other ***individuals living in the households***, and will be recorded as such.

In the subsequent study years/seasons, the cohort size will be maintained at ***a minimum of*** 500 subjects per site. Since subjects who prematurely terminate participation and/or lost to follow-up will be replaced, the final number of participants across the four study years may exceed 3600 subjects. Yearly, there will be an evaluation of active subjects. If the number of active subjects/site becomes < 500, there will be enrolment of replacement subjects to maintain a cohort size of at least 500 subjects/site. Enrolment will preferably take place during the low dengue transmission. Recruitment of replacement subjects could occur at the end of study year 1, 2 or 3. The recruitment approach will be the same as for the initial enrolment.

In order to better represent the Brazilian population distribution, subjects enrolled in the expansion cohort should be composed of at least 30% of subjects younger than 18 years old and should not exceed 20 % of adults 50 years or older. Subjects enrolled as replacement subjects for the initial and expansion cohort will be recruited to approach as much as possible a minimal representation of 30% of subjects younger than 18 years old and a maximal representation of 20% for adults 50 years or older.

Household visits may be scheduled to occur during weekends or at convenient times (like evenings) if deemed necessary.

Section 4.4 Inclusion criteria for enrolment

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

- Written, signed or thumb-printed informed consent (and assent when applicable) must be obtained from the subject or subject's parent(s)/LAR(s). If the subject/subject's parent(s)/LAR(s) are illiterate the consent form will be countersigned by a witness.
- Male or female at least 6 months of age at the time of enrolment.
- Subject and/or subject's parent(s)/LAR(s) who the study staff believes can comply with the requirements of the protocol (e.g., willingness to go to the hospital/clinic for visit/s if dengue, ***Zika and/or chikungunya*** is suspected, able to observe the signs of dengue, ***Zika and/or chikungunya*** and to understand how to take and report body temperature, etc.).
- Subject who plans, at the time of enrolment, to remain at same residence/study area during their study participation period.

Section 5.1 Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP), any applicable local guidelines, including the Document of the Americas, 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 Harmonized 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 from the appropriate regulatory agency, in accordance with applicable regulatory requirements, 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)/legally acceptable representative (LAR[s]) informed consent and subject informed assent, as appropriate
- Investigator reporting requirements as stated in the protocol.

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

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

GSK Biologicals will prepare a model Informed Consent Form (ICF) which will embody the applicable ICH GCP 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 Harmonized Tripartite Guidelines for Good Clinical Practice, those subjects who can only be enrolled in the study with the consent of the subject's parent(s)/LAR(s) (e.g. minors), should be informed about the study to the extent compatible with the subject's understanding and, if capable, the subject should sign and personally date a written informed assent. ***It*** is required ***to*** be signed by each subject, if capable, in addition to the informed consent that is to be signed by his/her parent or legal

representative. ***Requirement of assent*** should be assessed ***based*** on the age of the study population and the local requirements.

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

If a subject is unable to read or if a legally acceptable representative is unable to read, the informed consent will be obtained in the presence of an impartial witness, according to the International Conference on Harmonization (ICH) guidelines. For all participants unable to read for whatever reason, the subject's fingerprint must be obtained on the ICF.

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

Section 5.4 Outline of study procedures

Table 2 List of study procedures for initial cohort subjects

	Surveillance											Suspected Dengue/*Zika*/*Chikungunya* Visits (at hospital/clinic) as applicable		
Procedure	**Visit 1 Day 0**	**Monthly contact between visits**	**Visit 2 Month 6**	**Monthly contact between visits**	**Visit 3 ~ Month 12 (Year 1)**	**Monthly contact between visits**	**Visit 4 ~ Month 24 (Year 2)**	**Monthly contact between visits**	**Visit 5 ~ Month 36 (Year 3)**	**Monthly contact between visits**	**Visit 6 ~ Month 48 (Year 4)**	**First visit Day 0**	**Return visit** [2]	**Follow-up visit Day 21**
Informed consent (and assent if applicable)	●													
Re-consent (and assent if applicable)@			●		●		●		●		●	●	●	●
Subject number and ***initial*** household number attribution	●													
Check inclusion/ exclusion criteria	●													
Record socio-demographic information ***or updates (including household characteristics if applicable)***	●						●		●		●			
Medical history ***including*** YF ***and dengue*** vaccination history or updates	●		●		●		●		●		●			
Distribute subject ID card distribution and suspected dengue instruction kit	O													
Blood sample for serology (5 mL)	●		●		●		●		●		●			
Instruct/remind subjects/subject's parent(s)/LAR(s) on suspected dengue, ***chikungunya and Zika*** assessment procedures (where applicable)	O	O	O	O	O	O	O	O	O	O				

	Surveillance											Suspected Dengue/*Zika*/*Chikungunya* Visits (at hospital/clinic) as applicable		
Procedure	**Visit 1 Day 0**	**Monthly contact between visits**	**Visit 2 Month 6**	**Monthly contact between visits**	**Visit 3 ~ Month 12 (Year 1)**	**Monthly contact between visits**	**Visit 4 ~ Month 24 (Year 2)**	**Monthly contact between visits**	**Visit 5 ~ Month 36 (Year 3)**	**Monthly contact between visits**	**Visit 6 ~ Month 48 (Year 4)**	**First visit Day 0**	**Return visit** [2]	**Follow-up visit Day 21**
Issue diary logs to parents/LARs to be filled out in the event of a suspected dengue, ***chikungunya and Zika*** case[1]	O		O		O		O		O			O		
Contact subject regarding any dengue, ***chikungunya and Zika*** symptoms and remind subject of procedures for suspected dengue, ***chikungunya and Zika***		O		O		O		O		O				
Physical examination/record current medical conditions(see Section 5.6)												●	●	●
Blood sample for dengue/***zika/chikungunya*** infection ***differential*** diagnosis[3]												●		●
Blood sample for CBC and hematocrit ***(mandated for subjects presenting within 14 days following symptoms[7] onset)***												●		
Record body temperature												●		
Report SAEs related to study procedures[4]	●		●		●		●		●		●	●	●	●
Collect or verify diary logs if applicable												O	O	O
Study conclusion[5]											●			●

● is used to indicate a study procedure that requires documentation in the individual eCRF.
○ is used to indicate a study procedure that does not require documentation in the individual eCRF.
[1] Diary logs given at scheduled visits are only to be filled out in the event that the subject has fever ***and/or rash according to the case definition for*** suspected dengue***/chikungunya/Zika***. See Section 5.5.2.5 for details with regard to the diary log.
[2] A return visit is a visit linked to suspected dengue***/chikungunya/Zika***, occurring if the evolution of the subject's physical condition necessitates medical evaluation, according to local medical practice. The investigator will determine whether this return visit is necessary and whether it is considered as part of the same episode. See Section 5.6 for details.
[3] ***Blood sampling for dengue diagnosis which includes*** testing for RT-qPCR, dengue IgG and IgM ELISA, NS1 ELISA, neutralizing antibodies to dengue and ***virological/humoral***, ***testing for Zika and chikungunya infection***.
[4] SAE = Serious adverse event. See Section 6.1.1 for definition.
[5] The study conclusion information in the eCRF is collected once for each subject at the last scheduled visit or at the last follow-up contact for a suspected dengue***/zika/chikungunya*** case, should this case occur during the last scheduled visit or earlier if the subject terminates study participation or is lost to follow-up.
[7] ***Symptoms are fever for dengue, Zika and chikungunya, and rash for Zika.***
@ ***The field staff will re-consent at the first opportunity***

Table 3 List of study procedures for expansion cohort subjects

	Surveillance							Suspected Dengue/*Zika*/*Chikungunya* Visits (at hospital/clinic) as applicable		
Procedure	**Visit 1 # Day 0**	**Monthly contact between visits**	**Visit 2**	**Monthly contact between visits**	**Visit 3**	**Monthly contact between visits**	**Visit 4**	**First visit Day 0**	**Return visit [2]**	**Follow-up visit21**
Informed consent (and assent if applicable)	●									
Re-consent (and assent if applicable)@			●		●		●	●	●	●
Subject number and ***initial*** household number attribution	●									
Check inclusion/ exclusion criteria	●									
Record socio-demographic information ***or updates (including household characteristics if applicable)***	●		●		●		●			
Medical history, ***including*** YF and ***dengue*** vaccination history or updates	●		●		●		●			
Distribute subject ID card distribution and suspected dengue instruction kit	○									

	Surveillance							Suspected Dengue/*Zika/Chikungunya* Visits (at hospital/clinic) as applicable		
Procedure	**Visit 1 # Day 0**	**Monthly contact between visits**	**Visit 2**	**Monthly contact between visits**	**Visit 3**	**Monthly contact between visits**	**Visit 4**	**First visit Day 0**	**Return visit [2]**	**Follow-up visit21**
Blood sample for serology (5 mL)[6]	●		●		●		●			
Instruct/remind subjects/subject's parent(s)/LAR(s) on suspected dengue, ***chikungunya and Zika assessments*** procedures (where applicable)	O	O	O	O	O	O				
Issue diary logs to parents/LARs to be filled out in the event of a suspected dengue, ***chikungunya and Zika*** case [1]	O		O		O			O		
Contact subject regarding any dengue ***chikungunya and Zika*** symptoms and remind subject of procedures for suspected dengue, ***chikungunya and Zika***		O		O		O				
Physical examination/record current medical conditions (see Section 5.6)								●	●	●
Blood sample for dengue ***Zika/chikungunya*** infection ***differential*** diagnosis[3,]								●		●
Blood sample for CBC and hematocrit ***(mandated for subjects presenting within 14 days following symptoms[7] onset)***								●		
Record body temperature								●		
Report SAEs related to study procedures [4]	●		●		●		●	●	●	●
Collect or verify diary logs if applicable								O	O	O
Study conclusion [5]							●			●

● 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.
Enrolment of expansion cohort subjects will start preferably during study Year 2.

[1] Diary logs given at scheduled visits are only to be filled out in the event that the subject has fever ***and/or rash according to the case definition for*** suspected dengue/***chikungunya/Zika***.

[2] A return visit is a visit linked to suspected dengue***/Zika/chikungunya***, occurring if the evolution of the subject's physical condition necessitates medical evaluation, according to local medical practice. The investigator will determine whether this return visit is necessary and whether it is considered as part of the same episode. See Section 5.6 for details.

[3] ***Blood sampling for dengue diagnosis which includes*** testing for RT-qPCR, dengue IgG and IgM ELISA, NS1 ELISA, neutralizing antibodies to dengue and ***virological/humoral***, ***testing for Zika and chikungunya infection***.

[4] SAE = Serious adverse event. See Section 6.1.1 for definition.

[5] The study conclusion information in the eCRF is collected once for each subject at the last scheduled visit or at the last follow-up contact for a suspected dengue***/Zika/chikungunya*** case, should this case occur during the last scheduled visit or earlier if the subject terminates study participation or is lost to follow-up.

[7] ***Symptoms are fever for dengue, Zika and chikungunya, and rash for Zika.***

@ ***The field staff will re-consent at the first opportunity***

Table 4 List of study procedures for replacement subjects

	Surveillance							Suspected Dengue/*Zika/Chikungunya* Visits (at hospital/clinic) as applicable		
Procedure	**Visit 1 Day 0**	**Monthly contact between visits**	**Visit 2**	**Monthly contact between visits**	**Visit 3 as applicable**	**Monthly contact between visits**	**Visit 4 as applicable**	**First visit Day 0**	**Return visit[2]**	**Follow-up visit Day 21**
Informed consent (and assent if applicable)	●									
Re-consent (and assent if applicable)@			●		●		●	●	●	●
Subject number and ***initial*** household number attribution	●									
Check inclusion/ exclusion criteria	●									
Record/ update socio-demographic information ***or updates (including household characteristics if applicable)***	●		●		●		●			
Medical history, ***including*** YF and ***dengue*** vaccination history or updates	●		●		●		●			
Distribute subject ID card distribution and suspected dengue instruction kit	O		O		O					
Blood sample for serology (5 mL)	●		●		●		●			
Instruct/remind subjects/subject's parent(s)/LAR(s) on suspected dengue, ***chikungunya and Zika*** assessment procedures (where applicable)	O	O	O	O	O	O				
Issue diary logs to parents/LARs to be filled out in the event of a suspected dengue***, chikungunya and Zika*** case [1]	O		O		O			O		
Contact subject regarding any dengue, ***chikungunya and Zika*** symptoms and remind subject of procedures for suspected dengue***, chikungunya and Zika***		O		O		O				

	Surveillance							**Suspected Dengue/*Zika*/*Chikungunya* Visits (at hospital/clinic) as applicable**		
Procedure	**Visit 1 Day 0**	**Monthly contact between visits**	**Visit 2**	**Monthly contact between visits**	**Visit 3 as applicable**	**Monthly contact between visits**	**Visit 4 as applicable**	**First visit Day 0**	**Return visit[2]**	**Follow-up visit Day 21**
Physical examination/record current medical conditions (see Section 5.6)								●	●	●
Blood sample for dengue, ***Zika/chikungunya*** infection ***differential*** diagnosis[3]								●		●
Blood sample for CBC and hematocrit ***(mandated for subjects presenting within 14 days following symptoms[7] onset)***								●		
Record body temperature								●		
Report SAEs related to study procedures [4]	●		●		●		●	●	●	●
Collect or verify diary logs if applicable								O	O	O
Study conclusion [5]							●			●

● 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.

[1] Diary logs given at scheduled visits are only to be filled out in the event that the subject has fever ***and/or rash according to the case definition for*** suspected dengue ***chikungunya/Zika***. See Section 5.5.2.5 for details with regard to the diary log.

[2] A return visit is a visit linked to suspected dengue***/Zika/chikungunya***, occurring if the evolution of the subject's physical condition necessitates medical evaluation, according to local medical practice. The investigator will determine whether this return visit is necessary and whether it is considered as part of the same episode. See Section 5.6 for details.

[3] ***Blood sampling for dengue diagnosis which includes*** testing for RT-qPCR, dengue IgG and IgM ELISA, NS1 ELISA, neutralizing antibodies to dengue and ***virological/humoral***, ***testing for Zika and chikungunya infection***.

[4] SAE = Serious adverse event. See Section 6.1.1 for definition.

[5] The study conclusion information in the eCRF is collected once for each subject at the last scheduled visit or at the last follow-up contact for a suspected dengue***/Zika/chikungunya*** case, should this case occur during the last scheduled visit or earlier if the subject terminates study participation or is lost to follow-up.

@ ***The field staff will re-consent at the first opportunity***

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Table 5 Intervals between study visits/contacts for INITIAL cohort

Study Year*	Visits Interval	Optimal length of interval *	Allowed interval**
Year 1	Visit 1 (Day 0) → Visit 2 (Month 6)	6 months	± 28 days
	Visit 2 (Month 6) → Visit 3 (Month 12)	6 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	
Year 2	Visit X → Visit X+1¶ Note: Visit X ***is*** visit 3 for subjects enrolled in study year 1. For subjects enrolled in study year 2, visit X would be visit 1.	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	
Year 3	Visit X+1 → Visit X+2	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	
Year 4	Visit X → Visit X+3	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	

*Whenever possible the investigator should arrange study visits/contacts within this interval
**Subjects might not be eligible for inclusion in the ATP cohort for analysis if they make the study visit/contact outside this interval

Table 7 Interval between visits for suspected dengue case (Amended 16 October 2017)

Interval	Optimal length of interval*	Allowed interval**
Visit 1 (Day 0) → Visit 2 (Year 1)	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	±28 days
Visit 2 (Year 1) → Visit 3 (Year 2)	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	±28 days
Visit 3 (Year 2) → Visit 4 (Year 3)	12 months The interval may be adapted to allow scheduling the visit in a period of generally low dengue transmission	±28 days
~~Interval between visit for a suspected dengue case and the final follow-up visit for a case~~	~~21 days~~	~~+7 days~~

Table 7 Interval between visits for suspected dengue/chikungunya/Zika case

	Optimal length of time	Allowed interval
Suspected dengue/***chikungunya/Zika*** case: Interval between first and follow-up visit	21 days	+ 7 days

Note that this interval applies to all subjects enrolled regardless of the year of enrolment.

Section 5.5.2 Procedures at scheduled Visit 1 (enrolment visit)

Ideally, Visit 1 (the enrolment visit) will be scheduled before the peak incidence of dengue based on previous years.

A subject number and ***initial*** household number will be attributed at Visit 1.

Section 5.5.2.2 Collect specific medical history data

A baseline medical history, including history of dengue infection, ~~YF vaccination history will be taken and results~~ , ***of dengue, Zika and chikungunya infection, and YF and dengue vaccination history will be taken. Results*** will be recorded in the subject's source document for subsequent recording in the eCRF.

YF ***and dengue*** vaccination status will be recorded, along with the source of the information (either written or oral record). A vaccination record, if available, is preferable but self-reported history will be recorded if the vaccination record is not available.

Information on medical history (e.g., diabetes, cardiovascular diseases, asthma, cancers, hematologic diseases, genetic disorders) will be collected.

Section 5.5.2.3 Collect blood sample

- A blood sample for serology will be collected from all enrolled subjects (see Section 5.8 for details regarding sample collection). For children <2 years of age, the total amount of blood collected will not exceed 5 mL at any visit.

Section 5.5.2.4 Instruction on enhanced passive dengue, *chikungunya and Zika* surveillance by the subject or subject's parent(s)/LAR(s)

Study personnel will train subjects/subject's parent(s)/LAR(s) to recognize dengue ***Zika and chikungunya*** symptoms and will instruct them to contact the study personnel or come to a designated study hospital/clinic for medical evaluation within 5 days of the occurrence of ***fever and/or rash*** that may be associated with suspected dengue, ***chikungunya or Zika*** (defined in Section 5.7.1, 5.7.2 and 5.7.3).

Each household will also be given at least one dengue kit, as applicable, which includes thermometers, study contact information (phone numbers) and an instruction card with information about dengue, ***Zika and chikungunya*** symptoms***. This card will also provide instructions on who to contact and what to do if any of these infections are suspected.***

Participants or LAR will be instructed on how to take body temperature and how to record the temperatures in a diary log.

See Section 5.6 for details regarding management of suspected dengue, ***chikungunya and Zika*** cases.

5.5.2.4.1 Diary log distribution

A diary log will be given to all subjects in the household to be used in the event of suspected dengue***, chikungunya and/or Zika illness***. Diary logs will be distributed at the first scheduled visit and if they are needed, at subsequent visits, ***if needed*** (if the subject no longer has one, e.g., the previous one was lost or used). A new diary log will be issued by the study staff whenever necessary.

The subject will be instructed to start recording any symptoms and body temperature any time dengue, ***chikungunya and/or Zika infections are*** suspected. Refer to Section 5.6.1 for details.

5.5.2.4.2 Recording of serious adverse events related to a study procedure

The subject/subject's parent(s)/LAR(s) will be instructed to contact the study staff should any serious adverse event related to a study procedure occur during the study.

Refer to Section 8.2 for procedures for the Investigator to record SAEs that are related to study participation and for guidelines on how to report these SAEs to GSK Biologicals.

5.5.2.4.2 Procedures at subsequent scheduled visits (2-6, as applicable)

- For initial cohort subjects, Visit 2 will occur approximately 6 months after Visit 1 and Visit 3 approximately 6 months after Visit 2. Initial cohort subjects willing to extend their participation to three additional years will be re-consented at Visit 3 (i.e. at the end of study Year 1).
- For expansion cohort subjects, scheduled visits should ideally be performed at yearly intervals, preferably during periods of usually low dengue transmission. Expansion cohort subjects will have at most 4 scheduled visits, depending on the time of enrolment.
- For replacement subjects enrolled in the subsequent study years, scheduled visits should ideally be performed at yearly intervals, preferably during periods of usually low dengue transmission.
- A blood (serum) sample will be collected for serology.

  Note: Indirect DENV IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for DENV IgG at the previous scheduled visit. Even if the test is not repeated, the volume of the blood sample remains unchanged.
- ***Socio-demographic and household information will be updated in eCRF (for e.g. change of home location) (for all scheduled visits planned after approval of protocol amendment 4).***
- Medical and vaccination histories will be updated in the eCRF.
- The investigator will remind the subjects about the procedures in case of signs and symptoms of dengue, ***Zika and chikungunya***.

- A new diary log will be distributed for continued dengue, ***chikungunya and Zika*** surveillance if needed, and any completed diary logs will be verified, as applicable. Subjects will be asked to contact the study staff in the event of the occurrence of a symptom that may be associated with suspected dengue ***or Zika*** (see Section 5.7.1.1 for suspected dengue definition).

- The subject will be instructed to start recording any symptoms and body temperature any time dengue***, chikungunya and/or Zika*** is suspected. Refer to Section 5.6.1 for details.

- Any SAEs related to study procedures will be recorded.

Section 5.5.2.5 Diary log distribution

A diary log will be given to all subjects in the household to be used in the event of suspected dengue***, chikungunya and/or Zika illness***. Diary logs will be distributed at the first scheduled visit and if they are needed, at subsequent visits (if the subject no longer has one, e.g., the previous one was lost or used). A new diary log will be issued by the study staff whenever necessary.

Section 5.5.3 Procedures at subsequent scheduled visits (2-6, as applicable)

- For initial cohort subjects, Visit 2 will occur approximately 6 months after Visit 1 and Visit 3 approximately 6 months after Visit 2. Initial cohort subjects willing to extend their participation to three additional years will be re-consented at Visit 3 (i.e. at the end of study Year 1).
- For expansion cohort subjects, scheduled visits should ideally be performed at yearly intervals, preferably during periods of usually low dengue transmission. Expansion cohort subjects will have at most 4 scheduled visits, depending on the time of enrolment.
- For replacement subjects enrolled in the subsequent study years, scheduled visits should ideally be performed at yearly intervals, preferably during periods of usually low dengue transmission.
- A blood (serum) sample will be collected for serology.

  Note: Indirect DENV IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for DENV IgG at the previous scheduled visit.

  Zika IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for Zika IgG at the previous scheduled visit.

  Chikungunya IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for chikungunya IgG at the previous scheduled visit.

  Even if the tests are not repeated, the volume of the blood sample remains unchanged.

- ***Socio-demographic and household information will be updated in eCRF (for e.g. change of home location).***
- Medical and vaccination histories will be updated in the eCRF.
- The investigator will remind the subjects about the procedures in case of signs and symptoms of dengue, ***Zika and chikungunya***.
- A new diary log will be distributed for continued dengue, ***chikungunya and Zika*** surveillance if needed, and any completed diary logs will be verified, as applicable. Subjects will be asked to contact the study staff in the event of the occurrence of a symptom that may be associated with suspected dengue, ***chikungunya or Zika*** (see Section 5.7.1.1, 5.7.2 and 5.7.3 for suspected dengue, ***chikungunya or Zika*** definition).
- The subject will be instructed to start recording any symptoms, ***including rash*** and body temperature any time dengue***, chikungunya and/or Zika*** is suspected. Refer to Section 5.6.1 for details.
- Any SAEs related to study procedures will be recorded.

Section 5.5.4 Study conclusion

The Study Conclusion screen page in the eCRF will be completed at the last study contact. This last contact could occur at the last scheduled visit or follow up visit for a suspected dengue***, chikungunya or Zika*** case (if it is on-going at the last scheduled visit) or earlier if the subject terminates study participation or is lost-to-follow-up.

The study staff will review data collected to ensure accuracy and completeness and will complete the Study Conclusion screen page 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. If so, the investigator will ask each subject/subject's parent(s)/LAR(s) if he or she would be willing to participate (or let their child participate) in a long-term follow-up study and subject will be asked to sign a new consent form.

Section 5.5.5 Dengue, chikungunya and Zika case detection

Suspected cases (defined in Section 5.7.1, 5.7.2 and 5.7.3) in the study cohort may arise from three sources: 1) referred by study personnel during scheduled home visits; 2) through enhanced passive surveillance; and 3) as a result of active surveillance between scheduled visits.

Section 5.5.5.1 Case detection during scheduled home visits

If a subject is identified as suspected case ***of dengue, chikungunya and/or Zika*** during any home visit, he or she will be referred to the designated study hospital/clinic for medical evaluation (refer to Section 5.6).

Section 5.5.5.2 Case detection through enhanced passive surveillance

The subject/subject's parent(s)/LAR(s) will be instructed to contact the study staff (the local study coordinator) at any time dengue ***or chikungunya*** is suspected (i.e., body temperature ≥38°C measured (by any route) for at least two consecutive days) ***or if Zika is suspected (fever [i.e., body temperature ≥38°C measured (by any route) for at least two consecutive days] and/or rash)***.

The subject/subject's parent(s)/LAR(s) will be instructed to contact the hospital or study clinic should the subject manifest any signs or symptoms they/the subject's parent(s)/LAR(s) perceive as an emergency or severe.

The phone contact will be preferably made during working hours/days for logistical reasons. A reasonable schedule for phone contacts to the local study coordinator is from Monday to Saturday, 7:00 AM to 8:00 PM. ***In the case of an emergency outside of the defined hours, the patient should contact or present to their local emergency room, and contact the study staff point of contact as soon as convenient to the subject/subject's parent(s)/LAR(s).***

When a subject/subject's parent(s)/LAR(s) contacts the study staff, the local study coordinator will then arrange for an appointment at the designated study hospital/clinic. The subject/subject's parent(s)/LAR(s) will be instructed to record ***any symptom including rash and*** body temperature on the diary log daily until the appointment.

The visit should be arranged as soon as possible, preferably during week days for logistical reasons. If the subject contacts the study staff during the weekend, the visit should be scheduled for the following Monday. All efforts should be made to guarantee that the subject be seen by the fifth day of disease onset, at latest.

During holidays, arrangements will be made with study physicians and the subjects should be seen at the designated study hospital/clinic.

Although subjects will be instructed to contact the study staff in the event of suspected dengue***, chikungunya and/or Zika***, there may be cases where the subject is taken directly to the hospital or clinic. If this occurs when the study physician is not available, the staff at the designated hospital should notify the local study coordinator and the study physician.

The study staff should ensure that:

- the acute sample will be properly collected,
- all clinical information will be retrievable,
- a return visit (if applicable) will be scheduled
- and a follow-up visit will be scheduled.

See Section 5.6 for cases that present to a non-study hospital/clinic.

Section 5.5.5.3 Case detection through active surveillance

Telephone calls (or home visits when applicable, if a phone call is not feasible) will be conducted at least monthly and more frequently if needed. During the phone call or visit, a structured script will be used to inquire about dengue, ***chikungunya and Zika*** symptoms since the last contact.

An example of questions to be included in the structured script is provided in APPENDIX B.

If dengue, ***chikungunya and/or Zika*** is suspected during active surveillance, an appointment will be arranged at the designated study hospital/clinic, and at least one additional visit will be required for case follow-up (see Section 5.6).

Subjects whose status cannot be ascertained during active surveillance will be tracked by the study personnel through an active tracking algorithm.

Figure 2 Active tracking algorithm

Section 5.6 Management of suspected dengue, *chikungunya* and Zika cases

All study subjects with suspected dengue***, chikungunya and/or Zika*** should be seen at a designated study hospital or clinic by the study physician.

The first visit to the study hospital/clinic will include a detailed clinical examination to assess the subject's general condition, body temperature, height and weight, cardiac and respiratory rates, blood pressure, dengue, ***chikungunya and Zika*** associated clinical signs/symptoms and relevant clinical signs/symptoms (e.g., fatigue, headache, pain behind the eyes, abdominal pain, nausea, vomiting, muscle ache, joint ache, diffuse rash, photophobia and pruritus).

A blood sample should be collected by the hospital/clinic for laboratory confirmation of dengue***, chikungunya, Zika and differential diagnosis***. This sample is referred to as the 'acute' serum sample. In addition, a blood sample for haematology (CBC and HCT) will be collected ***(mandated procedure for subjects presenting within the first 14 days following onset of fever and/or rash)***. ***If blood chemistry is conducted according to local clinical practice, these data may also be collected.***

If dengue***, chikungunya or Zika*** is still suspected after the first visit to the study hospital/clinic, a return visit for suspected dengue***, chikungunya and/or Zika*** may be needed and will be conducted as directed by National guidelines by the study physician.

A return visit is a visit linked to suspected dengue, ***chikungunya and/or Zika***, occurring if the evolution of 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. If a return visit occurs, the diary log will be verified and possibly used by the physician to complete the clinical symptoms in the eCRF.

Medical data related to any suspected dengue***, chikungunya and/or Zika*** case will be collected for hospitalized study subjects and for those followed in the outpatient setting at the return visit. Treatment will be given according to local standard routines, following national guidelines.

Approximately 21(+7) days after the first visit for suspected dengue***, chikungunya and/or Zika***, a follow-up (convalescent) visit will be required. A physical examination will be conducted and a 'convalescent' serum sample (i.e., a blood sample collected after the acute phase for dengue***, chikungunya and/or Zika***) should be collected at this follow-up visit. Ideally, this collection should be done at the study hospital/clinic but may be done at the subject's home if directed by the study physician or delegated study staff, if the subject is unable to come to the hospital/ clinic.

Subjects who, for any reason, seek medical assistance at any non-study health care facility**,** will be identified through active surveillance, and clinical data will be retrospectively collected on the eCRF. The study physician will be responsible for collecting the retrospective data and informing the local study coordinator for appropriate follow-up.

Section 5.6.1 Diary log instructions for suspected dengue and Zika cases

The subject/subject's parent(s)/LAR(s) will be instructed to record body temperature ***and rash*** on the diary log daily until the appointment. See Section 5.6 for subjects who do not reach the study staff and go directly to the hospital/clinic. The subject will be instructed to start recording any symptoms, ***including rash*** and body temperature any time dengue***, chikungunya and/or Zika virus infection*** is suspected.

The information collected in the diary log will be provided to the study physician as a tool in the medical evaluation of the subject. If the physician considers that the subject meets the criteria for suspected dengue***, chikungunya and/or Zika***, he could use the information contained in the diary log to complete the description of first clinical symptoms in the eCRF.

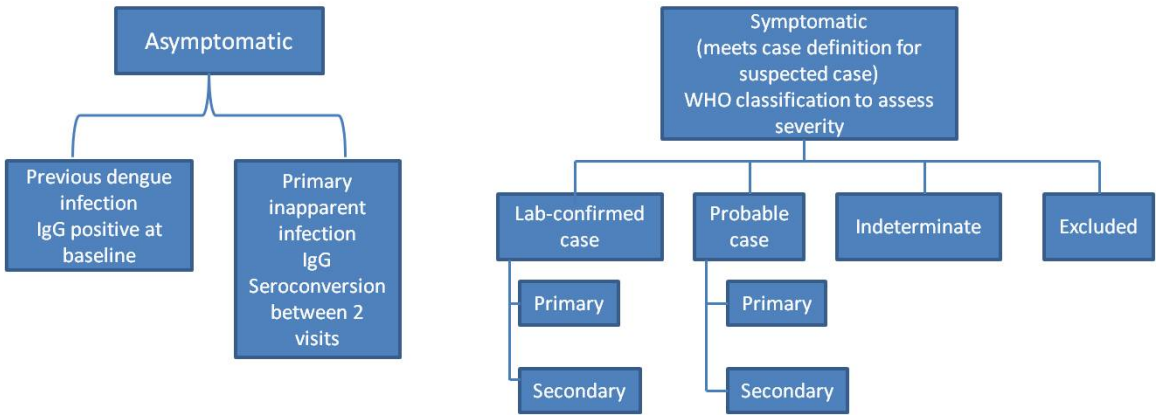
If dengue***, chikungunya and/or Zika*** is suspected, the diary log will be verified by the investigator or designee at the ***follow-up*** visit if the subject is not hospitalized. If the subject is hospitalized, information with regard to dengue ***chikungunya and/or Zika*** infection will be recorded in the hospital record. Information from diary log and hospital records can be used to update clinical data in the eCRF.

Section 5.7 Case definitions

Section 5.7.1 Dengue

The following classification of dengue cases will be used in this study.

Figure 3 Classification of dengue cases



Section 5.7.1.1 Suspected symptomatic dengue case

Febrile illness with body temperature ≥38°C measured (by any route) on at least two consecutive days and less than 14 days with or without the presence of other dengue symptoms or signs, without an obvious aetiology unrelated to dengue, based on investigator's judgement.

Although subjects are asked to come to a study hospital if fever (body temperature ≥ 38°C) ***is*** sustained for two consecutive days, a subject ***could*** present on the first day of fever. In this case the physician may still consider the subject as a suspected dengue case based on medical judgement and collect a blood sample for laboratory diagnosis.

Subjects should be examined at the health care facility during the 5 days following fever onset, as this is a requirement for RT-qPCR diagnosis of dengue infection and serotype identification.

However, subjects presenting until 30th (included) day of fever onset will be considered for investigation. After the 30th day, subjects will not be considered as suspected dengue cases.

A suspected dengue case presenting at the health care facility within 5 days following the onset of fever will be defined as an 'early presenter'.

Subjects presenting for care ***between sixth and 30th day*** of fever onset will be defined as 'late presenters'.

An example of other signs and symptoms of dengue, associated with fever, include but are not limited to: fatigue, headache, pain behind the eyes, abdominal pain, nausea, vomiting, muscle ache, joint ache, diffuse rash on the trunk, abnormal sensitivity to light (photophobia) and itching of skin (pruritus).

Section 5.7.1.2 Laboratory-confirmed symptomatic dengue case

Early presenter:

At least one of the following findings must be met for a laboratory-confirmed dengue case:

- Dengue virus identification through RT-qPCR on the acute serum sample
- Dengue virus NS1 positive on acute serum sample through ELISA.
- Anti-dengue IgM seroconversion between acute and convalescent serum samples through ELISA.

Late presenter:

Anti-dengue IgM seroconversion between acute and convalescent serum samples through ELISA.

Section 5.7.1.5 Previous dengue infection

~~Dengue seroprevalence measured at baseline will be defined as:~~

A subject will be considered as having previous dengue infection at visit 1(baseline) if:

- Dengue IgG positive at visit 1 (baseline)

or

- Laboratory-confirmed symptomatic dengue case detected at visit 1 (baseline)

A subject will be considered as having previous dengue infection at any time after visit 1 (baseline), if:

- ***Dengue IgG positive at previous schedule visit(s)***

or

Laboratory-confirmed symptomatic dengue case detected previously at study surveillance

Section 5.7.1.7 Primary symptomatic dengue case

A primary symptomatic dengue case is a subject with ***laboratory*** confirmed ***or probable*** symptomatic dengue infection without evidence of ~~past~~ ***previous*** dengue ***infection*** (~~presence of~~ ***absence of*** Ig G antibodies at the previous scheduled visit ***(s)*** or ***absence of*** laboratory-confirmed symptomatic case ~~at baseline~~ ***detected previously at study surveillance***).

Section 5.7.1.10 Secondary symptomatic dengue case

A secondary symptomatic dengue case is a subject with ***laboratory*** confirmed or ***probable*** symptomatic dengue infection with evidence of ~~past~~ ***previous*** dengue infection

(presence of Ig G antibodies at the previous scheduled visit or laboratory-confirmed symptomatic case ~~at baseline~~ ***detected previously at study surveillance***).

Section 5.7.1.6 Primary inapparent dengue infection

This condition is defined as a documented seroconversion (anti-dengue IgG antibodies) ~~to one or more dengue types~~ between two sequential sera samples obtained during the scheduled visits without clinical suspicion of dengue (identified during the time period in which seroconversion occurred).

Section 5.7.1.9 Probable dengue case

- ~~A suspected dengue case, based on strong clinical suspicion or a clinical diagnosis, in a late presenter (i.e., RT-qPCR not performed), and:~~
- ~~Anti-dengue IgM or anti-dengue IgG* positivity on at least one sample (on either the acute or convalescent blood sample)~~

~~*and*~~

- ~~NS1 negative on the acute sample~~

~~*and*~~

- ~~No evidence of anti-dengue IgM seroconversion between the acute and the convalescent sample~~

~~**The ELISA test allows the detection of dengue IgG levels characteristic of acute secondary infections.*~~

- ***For early presenters, a probable case will be that case without laboratory confirmation, presenting IgG positive in the convalescent sample.***
- ***For late presenters, a probable case will be the case without seroconversion of IgM, presenting at least one IgG positive in one sample (acute or convalescent).***

Section 5.7.1.10 Negative dengue case

For early and late presenters, a negative dengue case is a participant with a negative result for all planned laboratory tests on both acute and convalescent samples.

Section 5.7.1.11 Indeterminate dengue case

~~A case is considered as indeterminate when RT-PCR or NS1 is negative (early presenters) or not performed (late presenters) and a convalescent serum is missing to confirm or rule out dengue.~~

An indeterminate dengue case is a participant evaluated as an SDC (Section 5.7.1.1.) and not classified as laboratory confirmed case (Section 5.7.1.2), probably case (Section 5.7.1.9) or negative case (Section 5.7.1.11).

Section 5.7.2 Chikungunya

Section 5.7.2.1 Suspected symptomatic chikungunya case

Febrile illness with body temperature ≥ 38°C measured (by any route) on at least two consecutive days and less than 14 days with or without the presence of other chikungunya symptoms or signs, without an obvious aetiology unrelated to chikungunya, based on investigator's judgement.

Although subjects are asked to come to a study hospital if fever (body temperature ≥ 38°C) is sustained for two consecutive days, a subject could present on the first day of fever. In this case the physician may still consider the subject as a suspected chikungunya case based on medical judgement and collect a blood sample for laboratory diagnosis.

Subjects should be examined at the health care facility during the 5 days following fever onset, as this is a requirement for RT-qPCR diagnosis of dengue infection in the case of co-infection

However, subjects presenting until 30th (included) day of fever onset will be considered for investigation. After the 30th day, subjects will not be considered as suspected chikungunya cases.

A suspected chikungunya case presenting at the health care facility within 5 days following the onset of fever will be defined as an 'early presenter'.

Subjects presenting for care between sixth and 30th day of fever onset will be defined as 'late presenters'.

An example of other signs and symptoms of chikungunya, associated with fever, include but are not limited to:

- ***Polyarthralgia is usually bilateral and symmetric, and can be debilitating***
- ***Other symptoms may include headache, myalgia, arthritis, conjunctivitis, nausea/vomiting, or maculopapular rash.***

Rare complications include uveitis, retinitis, myocarditis, hepatitis, nephritis, bullous skin lesions, hemorrhage, meningoencephalitis, myelitis, Guillain-Barré syndrome, and cranial nerve palsies.

Section *5.7.2.2 Laboratory-confirmed symptomatic chikungunya case*

Early presenter:

At least one of the following findings must be met for a laboratory-confirmed chikungunya case:

- ***Chikungunya virus identification through RT-qPCR on the acute serum sample***
- ***Anti-chikungunya IgM seroconversion between acute and convalescent serum samples through ELISA.***

Late presenters:

- ***Anti-chikungunya IgM seroconversion between acute and convalescent serum samples through ELISA.***

Section *5.7.2.3* ***Virologically confirmed symptomatic chikungunya infection***

A virologically confirmed symptomatic chikungunya infection is defined as a chikungunya case confirmed by RT-qPCR.

Section *5.7.2.4* ***Previous chikungunya infection at baseline***

A subject will be considered as having previous chikungunya infection at baseline (enrolment visit/Visit 1), if:

- ***Chikungunya IgG positive at baseline visit OR***
- ***Laboratory-confirmed symptomatic chikungunya case at baseline***

Section 5.7.2.5 Inapparent chikungunya infection

This condition is defined as a documented seroconversion (anti-chikungunya IgG antibodies) between two sequential sera samples obtained during the scheduled visits without clinical suspicion of chikungunya (identified during the time period in which seroconversion **occurred).**

Section 5.7.2.7 Probable chikungunya case

For early and late presenters, a probable chikungunya case will be that case without a lab-confirmed criteria, presenting at least one IgG positive in one sample (acute or convalescent).

Section 5.2.7.8 Negative chikungunya case

For early and late presenters, a negative chikungunya case is a participant with a negative result for all planned laboratory tests on both acute and convalescent samples.

Section 5.7.2.6 Indeterminate chikungunya case

An indeterminate chikungunya case is a participant evaluated as an suspected chikungunya case (Section 5.7.2.1.) and not classified as laboratory confirmed case (Section 5.7.2.2), probably case (Section 5.7.2.6) or negative case (Section 5.7.2.7).

***Section* 5.7.3 Zika**

Section 5.7.3.1 Suspected symptomatic Zika case

Rash and/or febrile illness on at least two consecutive days and less than 14 days with or without the presence of other Zika symptoms or signs, without an obvious aetiology unrelated to Zika, based on investigator's judgement.

Although subjects are asked to come to a study hospital if fever and/or rash is sustained for two consecutive days, a subject could present on the first day of fever and/or rash. In this case the physician may still consider the subject as a suspected Zika case based on medical judgement and collect a blood sample for laboratory diagnosis.

Subjects should be examined at the health care facility during the 5 days following fever and/or rash onset, as this is optimal for RT-qPCR diagnosis of Zika infection.

However, subjects presenting until 30th (included) day of fever and/or rash onset will be considered for investigation. After the 30th day, subjects will not be considered as suspected Zika cases.

A suspected Zika case presenting at the health care facility within 5 days following the onset of fever and/or rash will be defined as an 'early presenter'. Subjects presenting for care between sixth and 30th day of fever and/or rash onset will be defined as 'late presenters'. At the clinical presentation of the first visit, fever will take priority in the classification of "early" or "late" presenter if the participant reported both fever and rash are present. If the participant presents the rash without fever, the date of onset of rash will determine classification.

An example of other signs and symptoms include arthralgia, arthritis, or conjunctivitis (non-purulent/hyperaemic).

Section 5.7.3.2 Laboratory-confirmed symptomatic Zika case

Early presenters:

At least one of the following findings must be met for a laboratory-confirmed Zika case.

- ***Zika virus identification through RT-qPCR on the acute serum sample***
- ***Anti-Zika IgM seroconversion between acute and convalescent serum samples through ELISA and confirmed by Zika neutralization antibody testing.***

Late presenters:

- ***Anti-Zika IgM seroconversion between acute and convalescent serum samples through ELISA and confirmed by Zika neutralization antibody testing.***

Section 5.7.3.3 Virologically confirmed symptomatic Zika infection

A virologically confirmed symptomatic Zika infection is defined as a Zika suspected case confirmed by RT-qPCR.

Section 5.7.3.4 Previous Zika infection at baseline

A subject will be considered as having previous Zika infection at baseline (enrolment visit/Visit 1), if:

- ***Zika IgG positive at baseline as confirmed by neutralizing antibody tests visit OR***
- ***Laboratory-confirmed symptomatic Zika case at baseline***

Section 5.7.3.5 Inapparent Zika infection

This condition is defined as a documented seroconversion and confirmed by Zika neutralising antibody testing between two sequential sera samples obtained during the scheduled visits without clinical suspicion of Zika (identified during the time period in which seroconversion occurred).

Section 5.7.3.7 Probable Zika case

- ***For early and late presenters, a probable Zika case will be that case without a lab-confirmed criteria, presenting at least one IgG positive in one sample (acute or convalescent).***

Section 5.7.3.7 Negative Zika case

For early and late presenters, a negative Zika case is a participant with a negative result for all planned laboratory tests on both acute and convalescent samples.

Section 5.7.3.6 Indeterminate Zika case

An indeterminate Zika case is a participant evaluated as an suspected Zika case (Section 5.7.3.1.) and not classified as laboratory confirmed case (Section 5.7.3.2), probably case (Section 5.7.3.6) or negative case (Section 5.7.3.7).

Section 5.8.2.1 Laboratory assays

Table 8 Humoral Immunity

System	Component	Method	Kit / Manufacturer	Laboratory
Serum	IgM to Dengue	ELISA	Commercial kit Panbio or equivalent	Local# or ***GSK designated laboratory***
	IgG to Dengue	ELISA	Indirect IgG Panbio or equivalent (for scheduled visits)	Local# or ***GSK designated laboratory***
			IgG Capture PanBio or equivalent (for ***unscheduled visits***)	Local# or ***GSK designated laboratory***
Serum	Dengue virus types 1-4 neutralizing antibodies	Dengue neutralization assay	In-house	Laboratório de Tecnologia Virológica, Bio-Manguinhos, Fiocruz Rio de Janeiro or GSK designated laboratory.
Serum	***Antibodies against Zika and chikungunya***	***To be determined***	***To be determined***	***GSK designated laboratory***
Serum	YF virus neutralizing antibody*	YF neutralization assay	To be determined	GSK designated laboratory

*For subjects residing in a yellow fever endemic region or vaccinated against yellow fever
#***Testing performed according to local practices***

Table 9 Virology

System	Component	Method	Kit/Manufacturer	Laboratory
Serum	DENV RNA	RT-qPCR	In-house	Laboratório de Tecnologia Virológica, Bio-Manguinhos, Fiocruz Rio de Janeiro (GSK as back-up)
Serum	***Zika RNA***	***RT-PCR***	***To be determined***	***GSK designated laboratory***
Serum	***Chikungunya RNA***	***RT-PCR***	***To be determined***	***GSK designated laboratory***
Serum	NS1	ELISA	Commercial kit Biorad,Platelia	Local

Section 5.8.2.2 Laboratory read-outs

In case of insufficient blood sample volume to perform all assays, the samples will be analyzed according to priority ranking provided in Table 11, Table 12 and Table 13.

Table 11 Laboratory read-outs at each time point, and priority ranking for scheduled visits

SCHEDULED VISITS								
Blood sampling time point		No. Subjects (anticipated)	Subset*	Possible assays	Serum aliquot volume	*Aliquot* priority rank	*Test priority rank*	Aliquot number
Type of contact/ Amount of whole blood	Sampling time point							
Scheduled Visit 1 5 mL whole blood	(Day 0)	3600 (1800 from wave 1 & 2***)	-	Dengue IgG ELISA**	250 µL	1	***1***	1
			yes	Dengue virus 1-4 neutralizing antibodies	1 mL	2	***1***	2
				YF neutralization			***4***	
				Zika neutralization			***3***	
				Zika serology (IgG)#			***2***	
				Chikungunya serology (IgG)#				
			yes	***Chikungunya neutralization***	remaining volume	3	***1***	3
Scheduled Visit 2 5 mL whole blood	Visit 1+ 6 months for subject enrolled in Study Year 1; Visit 1+ 12 months for subject enrolled in beginning of Study Year 2, 3 or 4)	3190 (1660 from wave 1 and 1530 from wave 2)	yes	Dengue IgG ELISA** (only for subjects IgG negative at the previous visit)	250 µL	1	***1***	1
			yes	Dengue virus 1-4 neutralizing antibodies	1 mL	2	***1***	2
				YF neutralization			***4***	
				Zika neutralization			***3***	
				Zika serology (IgG)#			***2***	
				Chikungunya serology (IgG)#				
			yes	***Chikungunya neutralization***	remaining volume	3	***1***	3
Scheduled Visit 3 5 mL whole blood	(Visit 2+ 6 months for subject enrolled in Study Year 1; Visit 2+12 months for	3030 (1530 from wave 1 and 1500 from wave 2)	yes	Dengue IgG ELISA** (only for subjects IgG negative at the previous visit)	250 µL	1	***1***	1
			yes	Dengue virus 1-4 neutralizing antibodies	1 mL	2	***1***	2

SCHEDULED VISITS								
Blood sampling time point		**No. Subjects (anticipated)**	**Subset***	**Possible assays**	**Serum aliquot volume**	***Aliquot* priority rank**	***Test priority rank***	**Aliquot number**
Type of contact/ Amount of whole blood	**Sampling time point**							
	subject enrolled in beginning of Study Year 2 or 3)			***YF neutralization***			***4***	
				Zika neutralization			***3***	
				Zika serology (IgG)#				
				Chikungunya serology (IgG)#			***2***	
			yes	***Chikungunya neutralization***	remaining volume	3	***1***	3
Subsequent Yearly Visit(s) as applicable 5 mL whole blood	Yearly	3000 for Visit 4 and 1500 for visit 5 and 6 which applies only to subjects of the wave 1	yes	Dengue IgG ELISA** (only for subjects IgG negative at the previous visit)	250 µL	1	***1***	1
			yes	Dengue virus 1-4 neutralizing antibodies	1 mL	2	***1***	2
				YF neutralization			***4***	
				Zika neutralization			***3***	
				Zika serology (IgG)#			***2***	
				Chikungunya serology (IgG)#				
			yes	***Chikungunya neutralisation***	remaining volume	3	***1***	3

*tertiary (exploratory) analysis, number of subjects in the subset will be determined during analysis

- not applicable,

** Dengue IgG indirect ELISA

*** Wave 1 enrolment = initial cohort / Wave 2 enrolment = Expansion cohort

 # Indirect DENV IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for DENV IgG at the previous scheduled visit. Zika IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for Zika IgG at the previous scheduled visit; chikungunya IgG assay is repeated in the following scheduled visits (after enrolment) only if the subject was negative for chikungunya IgG at the previous scheduled visit

Table 12 Laboratory read-outs at each time point, and priority ranking for suspected dengue, chikungunya and/or Zika visits for EARLY presenters (Amended 16 October 2017)

SUSPECTED DENGUE, CHIKUNGUNYA AND/OR ZIKA VISITS – EARLY PRESENTERS								
Blood sampling time point		**No. Subjects**	**Subset**	**Component**	**Serum aliquot volume**	***Aliquot* priority rank**	***Test priority rank***	**Aliquot number**
Type of contact/ Amount of whole blood	**Sampling time point**							
First visit (amount of blood= 5 mL for < 2 years of age and 10 mL for all other subjects)	Sample collected ≤ 5 days of fever and/or rash	unknown	-	***Dengue*** RT-qPCR	750 µL	1	***1***	1
				Zika and chikungunya RT-qPCR			***2***	
				Dengue virus 1-4 neutralizing antibodies	750 µL	***3***	***1***	2
				Dengue IgM and NS1 ELISA	500 µL	2	***1***	3
				Zika neutralising antibodies	1 mL	4	***1***	4
				Chikungunya neutralizing antibodies and Serology for Zika (IgG/IgM) and chikungunya (IgG/IgM)	remaining volume	5	***1***	5
	Optional for severe cases			PCR	200 µL	-		
First visit (total amount of blood per local practice	***Sample collected before day 14 of onset of fever/rash***	unknown		CBC, HCT	No aliquot	-		
Suspected dengue follow-up visit (total amount of blood 5 mL)	Approximately 21 days after first visit	unknown	-	Dengue IgM and Dengue capture IgG ELISA	500 µL	1	**1**	1
				Dengue virus 1-4 neutralizing antibodies	1 mL	2	**1**	2
				Serology for Zika (IgM/IgG) and chikungunya (IgG/IgM)			**2**	

SUSPECTED DENGUE, CHIKUNGUNYA AND/OR ZIKA VISITS – EARLY PRESENTERS								
Blood sampling time point		**No. Subjects**	**Subset**	**Component**	**Serum aliquot volume**	***Aliquot* priority rank**	***Test priority rank***	**Aliquot number**
Type of contact/ Amount of whole blood	**Sampling time point**							
				Zika neutralizing antibodies			**3**	
				Chikungunya neutralizing antibodies	remaining volume	3	**1**	3

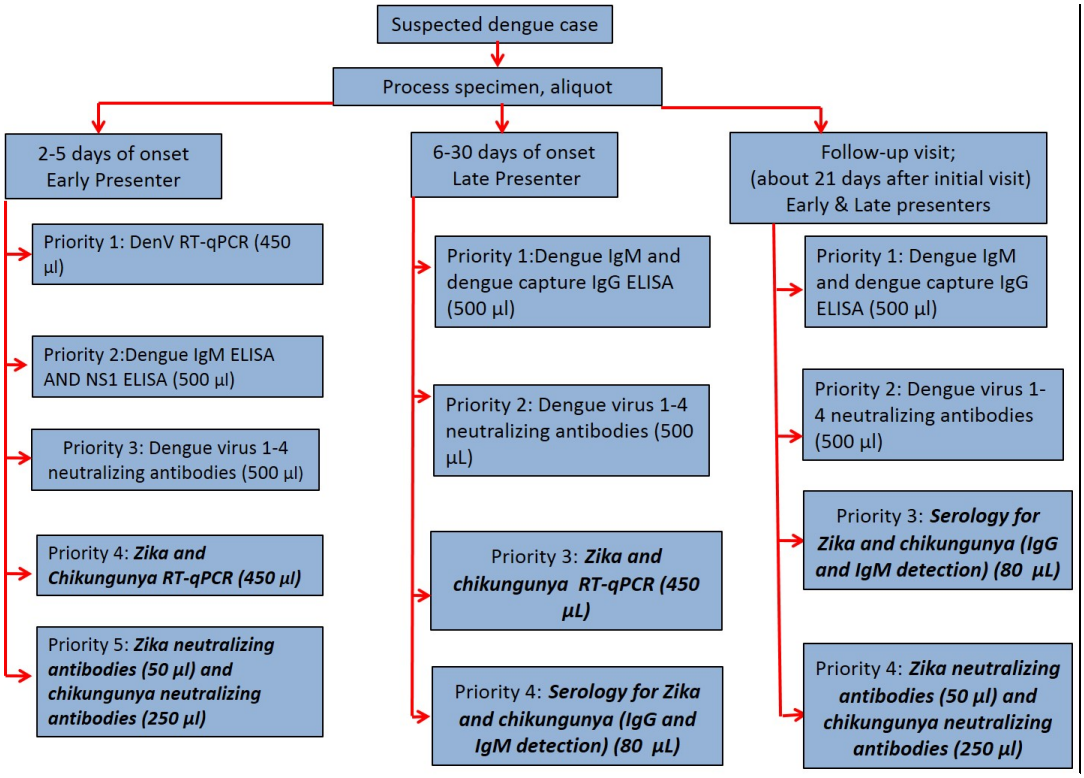
- not applicable

Table 13 Laboratory read-outs at each time point, and priority ranking for suspected dengue, chikungunya and/or Zika visits for LATE presenters

SUSPECTED DENGUE, CHIKUNGUNYA AND/OR ZIKA VISITS – LATE PRESENTERS								
Blood sampling time point		No. Subjects	Subset	Component	Serum aliquot volume	*Aliquot* priority rank	*Test priority rank*	Aliquot number
Type of contact/ Amount of whole blood	Sampling time point							
First visit (total amount of whole blood 5 mL)	Sample collected > 5 days or more after fever and/or rash	unknown	-	Dengue IgM and Dengue capture IgG ELISA	500 µL	1	***1***	1
				Dengue virus 1-4 neutralizing antibodies	1 mL	2	***1***	2
				Zika and chikungunya RT-qPCR			***2***	
				Serology for Zika (IgG/IgM) and chikungunya (IgG/IgM)	remaining volume	3	***1***	3
First visit (total amount of blood per local practice	***Sample collected before day 14 of onset of fever/rash***	unknown		CBC, HCT	No aliquot	-		
Suspected dengue, ***chikungunya and Zika*** follow-up visit (total amount of blood 5 mL)	Approximately 21 days after first visit	unknown	-	Dengue IgM and Dengue capture IgG ELISA	500 µL	1	***1***	1
				Dengue virus 1-4 neutralizing antibodies	1 mL	2	***1***	2
				Serology for Zika (IgG/IgM) and chikungunya (IgG/IgM)			***2***	
				Zika neutralizing antibodies			***3***	
				Chikungunya neutralizing antibodies	remaining volume	3	***1***	3

- not applicable

~~Figure 4: Flow chart of serum aliquot testing for suspected dengue cases~~



Section 6 Serious adverse events

In this prospective cohort study no test product/vaccine will be given. However blood samples will be collected at scheduled visits and ***unscheduled visits*** for suspected dengue, ***chikungunya and/or Zika***.

In order to fulfil international reporting obligations, serious adverse events (SAEs) that are related to study procedures (blood collection) will be collected and recorded from the time the subject consents to participate in the study until the study conclusion for each subject.

The investigators or site staff 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 or 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.

Section 7.2.3 Contact information for reporting serious adverse events and other events to GSK Biologicals

Study Contact for Reportable SAEs
During office hours: Local Name: Dr. PPD GlaxoSmithKline Biologicals PPD, PPD, PPD, Brazil CEP 22783-110 Email: PPD Phone: PPD Cell: PPD Name: PPD ***Clinical Safety Analyst*** ***Pharmacovigilance Department*** PPD ~~GlaxoSmithKline Biologicals~~ ***GlaxoSmithKline Brazil*** PPD, PPD, PPD, PPD, Brazil 22783-110 Email: PPD PPD Phone: PPD ~~Fax:~~ PPD
Back-up Study Contact for Reporting SAEs
24/24 hour and 7/7 day availability: GSK Biologicals Clinical Safety & Pharmacovigilance Fax: PPD or PPD

Section 8.3 Subject replacement

Enrolment of new subjects to replace:

- subjects not wishing to continue after amendment 2 (initial cohort), or
- subjects who prematurely terminate participation (initial and expansion cohorts) or
- subjects lost to follow-up will be done (initial and expansion cohorts).

The aim is to maintain a critical cohort size of ≥ 500 subjects/site. This replacement will be done at the beginning of each study year: during the scheduled visits starting at the end of study Year 1 up till the end of study Year 3.

The number of subjects to be recruited as replacements will be defined based on the number of subjects still active in the study. If at the end of each study year we have ≥500 subjects/site, no replacements will be needed.

In last three months of the of penultimate year of the study, each site will review the number of active subjects and drop-out rate.. If the risk of having fewer than the required number of active participants at the end of the study is high, recruitment for participants into the replacement cohort may be performed with sponsor approval. If the risk is high, recruitment into the replacement cohort should begin even if the number of active subjects is 500 or above 500.

The time window for the recruitment of replacements will be during the low dengue transmission***, preferably***.

Section 9.1.3 Tertiary endpoints

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

- ***Symptomatic laboratory-confirmed Zika virus infection***
- ***Symptomatic Zika virus infection (including laboratory-confirmed and probable cases).***
- ***Symptomatic laboratory-confirmed chikungunya infection***
- ***Symptomatic chikungunya infection (including laboratory-confirmed and probable cases).***
- Risk factors for dengue infection and disease.
- Neutralizing antibodies titers against DENV 1-4.
- Neutralizing antibody titers against YF virus.
- Spatial and temporal distribution of dengue cases (confirmed and probable symptomatic cases and inapparent infections).

- ***Spatial and temporal distribution of laboratory confirmed and probable Zika virus cases***
- ***Spatial and temporal distribution of patients with laboratory confirmed and probable chikungunya virus infection***
- ***Occurrence of laboratory confirmed or probable Zika and/or chikungunya infection in suspected dengue cases (differential diagnosis of dengue), retrospectively***
- ***Antibody titre against Zika and chikungunya virus at the scheduled visits***

Section 8.5.4 Analysis of tertiary objectives

Analysis of tertiary objectives is optional and may or may not be performed. If these analyses are performed, they will be conducted by the Research and Development Department. Statistical methods for tertiary objectives will be described in the SAP.

The neutralization assay, if performed, will only be done on blood samples from a subset of subjects. YF virus antibody testing will be performed retrospectively, for subjects in YF endemic region and who was tested positive for dengue.

Analysis of risk factors for symptomatic dengue

Poisson regression models will be used for exploring the risk factors (e.g. region, age and gender, etc.) for symptomatic dengue. The analyses will account for clustering of observations within households for the analyses by centre and also the stratification by centres for the overall analyses.

Incidence rates of symptomatic Zika infections

The following analyses will be performed by study site, gender and age-group, previous dengue exposure and previous Zika infection:

- ***Incidence rate of laboratory-confirmed or probable symptomatic Zika infection with 95% CI for each season separately: the numerator will be the number of subjects with lab-confirmed or probable symptomatic Zika infection during the season (between the visits scheduled before and after the season). The denominator will be the total person-years at risk, i.e. from the visit scheduled before the season until the first lab-confirmed or probable symptomatic Zika infection during the season, the next scheduled visit or the subject's withdrawal, whichever comes first.***
- ***Incidence rate of laboratory-confirmed or probable symptomatic Zika infection with 95% CI all seasons combined: the numerator will be the number of subjects with lab-confirmed or probable symptomatic Zika infection during the study Zika follow-up period. The denominator will be the total person-years at risk, i.e. from start of the study Zika follow-up period until the first lab-confirmed or probable symptomatic Zika infection, the end of the study or the subject's withdrawal, whichever comes first.***

Incidence rates of symptomatic chikungunya infections

The following analyses will be performed by study site, gender and age-group, previous dengue exposure and previous chikungunya infection:

- ***Incidence rate of laboratory-confirmed or probable symptomatic chikungunya infection with 95% CI for each season separately: the numerator will be the number of subjects with lab-confirmed or probable symptomatic chikungunya infection during the season (between the visits scheduled before and after the season). The denominator will be the total person-years at risk, i.e. from the visit scheduled before the season until the first lab-confirmed or probable symptomatic chikungunya infection during the season, the next scheduled visit or the subject's withdrawal, whichever comes first.***
- ***Incidence rate of laboratory-confirmed or probable symptomatic chikungunya infection with 95% CI all seasons combined: the numerator will be the number of subjects with lab-confirmed or probable symptomatic chikungunya infection during the study chikungunya follow-up period. The denominator will be the total person-years at risk, i.e. from start of the study chikungunya follow-up period until the first lab-confirmed or probable symptomatic chikungunya infection, the end of the study or the subject's withdrawal, whichever comes first.***

Analysis for Zika and chikungunya viruses in suspected dengue cases

The proportions and associated exact 2-sided 95% confidence intervals (CI)of the Zika and chikungunya viruses in suspected dengue cases will be summarized.

Analysis for seroprevalence of antibody titers against Zika and chikungunya viruses at selected timepoints

Seroprevalence at each selected timepoint will be calculated as a proportion i.e. the number of seropositive samples at each selected timepoint over the total number of samples with antibody results. An exact 95% CI will be computed.

Section 9.3 Archiving of data at study sites

Following closure of the study, the investigator must maintain all site study records in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g. audit or inspection), and, whenever feasible, to allow any subsequent review of data 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 for studies with an eCRF); however, caution needs to be exercised before such action is taken. The investigator must assure 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 there is an acceptable back-up of these reproductions and that an acceptable quality control process exists 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 that site for the study, 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~~ ***25*** years.

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

Section 11 References

Boletim Epidemiológico - Volume 48 - n 05 - 2017 - Secretaria de Vigilância em Saúde − Ministério da Saúde. Accessed at : http://combateaedes.saude.gov.br/images/pdf/2017-Dengue_Zika_Chikungunya-SE4.pdf***. Accessed on 22 August 2016.***

Siqueira, JB, Martelli CM, Maciel IJ, Oliveira RM, Ribeiro MG, Amorim FP, Moreira BC, Cardoso DD, Souza WV, Andrade AL. Household survey of dengue infection in Central Brazil: spatial point pattern analysis and risk factors assessment. *Am J Trop Med Hyg.* 2004; 71(4): 646-51.

Appendix A Study laboratories

GSK Biologicals' laboratories (for back up samples)

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, North America, Laval ***NEOMED Labs***~~	~~Biospecimen Reception - Clinical Serology 525 Cartier blvd West - Laval - Quebec - Canada - H7V 3S8~~
GSK Biologicals Global Vaccine Clinical Laboratory, Wavre-Nord Noir Epine	Avenue Fleming, 20 - B-1300 Wavre - Belgium

Appendix B Structured script

Are you aware of dengue, ***chikungunya or Zika*** cases in your neighborhood during the past month?

Any subject that meets the case definition for suspected dengue***, chikungunya or Zika*** the subject (and subject's parent(s)/LAR(s) as applicable) will be referred to the study hospital according to protocol