GlaxoSmithKline	**Statistical Analysis Plan**
Detailed Title:	A prospective, multicenter, multiyear, cohort study to determine dengue incidence in children and adults in communities from endemic regions in Brazil
eTrack study number and Abbreviated Title	116606 (EPI-DENGUE-006 BOD BR)
Scope:	All available data pertaining to the above study related to the final analysis
Date of Statistical Analysis Plan	Amendment 1 Final: 29 October 2019

APP 9000058193 Statistical Analysis Plan Template V4 (Effective date: 03 June 2019)

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LIST OF ABBREVIATIONS

ATP	According-To-Protocol
CI	Confidence Interval
CTRS	Clinical Trial Registry Summary
DE	Design Effect
DENV	Dengue Virus
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
FHP	Family Health Physician Program
GEE	Generalized Estimating Equations
GSK	GlaxoSmithKline
HCT	Hematocrit
ICC	Intra-cluster Correlation
IgG	Immunoglobulin type G
IgM	Immunoglobulin type M
LAR	Legally Acceptable Representative
LIRA	Larval Index Rapid Assay
LL	Lower Limit of the Confidence Interval
NS1	Non Structural 1
RT-qPCR	Reverse Transcriptase quantitative Polymerase Chain Reaction
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDC	Suspected Dengue Case
TFL	Tables Figures and Listing template annexed to SAP
UL	Upper Limit of the Confidence Interval
WHO	World Health Organization

1. DOCUMENT HISTORY

Date	Description	Protocol Version
29-OCT-2019	Amendment 1*	Amendment 4: 16 Oct 2017
14-JUN-2019	Final version	Amendment 3: 09 JAN 2015

* In 2017 GSK vaccines has decided to terminate the development of a dengue vaccine. Accordingly none of the tertiary objectives will be evaluated and the analyses additional to those planned in the protocol have been removed from the first statistical analysis plan. In addition in light of the low number of dengue episodes, the large number of positive anti-dengue IgG at visit 1 and the number of dengue cases reported outside the season,

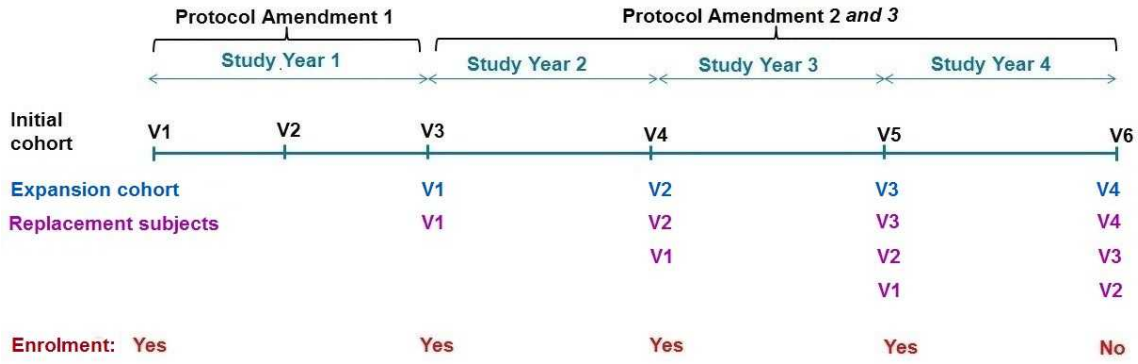
(1) The analyses of PCR confirmed cases (only 10 cases) and the analyses of secondary dengue infections were removed.
(2) The analyses by seroprevalence at each visit were removed,
(3) The analyses by season were removed. Only the analyses by calendar year are to be conducted
(4) Subjects without seroprevalence status at visit 1 were removed from the ATP cohort because serological status of symptomatic/inapparent dengue infections cannot be assessed properly for such subjects
(5) Planned analyses of medical and vaccination history were removed.
(6) The statistical method was updated to ignore the household cluster effect and to provide incidence rate with confidence interval in absence of dengue episodes in a calendar year for a study site .

2. STUDY DESIGN

2.1. Study design overview

- **Type of design:** Prospective, multicenter, community-based, household-sampling, cohort study in Brazil.
- **Duration of the study:** The study period per initial site, initially planned to be one year, was planned to be extended by additional three years (overall four years) to cover three additional dengue seasons. The study period for any new site was planned to be 3 years.
- **Enrolment wave:** There were 2 waves of enrolment for recruiting the planned 3600 subjects:
  - Initial Cohort = about 1800 subjects planned – four years follow-up planned – INITIAL sites: Rio (PPD), Manaus (PPD) and Salvador (PPD).
  - Expansion Cohort = about 1800 subjects planned – three years follow-up planned – planned NEW sites: Natal (PPD), Campo Grande (PPD), and Campinas (PPD).
- **Replacement:** replacement subjects were enrolled in order to compensate for subjects who did not wish to extend their participation for three additional years (for subjects from the initial cohorts), subjects who prematurely terminated participation or subjects who were lost to follow-up (for subjects from the initial and expansion cohorts).
- This was 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.

- **Schematic representation of the scheduled visits:**



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

- **Type of study:** self-contained.
- **Data collection**: Electronic Case Report Form (eCRF).
- **Study termination**: in December 2017, GSK decided to deprioritise the development of the dengue purified inactivated vaccine (DPIV) candidate and to terminate the study EPI-DENGUE-006 at the study visit planned after the 2018 dengue season. This decision was made due to the scientific challenges and development risks associated with the candidate vaccine. The planned study site in Campo Grande was not initiated before the decision of study termination was taken, so no subjects were enrolled in that site.

2.2. Study population

This study planned to enrol subjects (six months of age and older at the time of enrolment) from randomly selected households from different communities within the selected sites.

2.2.1. Selection of study sites

Five sites covering different regions of the country were 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. Other selection criteria of the sites include an extensive feasibility assessment that includes aspects related to the site structure including laboratory and local facilities, availability of the principal investigator and previous experience on Dengue.

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 to identify where there is a risk of mosquito reproduction, and has been widely implemented in Brazil [Pontes, 2000].

The study sites in Rio, Salvador, Natal and Campinas were selected mainly considering their experience in previous Dengue field research. Manaus was selected mainly for the experience of its principal investigator in dengue studies and the previous experience of the site in clinical trials with GSK.

At least one community of households within each site was selected.

2.2.2. Selection of communities

- Community selection was based on the following characteristics: 1) 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; 2) safe access by study personnel; 3) high population density; and 4) low migration rate.

2.2.3. Selection of households

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

The FHP or LIRA provided an exhaustive list of households to the study sites and served as the liaison between the study personnel and the household, and had not any other role in this study.

Sites with access to LIRA (i.e. Manaus, Natal and Campinas) 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 was not implemented but has FHP coverage, a random sample of households was to be selected from the FHP database. Note that none of the five study sites part of the final analysis had FHP coverage.

Where another type of household registry offers the only access to the community (i.e. Rio and Salvador), a random sample was to be drawn from the registry database.

Actually, for Rio, Salvador, Natal and Campinas, contact in the communities was performed to identify potential subjects who were interested to be enrolled. In addition, households were selected based on their location (in more safety areas and near to the Unit of Health Care).

2.2.4. Selection of subjects

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

Preferably the recruitment period was planned to occur outside of the peak dengue transmission season, and continued until each site has reached its foreseen target. Recruitment of replacement subjects were done during the low dengue transmission.

If the target household was found empty or all members refused to participate, the first household to the left was approached. Those households refusing to participate were recorded as such. A household refusal was characterized when all individuals in the household refused to participate in the study. Individual refusals in a given household were not preclude inclusion of other individuals living in the households, and were recorded as such. Information on refusal was kept at the study site and was not recorded in the screening/enrolment log.

In the subsequent study years/seasons, the cohort size was maintained at a minimum of 500 subjects per site. Since subjects who prematurely terminate participation and/or lost to follow-up were replaced, the final number of participants across the four planned study years may exceed 3600 subjects. Yearly, there was an evaluation of active subjects. If the number of active subjects/site became < 500, there were enrolment of replacement subjects to maintain a cohort size of at least 500 subjects per site. Recruitment of replacement subjects could have occurred at the end of the planned study year 1, 2 or 3. The recruitment approach was the same as for the initial enrolment. Replacement subjects were enrolled from households located in the same communities as the withdrawn subjects.

In order to better represent the Brazilian population distribution, subjects enrolled in the expansion cohorts 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 cohorts were 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. This strategy was implemented at enrolment in Salvador, Natal and Campinas and for replacement subject in Rio.

2.3. Dengue case detection

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

For the five study sites, the designated study hospitals/clinics are not the investigator sites. The designated study hospitals/clinics are working with the investigator sites and are located in the community where the subjects live.

Suspected cases in the study may arise from three sources:

1. Referred by study personnel during scheduled home visits
2. Through enhanced passive surveillance
3. As a result of active surveillance between scheduled visits

2.3.1. Case detection during scheduled home visits

If a subject was identified as suspected case during any home visit, he or she was referred to the designated study hospital/clinic for medical evaluation.

Note that the home visits are denoted as scheduled study visits in the list of study procedures in the protocol.

2.3.2. Case detection through enhanced passive surveillance

The subject/subject's parent(s)/legally acceptable representative (LAR[s]) was instructed to contact the study staff (the local study coordinator) at any time dengue was suspected (i.e., body temperature ≥38°C measured (by any route) for at least two consecutive days).

The subject/subject's parent(s)/LAR(s) was 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 local study coordinator then arranged for an appointment at the designated study hospital/clinic.

Although subjects were instructed to contact the study staff in the event of suspected dengue, there may have been cases where the subject was taken directly to the hospital or clinic. If this occurred when the study physician was not available, the staff at the designated hospital should have notified the local study coordinator and the study physician.

2.3.3. Case detection through active surveillance

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

If dengue was suspected during active surveillance, an appointment was arranged at the designated study hospital/clinic, and at least one additional visit was required for case follow-up.

2.4. Dengue seasons

Dengue season:

Region/study site name (site number)	Dengue season
Rio (PPD)	1st January to 30th June
Manaus (PPD)	1st January to 30th June
Salvador (PPD)	1st January to 30th June
Natal (PPD)	1st January to 30th June
Campinas (PPD)	1st January to 30th June

3. OBJECTIVES

3.1. Primary objective

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

3.2. 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.

3.3. Tertiary objectives (optional)

- 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 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.

4. ENDPOINTS

4.1. Primary endpoint

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

4.2. 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.
- Primary inapparent dengue infection.
- Severity of symptoms of symptomatic dengue (using the 2009 WHO guidelines).

4.3. Tertiary endpoints

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

- 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).

5. ANALYSIS SETS

5.1. Definition

The following analysis sets are defined:

Analysis Set	Description
Total cohort	All subjects enrolled in the study
Total cohort without subjects with code 900	All subjects from the Total cohort without elimination code 900
ATP cohort	All subjects from the Total cohort without subjects with code 900, who met all inclusion/exclusion criteria (code 2010) and had seroprevalence status at visit 1 (code 2020)

5.2. Criteria for eliminating data from Analysis Sets

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

5.2.1. Elimination from Total cohort without subjects with code 900

Code 900 (invalid informed consent or fraudulent data) will be used for identifying subjects eliminated from Total cohort without subjects with code 900.

5.2.2. Elimination from ATP cohort

Code 900 (invalid informed consent or fraudulent data) and code 2010 (Protocol violation linked to the inclusion/exclusion criteria) will be used for identifying subjects eliminated from ATP cohort.

5.3. Important protocol deviation not leading to elimination from Analysis sets

Refer to the Periodic Review of Protocol deviation documents stored in PPD

6. DENGUE CASE CLASSIFICATION

The following classification of suspected dengue case (SDC) will be used in this study.

6.1. Suspected symptomatic dengue case

As per protocol a suspected symptomatic dengue case was defined by 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.

Note that few cases were reported as suspected symptomatic dengue cases without documentation of fevers. These cases will be ignored from all analyses on symptomatic dengue infections.

Although subjects are asked to come to a study hospital if fever (body temperature ≥ 38°C by any route) 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.

A suspected dengue case presenting at the health care facility within 5 days following the onset of fever (i.e. day of fever onset and the next 4 days) is defined as an 'early presenter'.

A suspected dengue case presenting at the health care facility 6 days or more after the onset of fever 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).

The start date of a suspected symptomatic dengue case is defined by the date of fever or the date of first symptoms which ever appear last. In case both the date of fever and the date of first symptoms are missing, the start date will be the date of visit with symptoms.

6.2. 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.

Acute and convalescent serum samples are samples taken at the first and follow-up visits for suspected dengue case, respectively.

6.3. Virologically confirmed symptomatic dengue infection

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

6.4. Probable dengue case

- 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).

6.5. 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.

6.6. Indeterminate dengue case

An indeterminate dengue case is a participant evaluated as an SDC (Section 6.1) and not classified as laboratory confirmed case (Section 6.2), probable case (Section 6.4) or negative case (Section 6.5).

6.7. Primary symptomatic dengue case

A primary symptomatic dengue case is a subject with laboratory confirmed or probable symptomatic dengue infection and 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).

6.8. Secondary symptomatic dengue case

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

6.9. Primary inapparent dengue infection

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).

6.10. Previous dengue infection

A subject will be considered as having previous dengue infection at visit 1(baseline) based on seroprevalence at visit 1, namely if:

- Dengue IgG positive (ie reactive) 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 (ie reactive) at previous schedule visit(s) or
- Laboratory-confirmed symptomatic dengue case detected previously at study surveillance

This status will be considered as unknown for subjects without laboratory result at baseline Visit 1.

7. STATISTICAL ANALYSES

Note that data derivation rules are described in Section 11 and will not be repeated below.

Continuous variables will be summarized with number of non-missing observations, mean, standard deviation (SD), median, minimum, maximum, 25th and 75th percentiles. Categorical variables will be summarized with frequency tables; numbers and percentages for each level will be given.

If an elimination code 900 is attributed, then all the analyses planned to be done on the Total cohort will be done on the Total cohort without subjects with code 900, except Template 5 from EPI-DENGUE-006 BOD BR (116606) TFL (Final analysis) (14-JUN-2019) DRAFT.

The final analysis will be performed on the following study sites using data up to the specified study visits and dengue seasons covered up to study termination:

Region/Study sites name	Initial / Expansion / Replacement cohort	Subject numbers	Study visits covered up to study termination	Dengue season covered up to study termination
Rio	Initial	P – PP	Visit 1 to Visit 6	2015, 2016, 2017, 2018
	Replacement*	PP - PP	Visit 1 to Visit 4	2016, 2017, 2018
Manaus	Initial	PPD - PPD	Visit 1 to Visit 6	2015, 2016, 2017, 2018
	Replacement*	PPD - PPD	Visit 1 to Visit 2	2018
Salvador	Initial	PPD - PPD	Visit 1 to Visit 5	2016, 2017, 2018
Natal	Expansion	PPD - PPD	Visit 1 to Visit 3	2017, 2018
Campinas	Expansion	PPD - PPD	Visit 1 to Visit 2	2018

*only 1 replacement cohort

The following results will be available at the scheduled visits for the following tests:

- Dengue IgG ELISA will be available at Visit 1
- For Visit 2 onwards, Dengue IgG ELISA will be available for subjects with IgG negative at the previous visit.

The following results will be available in case of SDC, for the following tests:

		Early presenters		Late presenters	
Assays	**Result type**	**Acute sample (first visit)**	**Convalescent sample (follow-up visit)**	**Acute sample (first visit)**	**Convalescent sample (follow-up visit)**
RT-qPCR	Positive, negative or not typeable DENV type Copies/ml Final classification into positive or negative (used for the dengue case classification)	Yes	TNP	TNP	TNP
NS1 ELISA	Reactive or no reactive	Yes	TNP	Yes	TNP
Dengue IgM ELISA	Reactive or no reactive	Yes	Yes	Yes	Yes
Dengue capture IgG ELISA	Reactive or no reactive	TNP	Yes	Yes	Yes
Hematology / Biochemistry	Numerical results	Yes	TNP	Yes	TNP

TNP = test not performed on the sample as per protocol

Laboratory results 'Not reactive' and 'Reactive' for NS1, Dengue IgM and Dengue capture IgG ELISA tests will be considered as 'Negative' and 'Positive' respectively for the classification of the SDC (refer to Section 6).

If a blood sample is collected for more than 1 SDC, the following rule will be applied:

- The sample collected from start of the SDC (first symptom onset) and the day before onset of the next SDC will be used to associate the blood sample to a SDC.

7.1. Analysis of demographics/baseline characteristics

7.1.1. Analysis as planned per protocol

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.

7.1.2. Additional considerations

The number of subjects enrolled into the study will be tabulated by study site and overall on the Total cohort.

The number of subjects enrolled per household will be tabulated by study site and overall on the Total cohort.

The numbers of withdrawn subjects will be tabulated according to the reason for withdrawal by study site and overall on the Total cohort.

The distribution of the subjects in the ATP cohort and reasons for exclusion will be tabulated by study site and overall.

Demographic characteristics (age at Visit 1, age category at visit 1: < 1 year, 1-4 year, 5-8 year, 9-17 year, 18-49 year, >= 50 years, seroprevalence at visit 1 and gender) will be summarized using descriptive statistics by study site and overall on the ATP cohort.

Socio-economic characteristics of the households at Visit 1 (enrolment visit) will be tabulated by study site and overall on the ATP cohort.

The planned analysis of medical and vaccination history was removed.

7.2. Analysis of primary and secondary endpoints

7.2.1. Analysis as planned per protocol

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).

Endpoint numerators and denominators are described in Table 1 below:

Table 1 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.

7.2.2. Additional considerations

7.2.2.1. Incidence rate

In light of the low number of dengue episodes, the large number of positive anti-dengue IgG at visit 1 and the number of dengue cases reported outside the season,

1. The analyses of PCR confirmed cases (only 10 cases) and the analyses of secondary dengue infections were removed.
2. The analyses by seroprevalence at each visit were removed,
3. The analyses by season were removed. Only the analyses by calendar year are to be conducted

4. Subjects without seroprevalence status at visit 1 were removed from the ATP cohort because serological status of symptomatic/inapparent dengue infections cannot be assessed properly for such subjects
5. Planned analyses of medical and vaccination history were removed.
6. The statistical method was updated to ignore the household cluster effect and to provide incidence rate with confidence interval in absence of dengue episodes in a calendar year for a study site .

Regarding the last point, the expected annual incidence rate for a cohort of subjects followed over a full calendar will be computed using a log-linear model with the number of dengue events (all cases) as depend variable and the total number of subject-month as offset. The model will include the study site, year and month as independent categorical fixed effects (see below).

The expected annual incidence rate in a study site for each year will be estimated as the average of incidence rate over months

```
proc genmod ;
    class center month year;
    model evt= center year month /
              dist=poisson link=log offset=ln TYPE3;

      ods output Estimates=estimc;
      estimate 'center PPD  2014' intercept 12 center 12 0 0 0 0
                                  year 12 0 0 0 0
                                  Month 1 1 1 1 1 1 1 1 1 1 1 1;
      estimate 'center PPD  2014' intercept 12 center 0 12 0 0 0
                                  year 12 0 0 0 0
                                  Month 1 1 1 1 1 1 1 1 1 1 1 1;
      estimate 'center PPD  2014' intercept 12 center 0 0 12 0 0
                                  year 12 0 0 0 0
                                  Month 1 1 1 1 1 1 1 1 1 1 1 1;
      estimate 'center PPD  2014' intercept 12 center 0 0 0 12 0
                                  year 12 0 0 0 0
                                  Month 1 1 1 1 1 1 1 1 1 1 1 1;
      estimate 'center PPD  2014' intercept 12 center 0 0 0 0 12
                                  year 12 0 0 0 0
                                  Month 1 1 1 1 1 1 1 1 1 1 1 1;
      estimate 'overall 2014' intercept 12 center 2.4 2.4 2.4 2.4 2.4
                                  y 12 0 0 0 0
                                  Month 1 1 1 1 1 1 1 1 1 1 1 1;
      estimate 'center PPD  2015' intercept 12 center 12 0 0 0 0
                                  year 0 12  0 0 0
                                  Month 1 1 1 1 1 1 1 1 1 1 1 1;
      estimate 'center PPD  2015' intercept 12 center 0 12 0 0 0
                                  year 0 12  0 0 0
                                  Month 1 1 1 1 1 1 1 1 1 1 1 1;
      estimate 'center PPD  2015' intercept 12 center 0 0 12 0 0
                                  year 0 12  0 0 0
                                  Month 1 1 1 1 1 1 1 1 1 1 1 1;
      estimate 'center PPD  2015' intercept 12 center 0 0 0 12 0
                                  year 0 12  0 0 0
                                  Month 1 1 1 1 1 1 1 1 1 1 1 1;
      estimate 'center PPD  2015' intercept 12 center 0 0 0 0 12
                                  year 0 12  0 0 0
```

```
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'overall 2015' intercept 12 center 2.4 2.4 2.4 2.4 2.4
                                year 0 12  0 0 0
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
estimate 'center PPD  2016' intercept 12 center 12 0 0 0 0
                                year 0 0 12 0 0
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'center PPD  2016' intercept 12 center 0 12 0 0 0
                                year 0 0 12 0 0
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'center PPD  2016' intercept 12 center 0 0 12 0 0
                                year 0 0 12 0 0
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'center PPD  2016' intercept 12 center 0 0 0 12 0
                                year 0 0 12 0 0
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'center PPD  2016' intercept 12 center 0 0 0 0 12
                                year 0 0 12 0 0
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'overall 2016' intercept 12 center 2.4 2.4 2.4 2.4 2.4
                                y 0 0 12 0 0
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'center PPD  2017' intercept 12 center 12 0 0 0 0
                                year 0 0 0 12 0
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'center PPD  2017' intercept 12 center 0 12 0 0 0
                                year 0 0 0 12 0
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'center PPD  2017' intercept 12 center 0 0 12 0 0
                                year 0 0 0 12 0
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'center PPD  2017' intercept 12 center 0 0 0 12 0
                                year 0 0 0 12 0
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'center PPD  2017' intercept 12 center 0 0 0 0 12
                                year 0 0 0 12 0
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'overall 2017' intercept 12 center 2.4 2.4 2.4 2.4 2.4
                                year 0 0 0 12 0
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
estimate 'center PPD  2018' intercept 12 center 12 0 0 0 0
                                year 0 0 0 0 12
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'center PPD  2018' intercept 12 center 0 12 0 0 0
                                year 0 0 0 0 12
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'center PPD  2018' intercept 12 center 0 0 12 0 0
                                year 0 0 0 0 12
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'center PPD  2018' intercept 12 center 0 0 0 12 0
                                y 0 0 0 0 12
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'center PPD  2018' intercept 12 center 0 0 0 0 12
                                year 0 0 0 0 12
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
  estimate 'overall 2018' intercept 12 center 2.4 2.4 2.4 2.4 2.4
                                year 0 0 0 0 12
                                Month 1 1 1 1 1 1 1 1 1 1 1 1;
```

```
      estimate 'overall' intercept 60 center 12 12 12 12 12
                                   year 12 12 12 12 12
                                   Month 5 5 5 5 5 5 5 5 5 5 5 5;
      estimate 'overall center PPD ' intercept 60 center 60 0 0 0 0
                                    ear 12 12 12 12 12
                                   Month 5 5 5 5 5 5 5 5 5 5 5 5;
      estimate 'overall center PPD ' intercept 60 center 0 60 0 0 0
                                   year 12 12 12 12 12
                                   Month 5 5 5 5 5 5 5 5 5 5 5 5;
      estimate 'overall center PPD ' intercept 60 center 0 0 60 0 0
                                    ear 12 12 12 12 12
                                   Month 5 5 5 5 5 5 5 5 5 5 5 5;
      estimate 'overall center PPD ' intercept 60 center 0 0 0 60 0
                                    ear 12 12 12 12 12
                                   Month 5 5 5 5 5 5 5 5 5 5 5 5;
      estimate 'overall center PPD ' intercept 60 center 0 0 0 0 60
                                   year 12 12 12 12 12
                                   Month 5 5 5 5 5 5 5 5 5 5 5 5;
    run;


data estimc; set estimc;
      if label='overall' or index(label,'overall center') then do;
      estimate=exp(lbetaestimate/60);
      lower=exp(lbetalowercl/60);
      upper=exp(lbetauppercl/60);
        end;
      else do;
      estimate=exp(lbetaestimate/12);
      lower=exp(lbetalowercl/12);
      upper=exp(lbetauppercl/12);
      end;
   run;
```

In case of subgroup analysis by gender or by age at visit 1 (<=17years, 18-49 years and >= 50years) or in case the model does not allow estimating confidence interval (eg problem of convergence or situation where there is no event in a site, in a calendar year or in a month) CI will be computed using exact CI based on a Poisson Distribution.

The following case provides the SAS code for the exact CI for incidence rate per subject-year when there are 14 events and 400 subject-years:

```
data check;
   fu=400;
   n=14;
   Rate=n/fu;
LL_rate= GAMINV(0.025, n)/fu;
UL_rate= GAMINV(0.975, (n+1))/fu;
LL_rate_=cINV(0.025, 2*n)/(2*fu);
UL_rate_=cINV(0.975, 2*n+2)/(2*fu);
```

7.2.2.2. Seroprevalence

Seroprevalence will be summarized at visit 1 with the demography characteristics.

7.3. Analysis of symptoms and spectrum of dengue disease

7.3.1. Analysis as planned per protocol

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

The analyses will be performed on the ATP cohort.

7.3.2. Additional considerations

The following parameters will be accounted in the summary:

- Laboratory diagnosis (PCR confirmed, other serologically confirmed, negative, inapparent, undetermined)
- Laboratory schedule (Early/Late)
- Primary dengue diagnosis (Yes/No/missing)
- Clinical diagnosis including severity and hospitalization

7.4. Analysis of tertiary objectives

Tertiary objectives will not be analysed.

7.5. Analysis of serious adverse events

Serious adverse events related to study procedures reported during the study period will be listed for all enrolled subjects.

8. ANALYSIS INTERPRETATION

All analyses are descriptive.

9. CONDUCT OF ANALYSES

9.1. Sequence of analyses

The planned interim analysis was not performed.

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

Study progress reports were generated during the study conduct to follow study progress using specified tables and using the study database as source data.

Description	Analysis ID	Disclosure Purpose (CTRS=public posting, SR=study report, internal)	Dry run review needed (Y/N)	Study Headline Summary (SHS) requiring expedited communication to upper management (Yes/No)	Reference for TFL
Final analysis	E1_01	SR, CTRS	No	Yes	Refer to EPI-DENGUE-006 BOD BR (116606) TFL (Final analysis) (14-JUN-2019).docx
Study progress reports	E1_02	Monitoring	No	No	Refer to EPI-DENGUE-006 BOD BR (116606) Additional Analysis Request E01_02 Study progress.docx

9.2. Statistical considerations for interim analyses

The planned interim analysis was not performed.

10. CHANGES FROM PLANNED ANALYSES

All changes are provided in the additional consideration sections 7.1.2, 7.2.2, 7.3.2, 7 .4 and also summarized under section 1.

11. DATA DERIVATION RULES AND STATISTICAL METHODS

11.1. Data presentation

The following decimal description will be used for the analyses:

Display Table	Parameters	Number of decimal digits
Dengue cases	Incidence rate (in 1000 person-years) with LL & UL, person-years	1
Dengue cases	% of count, including LL & UL of CI	1
Dengue cases	Minimum, maximum, range	Number of decimals in the raw data
Dengue cases	Mean, median	Number of decimals in the raw data +1
Dengue cases	SD	Number of decimals in the raw data +2
Hematology/biochemistry	%	1
Demographic/baseline characteristics and changes between scheduled visits	Percentage, mean, median, standard deviation, minimum, maximum, Q1, Q3	1

CI = confidence interval
LL = lower limit of the confidence interval
SD = standard deviation
UL = upper limit of the confidence interval
Q1/Q3 = 25th and 75th percentiles

11.2. Handling missing data

No data handling will be performed in case of missing data.

11.3. Data derivation

11.3.1. Date derivation

Statistical Analyis Software (SAS date derived from a character date: in case day is missing, 15 is used. In case day and month are missing, 30 June is used.

11.3.2. Age

Age: age at the reference activity is computed as the number of units between the date of birth and the reference activity, expressed in months/years.

When age at reference activity is to be displayed in months/years, it will be calculated as the number of complete calendar months/years between the date of birth and the date of reference activity. For example:

- DOB = 10JUN2017, Date of vaccination = 09JUL2018 -> Age = 12 months
- DOB = 10JUN2017, Date of vaccination = 10JUL2018 -> Age = 13 months
- DOB = 10SEP1983, Date of reference = 09SEP2018 -> Age = 34 years
- DOB = 10SEP1983, Date of reference = 10SEP2018 -> Age = 35 years

11.3.3. Follow-up/duration

Follow-up time in a month will be computed as the total number of follow-up days in the month. For instance, a subject with follow-up start on 30 September (last day of the month) will contribute by 1 day. The follow-up will be converted in month by dividing by 12 or will be converted in year by dividing by 365.25.

Duration of an event is expressed in days. It is the number of days between the start and the stop dates + 1. Therefore, duration is 1 day for an event starting and ending on the same day.

11.3.4. Moderate to severe dengue cases

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 (i.e. criteria box ticked in the eCRF):

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

12. REFERENCES

Pontes RJ, Freeman J, Oliveira-Lima JW, Hodgson JC, Spielman A. Vector densities that potentiate dengue outbreaks in a Brazilian city. *Am J Trop Med Hyg.* 2000; 62: 378–383.

GlaxoSmithKline	**Statistical Analysis Plan**
Detailed Title:	A prospective, multicenter, multiyear, cohort study to determine dengue incidence in children and adults in communities from endemic regions in Brazil
eTrack study number and Abbreviated Title	116606 (EPI-DENGUE-006 BOD BR)
Scope:	All available data pertaining to the above study related to the final analysis
Date of Statistical Analysis Plan	Final: 14 June 2019

APP 9000058193 Statistical Analysis Plan Template V4 (Effective date: 03 June 2019)

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LIST OF ABBREVIATIONS

ATP	According-To-Protocol
CI	Confidence interval
CTRS	Clinical Trial Registry Summary
DE	Design effect
DENV	Dengue virus
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
FHP	Family Health Physician Program
GEE	Generalized estimating equations
GSK	GlaxoSmithKline
HCT	Hematocrit
ICC	Intra-cluster correlation
IgG	Immunoglobulin type G
IgM	Immunoglobulin type M
LAR	Legally Acceptable Representative
LIRA	Larval Index Rapid Assay
LL	Lower limit of the confidence interval
NS1	Non Structural 1
RT-qPCR	Reverse Transcriptase quantitative Polymerase Chain Reaction
SAP	Statistical analysis plan
SD	Standard deviation
SDC	Suspected dengue case
TFL	Tables Figures and Listing template annexed to SAP
UL	Upper limit of the confidence interval
WHO	World Health Organization

1. DOCUMENT HISTORY

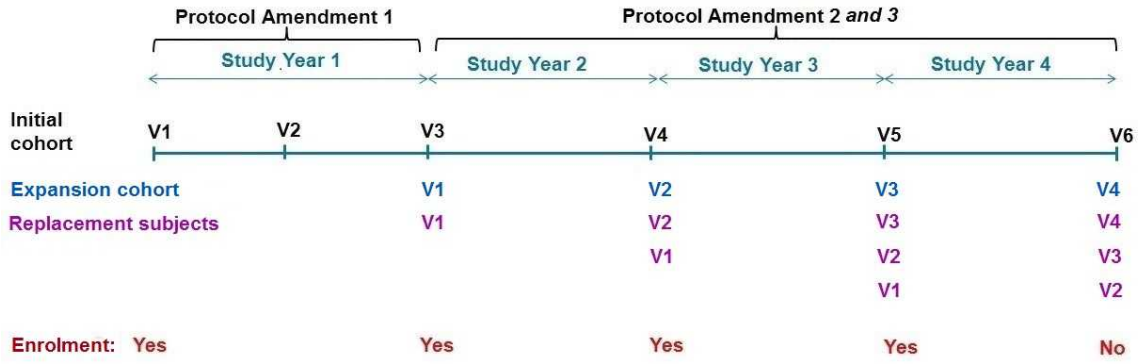
Date	Description	Protocol Version
14 JUN 2019	Final version	Amendment 3: 09 JAN 2015

2. STUDY DESIGN

2.1. Study design overview

- **Type of design:** Prospective, multicenter, community-based, household-sampling, cohort study in Brazil.
- **Duration of the study:** The study period per initial site, initially planned to be one year, was planned to be extended by additional three years (overall four years) to cover three additional dengue seasons. The study period for any new site was planned to be 3 years.
- **Enrolment wave:** There were 2 waves of enrolment for recruiting the planned 3600 subjects:
  - Initial Cohort = about 1800 subjects planned – four years follow-up planned – INITIAL sites: Rio (PPD), Manaus (PPD) and Salvador (PPD).
  - Expansion Cohort = about 1800 subjects planned – three years follow-up planned – planned NEW sites: Natal (PPD), Campo Grande (PPD), and Campinas (PPD).
- **Replacement:** replacement subjects were enrolled in order to compensate for subjects who did not wish to extend their participation for three additional years (for subjects from the initial cohorts), subjects who prematurely terminated participation or subjects who were lost to follow-up (for subjects from the initial and expansion cohorts).
- This was 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.

- **Schematic representation of the scheduled visits:**



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

- **Type of study:** self-contained.
- **Data collection**: Electronic Case Report Form (eCRF).
- **Study termination**: in December 2017, GSK decided to deprioritise the development of the dengue purified inactivated vaccine (DPIV) candidate and to terminate the study EPI-DENGUE-006 at the study visit planned after the 2018 dengue season. This decision was made due to the scientific challenges and development risks associated with the candidate vaccine. The planned study site in Campo Grande was not initiated before the decision of study termination was taken, so no subjects were enrolled in that site.

2.2. Study population

This study planned to enrol subjects (six months of age and older at the time of enrolment) from randomly selected households from different communities within the selected sites.

2.2.1. Selection of study sites

Five sites covering different regions of the country were 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. Other selection criteria of the sites include an extensive feasibility assessment that includes aspects related to the site structure including laboratory and local facilities, availability of the principal investigator and previous experience on Dengue.

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 to identify where there is a risk of mosquito reproduction, and has been widely implemented in Brazil [Pontes, 2000].

The study sites in Rio, Salvador, Natal and Campinas were selected mainly considering their experience in previous Dengue field research. Manaus was selected mainly for the experience of its principal investigator in dengue studies and the previous experience of the site in clinical trials with GSK.

At least one community of households within each site was selected.

2.2.2. Selection of communities

Community selection was based on the following characteristics: *1)* 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; *2)* safe access by study personnel; *3)* high population density; and *4)* low migration rate.

2.2.3. Selection of households

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

The FHP or LIRA provided an exhaustive list of households to the study sites and served as the liaison between the study personnel and the household, and had not any other role in this study.

Sites with access to LIRA (i.e. Manaus, Natal and Campinas) 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 was not implemented but has FHP coverage, a random sample of households was to be selected from the FHP database. Note that none of the five study sites part of the final analysis had FHP coverage.

Where another type of household registry offers the only access to the community (i.e. Rio and Salvador), a random sample was to be drawn from the registry database.

Actually, for Rio, Salvador, Natal and Campinas, contact in the communities was performed to identify potential subjects who were interested to be enrolled. In addition, households were selected based on their location (in more safety areas and near to the Unit of Health Care).

2.2.4. Selection of subjects

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

Preferably the recruitment period was planned to occur outside of the peak dengue transmission season, and continued until each site has reached its foreseen target. Recruitment of replacement subjects were done during the low dengue transmission.

If the target household was found empty or all members refused to participate, the first household to the left was approached. Those households refusing to participate were recorded as such. A household refusal was characterized when all individuals in the household refused to participate in the study. Individual refusals in a given household were not preclude inclusion of other individuals living in the households, and were recorded as such. Information on refusal was kept at the study site and was not recorded in the screening/enrolment log.

In the subsequent study years/seasons, the cohort size was maintained at a minimum of 500 subjects per site. Since subjects who prematurely terminate participation and/or lost to follow-up were replaced, the final number of participants across the four planned study years may exceed 3600 subjects. Yearly, there was an evaluation of active subjects. If the number of active subjects/site became < 500, there were enrolment of replacement subjects to maintain a cohort size of at least 500 subjects per site. Recruitment of replacement subjects could have occurred at the end of the planned study year 1, 2 or 3. The recruitment approach was the same as for the initial enrolment. Replacement subjects were enrolled from households located in the same communities as the withdrawn subjects.

In order to better represent the Brazilian population distribution, subjects enrolled in the expansion cohorts 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 cohorts were 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. This strategy was implemented at enrolment in Salvador, Natal and Campinas and for replacement subject in Rio.

2.3. Dengue case detection

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

For the five study sites, the designated study hospitals/clinics are not the investigator sites. The designated study hospitals/clinics are working with the investigator sites and are located in the community where the subjects live.

Suspected cases in the study may arise from three sources:

1. Referred by study personnel during scheduled home visits
2. Through enhanced passive surveillance
3. As a result of active surveillance between scheduled visits

2.3.1. Case detection during scheduled home visits

If a subject was identified as suspected case during any home visit, he or she was referred to the designated study hospital/clinic for medical evaluation.

Note that the home visits are denoted as scheduled study visits in the list of study procedures in the protocol.

2.3.2. Case detection through enhanced passive surveillance

The subject/subject's parent(s)/legally acceptable representative (LAR[s]) was instructed to contact the study staff (the local study coordinator) at any time dengue was suspected (i.e., body temperature ≥38°C measured (by any route) for at least two consecutive days).

The subject/subject's parent(s)/LAR(s) was 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 local study coordinator then arranged for an appointment at the designated study hospital/clinic.

Although subjects were instructed to contact the study staff in the event of suspected dengue, there may have been cases where the subject was taken directly to the hospital or clinic. If this occurred when the study physician was not available, the staff at the designated hospital should have notified the local study coordinator and the study physician.

2.3.3. Case detection through active surveillance

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

If dengue was suspected during active surveillance, an appointment was arranged at the designated study hospital/clinic, and at least one additional visit was required for case follow-up.

2.4. Dengue seasons

Dengue season:

Study site name	Dengue season
Rio	1st January to 30th June
Manaus	1st January to 30th June
Salvador	1st January to 30th June
Natal	1st January to 30th June
Campinas	1st January to 30th June

3. OBJECTIVES

3.1. Primary objective

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

3.2. 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.

3.3. Tertiary objectives (optional)

- 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 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.

4. ENDPOINTS

4.1. Primary endpoint

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

4.2. 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.
- Primary inapparent dengue infection.
- Severity of symptoms of symptomatic dengue (using the 2009 WHO guidelines).

4.3. Tertiary endpoints

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

- 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).

5. ANALYSIS SETS

5.1. Definition

The following analysis sets are defined:

Analysis Set	Description
Total cohort	All subjects enrolled in the study
Total cohort without subjects with code 900	All subjects from the Total cohort without elimination code 900
ATP cohort	All subjects from the Total cohort without subjects with code 900 and who met all inclusion/exclusion criteria

5.2. Criteria for eliminating data from Analysis Sets

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

5.2.1. Elimination from Total cohort without subjects with code 900

Code 900 (invalid informed consent or fraudulent data) will be used for identifying subjects eliminated from Total cohort without subjects with code 900.

5.2.2. Elimination from ATP cohort

Code 900 (invalid informed consent or fraudulent data) and code 2010 (Protocol violation linked to the inclusion/exclusion criteria) will be used for identifying subjects eliminated from ATP cohort.

5.3. Important protocol deviation not leading to elimination from Analysis sets

Refer to the Periodic Review of Protocol deviation documents stored in PPD

6. DENGUE CASE CLASSIFICATION

The following classification of suspected dengue case (SDC) will be used in this study.

6.1. 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 by any route) 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.

A suspected dengue case presenting at the health care facility within 5 days following the onset of fever (i.e. day of fever onset and the next 4 days) is defined as an 'early presenter'.

A suspected dengue case presenting at the health care facility 6 days or more after the onset of fever 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).

6.2. 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.
- *Acute and convalescent serum samples are samples taken at the first and follow-up visits for suspected dengue case, respectively.*

6.3. Virologically confirmed symptomatic dengue infection

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

6.4. Probable dengue case

- 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).

6.5. 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.

6.6. Indeterminate dengue case

An indeterminate dengue case is a participant evaluated as an SDC (Section 6.1) and not classified as laboratory confirmed case (Section 6.2), probable case (Section 6.4) or negative case (Section 6.5).

6.7. Primary symptomatic dengue case

A primary symptomatic dengue case is a subject with laboratory confirmed or probable symptomatic dengue infection and 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).

6.8. Secondary symptomatic dengue case

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

6.9. Primary inapparent dengue infection

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).

Note that subjects with a visit for suspicion of dengue case (even with a negative result to acute and convalescent blood sample) won't be considered for inapparent infection.

6.10. Previous dengue infection

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)
- *This status will be considered as unknown for subject without laboratory result at baseline visit and without laboratory-confirmed symptomatic dengue case Visit 1.*

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

7. STATISTICAL ANALYSES

Note that data derivation rule and statistical methods are described in Section 12 and will not be repeated below.

Continuous variables will be summarized with number of non-missing observations, mean, standard deviation (SD), median, minimum, maximum, 25th and 75th percentiles. Categorical variables will be summarized with frequency tables; numbers and percentages for each level will be given.

If an elimination code 900 is attributed, then all the analyses planned to be done on the Total cohort will be done on the Total cohort without subjects with code 900, except Template 5 from EPI-DENGUE-006 BOD BR (116606) TFL (Final analysis) (14-JUN-2019) DRAFT.

The final analysis will be performed on the following study sites using data up to the specified study visits and dengue seasons covered up to study termination:

Study sites name	Initial / Expansion / Replacement cohort	Subject numbers	Study visits covered up to study termination	Dengue season covered up to study termination
Rio	Initial	P - PP	Visit 1 to Visit 6	2015, 2016, 2017, 2018
	Replacement*	PP - PP	Visit 1 to Visit 4	2016, 2017, 2018
Manaus	Initial	PPD - PPD	Visit 1 to Visit 6	2015, 2016, 2017, 2018
	Replacement*	PPD - PPD	Visit 1 to Visit 2	2018
Salvador	Initial	PPD - PPD	Visit 1 to Visit 5	2016, 2017, 2018
Natal	Expansion	PPD - PPD	Visit 1 to Visit 3	2017, 2018
Campinas	Expansion	PPD - PPD	Visit 1 to Visit 2	2018

*only 1 replacement cohort

The following results (reactive or no reactive) will be available at the scheduled visits for the following tests:

- Dengue IgG ELISA will be available at Visit 1
- For Visit 2 onwards, Dengue IgG ELISA will be available for subjects with IgG negative at the previous visit.

The following results will be available in case of SDC, for the following tests:

		Early presenters		Late presenters	
Assays	**Result type**	**Acute sample (first visit)**	**Convalescent sample (follow-up visit)**	**Acute sample (first visit)**	**Convalescent sample (follow-up visit)**
RT-qPCR	Positive, negative or not typeable DENV type Copies/ml Final classification into positive or negative (used for the dengue case classification)	Yes	TNP	TNP	TNP
NS1 ELISA	Reactive or no reactive	Yes	TNP	Yes	TNP
Dengue IgM ELISA	Reactive or no reactive	Yes	Yes	Yes	Yes
Dengue capture IgG ELISA	Reactive or no reactive	TNP	Yes	Yes	Yes
Hematology / Biochemistry	Numerical results	Yes	TNP	Yes	TNP

TNP = test not performed on the sample as per protocol

Laboratory results 'Not reactive' and 'Reactive' for NS1, Dengue IgM and Dengue capture IgG ELISA tests will be considered as 'Negative' and 'Positive' respectively for the classification of the SDC (refer to Section 6).

If a blood sample is collected for more than 1 SDC, the following rule will be applied:

- The sample collected from start of the SDC (first symptom onset) and the day before onset of the next SDC will be used to associate the blood sample to a SDC.

7.1. Analysis of demographics/baseline characteristics and changes between scheduled visits

The number of subjects enrolled into the study will be tabulated by study site and overall on the Total cohort.

The number of subjects enrolled per household will be tabulated by study site and overall on the Total cohort.

The numbers of withdrawn subjects will be tabulated according to the reason for withdrawal by study site, age category and overall on the Total cohort.

The distribution of the subjects in each cohort and reasons for exclusion will be tabulated by study site and overall.

- Minimum and maximum visit dates will be tabulated by study site on the Total cohort.

Distribution of scheduled study visits by month will be displayed graphically by study site on the ATP cohort.

Time interval between scheduled study visits will be summarized by study site and overall on the ATP cohort.

Demographic characteristics (age at Visit 1 and gender) will be summarized using descriptive statistics by study site and overall on the Total cohort and on the ATP cohort.

Socio-economic characteristics of the households at Visit 1 (enrolment visit) will be tabulated by study site and overall on the ATP cohort.

General medical history, number of subjects with at least 2 consecutive days of fever within one month before Visit 1, dengue history and yellow fever vaccination history at Visit 1 will be summarized on the ATP cohort. Changes in general medical history and vaccination against yellow fever occurring during study conduct will be summarized at each scheduled study Visits at Month 6, Month 12, Month 24, Month 36 and Month 48.

7.2. Description of ad-hoc visits

The analyses will be performed on the ATP cohort.

The proportion of medical visits without suspicion of dengue reported without fever will be tabulated by study site and overall.

The duration of fever (0 day, 1 day, 2 to 13 days and 14 days or more) during the suspected dengue cases will be summarized by study site and overall.

SDC without fever and medical visits without suspicion of dengue reported without fever will be excluded from all analyses described below up to Section 7.4 included (i.e. Templates 17 to 51 from TFL). SDCs with a duration of fever of 1 day or 14 days or more will not be excluded from all analyses described below up to Section 7.4 included.

The distribution of ad-hoc visits with suspicion of dengue (stratified by laboratory outcome, i.e. laboratory-confirmed, probable, negative and indeterminate) and ad-hoc visits without suspicion of dengue will be tabulated by study site, age category and month. Distribution of ad-hoc visits by month and year will also be displayed graphically by study site.

A summary of characteristics (age category at visit, year quarter of visit, gender, diagnosis) of ad-hoc visits without suspicion of dengue will be tabulated by study site and overall. A summary of characteristics (age category at first visit, year quarter of first visit,

gender, laboratory outcome) of ad-hoc visits with suspicion of dengue will be tabulated by study site and overall.

The proportion of episodes of dengue suspicion having return visits, the number of visits (i.e. first, returned and follow-up visits), the time interval between the first visit and the first return visit and the time interval between the first visit and the follow-up visits will be tabulated by study site and overall. The number first and follow-up visits with a blood sample taken, the time interval between the visit and the blood sample, the time interval between fever onset and blood sample taken at first visit and the time interval between blood samples will be tabulated by study site and overall.

7.3. Characteristics of the suspected dengue cases

The analyses will be performed on the ATP cohort.

Distribution of suspected dengue cases type (early/late presenters) by month will be displayed graphically by study site.

The RT-qPCR (overall and by DENV type), NS1, IgM and IgG laboratory results for SDC will be summarized by presenter status (early versus late) and overall.

The distribution of SDC in the following categories will be computed for primary and secondary infections, and by calendar year:

- Cases confirmed by RT-qPCR only
- Cases confirmed by NS1 positivity only
- Cases confirmed by IgM seroconversion only
- Cases confirmed by both RT-qPCR and NS1 positivity, without IgM seroconversion
- Cases confirmed by both RT-qPCR and IgM seroconversion, without NS1 positivity
- Cases confirmed by both NS1 positivity and IgM seroconversion, without RT-qPCR positivity
- All laboratory confirmed cases
- All cases confirmed by RT-qPCR at least
- All probable cases
- All confirmed and probable cases
- Negative
- Indeterminate

The following characteristics will be summarized for each category of dengue cases (laboratory-confirmed, probable, negatives and indeterminate):

- Fever at onset: proportion of cases with fever being part of first symptoms
- Age at first symptoms onset, study site, IgG results at the preceding scheduled visit, surveillance source leading to Visit 1 (active/passive/scheduled visit), study hospital where the visit was performed, time interval between onset of fever and first visit for SDC, time interval between onset of fever (if fever is part of the first symptom) and first visit for SDC, time interval between onset of first symptom (if fever is not the first symptom) and first visit for SDC and time interval between first visit and follow-up visit for SDC
- Temperature at first visit: <37.5, ≥37.5, >38, >38.5, >39, >39.5, >40
- Clinical symptoms at onset, from first and returned visits, since onset of symptoms: frequency and proportion of cases with each symptom
- Vital signs at first visit (heart rate, systolic and diastolic blood pressure): number and proportion of subjects below, within and above the reference ranges of values (see 12.4.5 for reference ranges)
- Hematology/Biochemistry at first visit: number and proportion of subjects below, within and above the reference ranges of each test provided by the laboratory. Summary statistics of continuous variables for haemoglobin, haematocrit, platelets, white blood cells, neutrophils, monocytes, albumin, ASAT and ALAT.
- General medical history at scheduled visit before SDC
- Follow-up visits ('yes', 'phone contact', 'no')
- Final classification of the episode (duration of fever, most likely diagnosis according to investigator opinion (dengue, chikungunya, influenza, malaria rotavirus or other enteric infection, other infection disease or non-infectious disease), hospitalisation (yes/no, reason, duration), outcome, moderate to severe dengue episode (yes/no)).
- Severity criteria of SDC (each warning sign (yes/no), each criteria for severe dengue (yes/no))

7.4. Analysis of incidences

The analyses will be performed on the ATP cohort.

The following analyses will be performed overall and by DENV type:

- Incidence rate (expressed as number of cases/1000 person-years) of laboratory-confirmed symptomatic dengue infection with 95% CI (refer to Section 12.1 for the methodology for computing 95%CI) for each dengue season separately: the numerator will be the total number of laboratory-confirmed symptomatic dengue infections during the specific season (first of such event per subject during the specific season, using the date of first symptom onset). The denominator will be the total person-years at risk during the specific season, i.e. from the start of the specific season* until the first laboratory-confirmed symptomatic dengue infection during the

specific season, the end of the specific season or the subject's withdrawal, whichever comes first. Subjects withdrawn before the start of the specific season will not be included in the denominator.

- **From Visit 1 for subjects enrolled during the specific season. Subjects diagnosed with dengue between the start of the specific season up to before Visit 1 will not be included in the denominator for the specific season.*
- Incidence rate (expressed as number of cases/1000 person-years) of laboratory-confirmed symptomatic dengue infection with 95% CI for all dengue seasons combined: the numerator and denominator will be respectively the sum of each numerator and denominator used in the computation of incidence rates by season.

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

- Incidence rate (expressed as number of cases/1000 person-years) of laboratory-confirmed or probable symptomatic dengue infection with 95% CI for each dengue season separately: the numerator will be the total number of laboratory-confirmed or probable symptomatic dengue infections during the specific season (first of such event per subject during the specific season, using the date of first symptom onset). The denominator will be the total person-years at risk during the specific season, i.e. from the start of the specific season* until the first laboratory-confirmed or probable symptomatic dengue infection during the specific season, the end of the specific season or the subject's withdrawal, whichever comes first. Subjects withdrawn before the start of the specific season will not be included in the denominator.
- Incidence rate (expressed as number of cases/1000 person-years) of laboratory-confirmed or probable symptomatic dengue infection with 95% CI for all seasons combined: the numerator and denominator will be respectively the sum of each numerator and denominator used in the computation of incidence rate by season.
- Incidence rate (expressed as number of cases/1000 person-years) of probable symptomatic dengue infection with 95% CI for each dengue season separately: the numerator will be the total number of probable symptomatic dengue infections during the specific season (first of such event per subject during the specific season, using the date of first symptom onset). The denominator will be the total person-years at risk during the specific season, i.e. from the start of the specific season* until the first probable symptomatic dengue infection during the specific season, the end of the specific season or the subject's withdrawal, whichever comes first. Subjects withdrawn before the start of the specific season will not be included in the denominator.
- Incidence rate (expressed as number of cases/1000 person-years) of probable symptomatic dengue infection with 95% CI for all seasons combined: the numerator and denominator will be respectively the sum of each numerator and denominator used in the computation of incidence rate by season.

For calculation of incidence by age category, the time at risk will end at the day before the18th birthday for strata ≥9-17 years, and begin at the 18th birthday for strata ≥18-49 years. Same rule will be applied for the other age categories.

The same analysis as describe above will also be generated by calendar year. For the analyses by calendar years the analysis periods will begin on 01-Jan and end on 31-Dec of the considered year.

In addition, the number of subjects at risk, the number of households with at least one event (i.e. laboratory-confirmed symptomatic dengue cases, laboratory-confirmed or probable symptomatic dengue cases, probable symptomatic dengue cases) and the total number of events reported during the follow-up period at risk will also be tabulated along with the incidence rates.

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

- The seroprevalence of dengue infection with 95% CI (refer to Section 12.1 for the methodology for computing 95%CI) will be calculated at Visit 1 (i.e. the number of subjects tested positive for dengue IgG at Visit 1 divided by the total number of subjects with dengue IgG results at Visit 1).
- The incidence proportion of subjects with primary inapparent dengue infection with 95% CI (refer to Section 12.1 for the methodology for computing 95%CI) will be estimated for each dengue season and overall. It will be estimated as the number of documented seroconversion (anti-dengue IgG antibodies) without clinical suspicion of dengue (identified during the time period in which seroconversion occurred, using the blood sampling dates) per total number of subjects at risk. The total number of subjects at risk will be calculated using dengue IgG results at the scheduled visits. Only subjects with available test results for two consecutive scheduled visits will be considered in the denominator by season. Only subjects negative for anti-dengue IgG antibodies at one visit will contribute for the following period of analysis. For overall seasons, only subjects with negative result for anti-dengue IgG antibodies at the scheduled visit planned before their first season follow-up and with either available anti-dengue IgG results for the scheduled visit planned after the last season or with positive result for anti-dengue IgG antibodies at any scheduled visits planned in between will be included in the denominator.

7.5. Analysis of tertiary objectives

- Tertiary objectives will not be analysed.

7.6. Analysis of serious adverse events

Serious adverse events related to study procedures reported during the study period will be listed.

8. ANALYSIS INTERPRETATION

All analyses are descriptive.

9. CONDUCT OF ANALYSES

9.1. Sequence of analyses

The planned interim analysis was not performed (refer to Section 10).

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

Study progress reports were generated during the study conduct to follow study progress using specified tables and using the study database as source data.

Description	Analysis ID	Disclosure Purpose (CTRS=public posting, SR=study report, internal)	Dry run review needed (Y/N)	*Study Headline Summary (SHS)* requiring expedited communication to upper management (Yes/No)	Reference for TFL
Final analysis	E1_01	SR, CTRS	No	Yes	Refer to EPI-DENGUE-006 BOD BR (116606) TFL (Final analysis) (14-JUN-2019).docx
Study progress reports	E1_02	Monitoring	No	No	Refer to EPI-DENGUE-006 BOD BR (116606) Additional Analysis Request E01_02 Study progress.docx

9.2. Statistical considerations for interim analyses

The planned interim analysis was not performed.

10. CHANGES FROM PLANNED ANALYSES

The definitions of laboratory-confirmed, probable, negative, indeterminate, primary and secondary symptomatic dengue cases were clarified.

The cohorts were clarified and the Total cohort without subjects with code 900 was added.

Some tables for the analysis of demographics will be performed on ATP cohort.

The computation of incidence rates was clarified. Incidences rates will also be performed on calendar year.

The statistical methodology to estimate the CI for proportions and incidence rates accounting for clustering effect will not use stratification by center for the analyses on overall centers (refer to Section 12.1).

The clinical characteristics of symptomatic dengue infection (symptoms, hospitalizations, severity) will be presented by dengue classification (lab-confirmed, probable, indeterminate or negative) and not by DENV type (DENV 1-4), study site, gender and age category.

Due to study termination:

- The interim analysis planned in the protocol was not done
- Tertiary objectives will not be analysed
- The following laboratory tests will not be performed:
  - Dengue 1-4 Neutralizing assay
  - Yellow Fever Neutralizing assay

11. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

Refer to the document entitled EPI-DENGUE-006 BOD BR (116606) TFL (Final analysis) (14-JUN-2019) DRAFT.docx

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

- Age categories:

Group order in tables	Group label in tables	Group definition for footnote
1	6 months-<12 months	From the 6th month birthday up to and including the day before the 1st year birthday
2	1-4 years	From the 1st year birthday up to and including the day before the 5th year birthday
3	5-8 years	From the 5th year birthday up to and including the day before the 9th year birthday
4	9-17 years	From the 9th year birthday up to and including the day before the 18th year birthday
5	18-49 years	From the 18th year birthday up to and including the day before the 50th year birthday
6	≥50 years	From the 50th year birthday onwards

- Study sites (named center in the database):

Group order in tables	Group label in tables	Group definition for footnote
1	Rio	No footnote
2	Manaus	
3	Salvador	
4	Natal	
5	Campinas	

- Ad-hoc visits:

Group order in tables	Group label in tables	Group definition for footnote
1	Non SDC	Ad-hoc visits without suspicion of dengue, excluding those reported without fever
2	SDC	Ad-hoc visits with suspicion of dengue, excluding those reported without fever

- Symptomatic dengue confirmation status:

Group order in tables	Group label in tables	Group definition for footnote
1	Lab-confirmed	Laboratory-confirmed symptomatic dengue cases
2	Probable	Probable symptomatic dengue cases
3	Negative	Negative symptomatic dengue cases
4	Indeterminate	Indeterminate symptomatic dengue cases

- Early/late presenter classification:

Group order in tables	Group label in tables	Group definition for footnote
1	Early presenter	Suspected dengue cases presenting at the health care facility within 5 days of fever onset
2	Late presenter	Suspected dengue cases presenting at the health care facility 6 days or more after the onset of fever

- Calendar years:

Group order in tables	Group label in tables	Group definition for footnote
1	Year 2014	-
2	Year 2015	-
3	Year 2016	-
4	Year 2017	-
5	Year 2018	-

12. DATA DERIVATION RULE AND STATISTICAL METHODS

12.1. Methodology for computing CI

All confidence intervals (CIs) will be two-sided 95% CI.

This study will use cluster sampling (i.e. households within centers), inducing a potential clustering effect of the households and/or centers.

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. Similarly, subjects within a center may have a more similar risk of infection compared to individuals from different centers.

Therefore, CIs for proportions and incidence rates will account for clustering of observations.

12.1.1. Confidence interval for proportion / prevalence

The 95% CIs will be based on generalized estimating equations (GEE) logistic regression model with robust variance estimate [Liang, 1986]:

- For an analysis by center, households will be considered as the clusters in the model.
- For an analysis on overall centers, the model with the highest estimated design effect (DE) among those using either households or centers as clusters will be used.
- SAS code:

```
PROC GENMOD DATA=xxx descending;
  CLASS cluster;
  MODEL event = / dist=bin link=logit;
  REPEATED subject= cluster / type=exch;
RUN;
```

Note that if the DE estimated from the selected GEE model is less than 1, then the classical logistic regression model will be used (GENMOD without cluster statement).

The design effect (DE), assessing the impact of clustered data on the variance of the estimated proportions (thus on the the precision of the estimated proportions), will be estimated as follows:

$$DE = \frac{\text{estimated variance accounting for the cluster sampling from GEE model}}{\text{variance estimated under the assumption of simple random sampling}}$$

If the proportion is 0% or 100%, or if all subjects used to compute the proportion are from the same cluster, then ICC, DE and 95%CI will not be estimated.

12.1.2. Confidence interval for incidence rate

Because of the potential clustering effect of the households/centers, such data often present overdispersion, defined as the variance of the incidence rate exceeding its mean which is expected to be equal under the Poisson distribution assumption. If ten events or more are observed for the estimation of the incidence rate, a normal approximation will be used and the variance will be based on Taylor series (PROC SURVEYMEANS in SAS with CLUSTER statement). If less than ten events are observed, the variance will be corrected for overdispersion with Pearson's X^2 for goodness of fit divided by its degrees of freedom (PROC GENMOD with SCALE=PEARSON) [McCullagh, 1989].

For an analysis by center, households will be considered as the clusters in the model.

For an analysis on overall centers, the model with the highest estimated design effect (DE) among those using either households or centers as clusters will be used.

SAS code:

```
proc surveymeans data= xxx;
   cluster yyy;
   var ind_inc;
   weight  timetevt;
run;

   proc genmod data= xxx;
model event= / d=poisson offset=lgtte scale=pearson COVB;
   run;
```

12.2. Data presentation

The following decimal description will be used for the analyses:

Display Table	Parameters	Number of decimal digits
Dengue cases	Incidence rate (in 1000 person-years) with LL & UL, person-years	1
Dengue cases	% of count, including LL & UL of CI	1
Dengue cases	Minimum, maximum, range	Number of decimals in the raw data
Dengue cases	Mean, median	Number of decimals in the raw data +1
Dengue cases	SD	Number of decimals in the raw data +2
Hematology/biochemistry	%	1
Hematology/biochemistry	Minimum, maximum, range	Number of decimals in the raw data
Hematology/biochemistry	SD	Number of decimals in the raw data +2
Demographic/ baseline characteristics and changes between scheduled visits	Percentage, mean, median, standard deviation, minimum, maximum, Q1, Q3	1

CI = confidence interval
LL = lower limit of the confidence interval
SD = standard deviation
UL = upper limit of the confidence interval
Q1/Q3 = 25th and 75th percentiles

12.3. Handling missing data

No data handling will be performed in case of missing data.

12.4. Data derivation

12.4.1. Date derivation

SAS date derived from a character date: in case day is missing, 15 is used. In case day and month are missing, 30 June is used.

12.4.2. Age

Age: age at the reference activity is computed as the number of units between the date of birth and the reference activity, expressed in months/years.

When age at reference activity is to be displayed in months/years, it will be calculated as the number of complete calendar months/years between the date of birth and the date of reference activity. For example:

- DOB = 10JUN2017, Date of vaccination = 09JUL2018 -> Age = 12 months
- DOB = 10JUN2017, Date of vaccination = 10JUL2018 -> Age = 13 months
- DOB = 10SEP1983, Date of reference = 09SEP2018 -> Age = 34 years
- DOB = 10SEP1983, Date of reference = 10SEP2018 -> Age = 35 years

12.4.3. Time interval

Time interval between 2 visits is expressed in days. It is the number of days between the 2 visit dates. Therefore, the time interval is of 0 day if the 2 visit dates are the same.

Time interval expressed in months will be computed as follows: time interval expressed in days/30.4375.

Time interval expressed in years will be computed as follows: time interval expressed in days/365.25.

12.4.4. Duration

Duration of an event is expressed in days. It is the number of days between the start and the stop dates + 1. Therefore, duration is 1 day for an event starting and ending on the same day.

12.4.5. Reference values for vital signs

Reference* range of values for heart rate:

Age	Heart rate (beats/min)
0 - 6 months	110-160
7 months - < 1 year	90-160
1 - 3 years	80-150
4 - 6 years	70-120
7 - 12 years	60-110
>12 years	60-100

*PEDIATRIC ADVANCED LIFE SUPPORT from American Heart Association

Reference* range of values for blood pressure:

Age	Systolic (mmHg)		Diastolic (mmHg)	
	Female	Male	Female	Male
6months - <1 year	82-102	87-105	46-66	48-68
1 year	86-104	85-103	40-58	37-56
2 years	88-105	88-106	45-63	42-61
3 years	89-107	91-109	49-67	46-65
4 years	91-108	93-111	52-70	50-69
5 years	93-110	95-112	54-72	53-72
6 years	94-111	96-114	56-74	55-74
7 years	96-113	97-115	57-75	57-76
8 years	98-115	99-116	58-76	59-78
9 years	100-117	100-118	59-77	60-79
10 years	102-119	102-119	60-78	61-80
11 years	103-121	104-121	61-79	61-80
12 years	105-123	106-123	62-80	62-81
13 years	107-124	108-126	63-81	62-81
14 years	109-126	111-128	64-82	63-82
>15 years	110-127	113-131	65-83	64-83

*Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents, U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

12.4.6. Hematology and Biochemistry

The following unit conversions will be applied:

Laboratory test	Local laboratory unit	Reference unit (international unit)
Albumin	1 G/100ML	10 G/L
	1 UMOL/L	0.069010 G/L
Hemoglobin	1 G/100ML	10 G/L
	1 MG/100ML	0.01 G/L
	1 MG/DL	0.01 G/L
Monocytes / Neutrophils / Platelets / White Blood Cells	1 /MM3	0.001 10E9/L
	1 CELL/MM3	0.001 10E9/L
	1 10E3/MM3	1 10E9/L

12.4.7. Moderate to severe dengue cases

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 (i.e. criteria box ticked in the eCRF):

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

12.4.8. Fever at the visit

The status of fever at the visit for suspicion of dengue will be derived from the fever tick box of the eCRF from the list of main signs.

12.4.9. Safety

For analysis of serious adverse events related to study procedures, all enrolled subjects will be considered. Subjects who did not report the event will be considered as subjects without the event.

13. REFERENCES

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McCullagh P, Nelder JA. Generalized Linear Models, 2nd ed. London: Chapman and Hall, 1989

Pontes RJ, Freeman J, Oliveira-Lima JW, Hodgson JC, Spielman A. Vector densities that potentiate dengue outbreaks in a Brazilian city. *Am J Trop Med Hyg.* 2000; 62: 378–383.

Statistical Analysis Plan

Study alias & e-track number(s): EPI-DENGUE-006 BOD BR (116606)

Detailed Title:	A prospective, multicenter, multiyear, cohort study to determine dengue incidence in children and adults in communities from endemic regions in Brazil
SAP version	Version 1
SAP date	16-NOV-2017
Scope:	All available data pertaining to the above study related to the planned interim analysis on the primary and secondary objectives
Co-ordinating author:	PPD (Statistician)
Other author(s):	PPD (Statistician)
Reviewed by:	PPD (Lead statistician) PPD (Statistical analyst) PPD (Epidemiologist, Director) PPD (Epidemiologist, Brazil) PPD (Clinical and Epidemiology Project Lead) PPD (Scientific Writer) PPD (Study Delivery Lead) PPD (Clin and EPI Manager Biomanguinhos, Fiocruz) PPD (peer reviewer statistician)
Approved by:	PPD (Lead Biostatistician) PPD (Lead statistical analyst) PPD (Epidemiologist, Director) PPD (Clinical and Epidemiology Project Lead) PPD (Scientific Writer) PPD (Statistician)

Name (function)	Signature	dd-mmm-yyyy
PPD (Clin and EPI Manager Biomanguinhos, Fiocruz)		

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LIST OF ABBREVIATIONS

ATP	According-To-Protocol
CI	Confidence interval
DENV	Dengue virus
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
FHP	Family Health Physician Program
GEE	Generalized estimating equations
GSK	GlaxoSmithKline
HCT	Hematocrit
IgG	Immunoglobulin type G
IgM	Immunoglobulin type M
LAR	Legally Acceptable Representative
LIRA	Larval Index Rapid Assay
LL	Lower limit of the confidence interval
NS1	Non Structural 1
RT-qPCR	Reverse Transcriptase quantitative Polymerase Chain Reaction
SAP	Statistical analysis plan
SCTM	Study Core Team Meeting
SD	Standard deviation
SDC	Suspected dengue case
TFL	Tables Figures and Listing template annexed to SAP
UL	Upper limit of the confidence interval
WHO	World Health Organization

1. DOCUMENT HISTORY

Date	Description	Protocol Version
16-NOV-2017	Version 1	Amendment 4: 16 October 2017

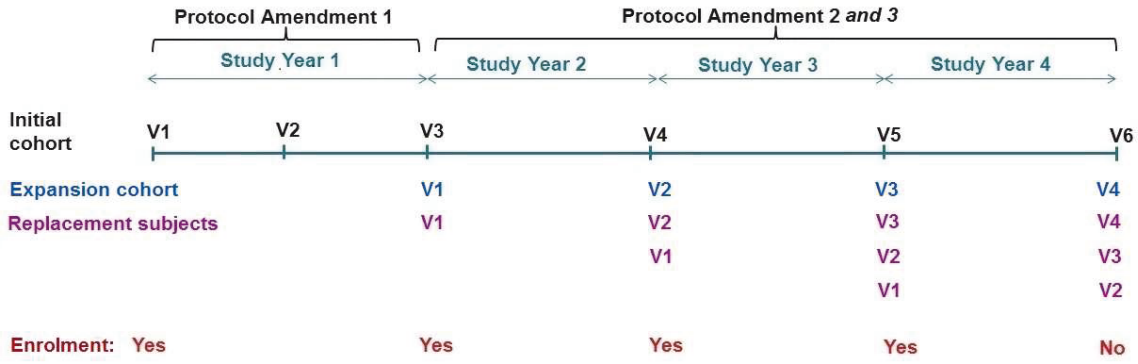
2. STUDY DESIGN

2.1. Study design overview

- **Type of design**: Prospective, multicenter, community-based, household-sampling, cohort study in Brazil.
- **Duration of the study:** The study period per initial site, initially planned to be one year, has been extended by additional three years (overall four years) to cover three additional dengue seasons. The study period per new site is 3 years.
- **Enrolment wave:** There will be 2 waves of enrolment for recruiting 3600 subjects:
  - Initial Cohort = about 1800 subjects – four years follow-up – INITIAL sites: Rio (PPD), Manaus (PPD) and Salvador (PPD). These initial sites will be included in the interim analysis.
  - Expansion Cohort = about 1800 subjects – three years follow-up – NEW sites: Natal (PPD), Campo Grande (PPD), and Campinas (PPD). These new sites will not be included in the interim analysis.
- **Replacement:** 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 cohorts), subjects who prematurely terminate participation or subjects who are lost to follow-up (for subjects from the initial and expansion cohorts).

  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.

- **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 of each study site
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).

2.2. Study population

This study plans to enrol subjects (six months of age and older at the time of enrolment) from randomly selected households from different communities within the selected sites.

2.2.1. Selection of study sites

Six sites covering different regions of the country were 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. Other selection criteria of the sites include an extensive feasibility assessment that includes aspects related to the site structure including laboratory and local facilities, availability of the principal investigator and previous experience on Dengue.

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 to identify where there is a risk of mosquito reproduction, and has been widely implemented in Brazil [Pontes, 2000].

The study sites included in the interim analysis of Rio and Salvador were selected mainly considering their experience in previous Dengue field research. Manaus was selected mainly for the experience of its principal investigator in dengue studies and the previous experience of the site in clinical trials with GSK.

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

2.2.2. Selection of communities

Community selection is based on the following characteristics: *1)* 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; *2)* safe access by study personnel; *3)* high population density; and *4)* low migration rate.

2.2.3. Selection of households

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

The FHP or LIRA provides an exhaustive list of households to the study sites and serves 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 (i.e. Manaus) 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 is selected from the FHP database. Note that none of the three study sites part of the interim analysis has FHP coverage.

Where another type of household registry offers the only access to the community (i.e. Rio and Salvador), a random sample is to be drawn from the registry database. Actually, for Rio and Salvador, contact in the communities was performed to identify potential

subjects who were interested to be enrolled. In addition, households were selected based on their location (in more safety areas and near to the Unit of Health Care).

2.2.4. Selection of subjects

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 per site in order to maintain at least 500 subjects per site at the beginning of each dengue season.

Preferably the recruitment period will occur outside of the peak dengue transmission season, and will continue until each site has reached its foreseen target. Recruitment of replacement subjects will be done during the low dengue transmission.

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. Information on refusal will be kept at the study site and will not be recorded in the screening/enrolment log.

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 per site. 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. Replacement subjects will be enrolled from households located in the same communities as the withdrawn subjects.

In order to better represent the Brazilian population distribution, subjects enrolled in the expansion cohorts 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 cohorts 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. This strategy was implemented at enrolment in Salvador and for replacement subject in Rio for subjects included in the interim analysis.

2.3. Dengue case detection

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

For Rio, Salvador and Manaus, the designated study hospitals/clinics are not the investigator sites. The designated study hospitals/clinics are working with the investigator sites and are located in the community where the subjects live.

Suspected cases in the study may arise from three sources:

1) Referred by study personnel during scheduled home visits
2) Through enhanced passive surveillance
3) As a result of active surveillance between scheduled visits

2.3.1. Case detection during scheduled home visits

If a subject is identified as suspected case during any home visit, he or she is referred to the designated study hospital/clinic for medical evaluation.

Note that the home visits are denoted as scheduled study visits in the list of study procedures in the protocol.

2.3.2. Case detection through enhanced passive surveillance

The subject/subject's parent(s)/legally acceptable representative (LAR[s]) is instructed to contact the study staff (the local study coordinator) at any time dengue is suspected (i.e., body temperature ≥38°C measured (by any route) for at least two consecutive days).

The subject/subject's parent(s)/LAR(s) is 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 local study coordinator then arranges for an appointment at the designated study hospital/clinic.

Although subjects are instructed to contact the study staff in the event of suspected dengue, 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.

2.3.3. Case detection through active surveillance

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

If dengue is suspected during active surveillance, an appointment is arranged at the designated study hospital/clinic, and at least one additional visit is required for case follow-up.

2.4. Dengue seasons

Dengue season for the study sites included in the interim analysis:

Study site number	Study site name	Dengue season
PPD	Rio	1st January to 30th June
PPD	Manaus	1st January to 30th June
PPD	Salvador	1st January to 30th June

3. OBJECTIVES

3.1. Primary objective

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

3.2. 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.

3.3. Tertiary objectives (optional)

- Tertiary objectives are not addressed in this SAP. Refer to the protocol for the list of tertiary objectives.

4. ENDPOINTS

4.1. Primary endpoint

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

4.2. 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.
- Primary inapparent dengue infection.
- Severity of symptoms of symptomatic dengue (using the 2009 WHO guidelines).

4.3. Tertiary endpoints

- Tertiary endpoints are not addressed in this SAP. Refer to the protocol for the list of tertiary endpoints.

5. ANALYSIS SETS

5.1. Definition

Three cohorts are defined for the purpose of the interim analysis:

- Total cohort
- Total cohort without subjects with code 900
- ATP cohort

5.1.1. Total cohort

The Total cohort will include all subjects enrolled in the study from the three sites included in the interim analysis (i.e. Rio, Manaus and Salvador).

5.1.2. Total cohort without subjects with code 900

The Total cohort without subjects with code 900 will include all subjects from the Total cohort with valid informed consent.

5.1.3. According-to-protocol cohort

The According-To-Protocol (ATP) cohort will include all subjects from the Total cohort with valid informed consent and who meet all inclusion/exclusion criteria.

5.2. Criteria for eliminating data from Analysis Sets

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

5.2.1. Elimination from Total cohort without subjects with code 900

Code 900 (invalid informed consent) will be used for identifying subjects eliminated from Total cohort without subjects with code 900.

5.2.2. Elimination from ATP cohort

Code 900 (invalid informed consent) and code 2010 (Protocol violation linked to the inclusion/exclusion criteria) will be used for identifying subjects eliminated from ATP cohort.

6. DENGUE CASE CLASSIFICATION

The following classification of suspected dengue case (SDC) will be used in this study.

6.1. 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 by any route) 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.

A suspected dengue case presenting at the health care facility within 5 days following the onset of fever (i.e. day of fever onset and the next 4 days) 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).

6.2. 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.
- *Acute and convalescent serum samples are samples taken at the first and follow-up visits for suspected dengue case, respectively.*

6.3. Virologically confirmed symptomatic dengue infection

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

6.4. Probable dengue case

- 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).

6.5. 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.

6.6. Indeterminate dengue case

An indeterminate dengue case is a participant evaluated as an SDC (Section 6.1) and not classified as laboratory confirmed case (Section 6.2), probably case (Section 6.4) or negative case (Section 6.5).

6.7. Primary symptomatic dengue case

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).

6.8. Secondary symptomatic dengue case

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

6.9. Primary inapparent dengue infection

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).

Note that subjects with a visit for suspicion of dengue case (even with a negative result to acute and convalescent blood sample) won't be considered for inapparent infection.

6.10. Previous dengue infection

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)
- *This status will be considered as unknown for subject without laboratory result at baseline visit and without laboratory-confirmed symptomatic dengue case Visit 1.*

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

7. GROUPS DEFINITION

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

Age categories:

Group order in tables	Group label in tables	Group definition for footnote
1	6 months-<12 months	From the 6 months birthday up to and including the day before the 1st year birthday
2	1-4 years	From the 1st year birthday up to and including the day before the 5th years birthday
3	5-8 years	From the 5th years birthday up to and including the day before the 9th year birthday
4	9-17 years	From the 9th years birthday up to and including the day before the 18th years birthday
5	18-49 years	From the 18th years birthday up to and including the day before the 50th years birthday
6	≥50 years	From the 50th years birthday up to any age

Study sites (named center in the database):

Group order in tables	Group label in tables	Group definition for footnote
1	Rio	Study site PPD
2	Manaus	Study site PPD
3	Salvador	Study site PPD

Suspected symptomatic dengue status:

Group order in tables	Group label in tables	Group definition for footnote
1	Non SDC	Ad-hoc visits without suspicion of dengue
2	SDC	Ad-hoc visits with suspicion of dengue

Symptomatic dengue confirmation status:

Group order in tables	Group label in tables	Group definition for footnote
1	Lab-confirmed	Laboratory-confirmed symptomatic dengue cases
2	Probable	Probable symptomatic dengue cases
3	Negative	Negative symptomatic dengue cases
4	Indeterminate	Indeterminate symptomatic dengue cases

Early/late presenter classification:

Group order in tables	Group label in tables	Group definition for footnote
1	Early presenter	Suspected dengue cases presenting at the health care facility within 5 days of fever onset
2	Late presenter	Suspected dengue cases presenting at the health care facility between sixth and 30th day of fever onset

Calendar years:

Group order in tables	Group label in tables	Group definition for footnote
1	Year 2014	-
2	Year 2015	-
3	Year 2016	-
4	Year 2017	-

Dengue seasons:

Group order in tables	Group label in tables	Group definition for footnote
1	Season 2015	1st January to 30th June 2015
2	Season 2016	1st January to 30th June 2016

8. STATISTICAL ANALYSES

SAS software version 9.3 will be used for statistical analysis.

Continuous variables will be summarized with number of non-missing observations, mean, standard deviation (SD), median, minimum and maximum. Categorical variables will be summarized with frequency tables; numbers and percentages for each level will be given.

The interim analysis on primary and secondary objectives on available data as cleaned as possible will be performed on the following study sites using data up to the specified study visit:

Study sites number	Study sites name	Study visits
PPD	Rio	Visit 1 to Visit 4 Replacement: Visit 1 to Visit 2
PPD	Manaus	Visit 1 to Visit 4 Replacement: not included in the interim analysis
PPD	Salvador	Visit 1 to Visit 3 Replacement: not included in the interim analysis

8.1. Analysis of demographics/baseline characteristics and changes between scheduled visits

The number of subjects enrolled into the study will be tabulated by study site and overall on the Total cohort.

The numbers of withdrawn subjects will be tabulated according to the reason for withdrawal by study site, age category and overall on the Total cohort.

The distribution of the subjects in each cohort and reasons for exclusion will be tabulated by study site and overall.

Distribution of scheduled study visits by calendar month will be tabulated by study site and overall on the ATP cohort.

Time interval between scheduled study visits will be summarized by study site and overall on the ATP cohort.

Demographic characteristics (age and gender) will be summarized using descriptive statistics by study site and overall on the Total cohort without subjects with code 900 and on the ATP cohort.

Socio-economic characteristics of the households at Visit 1 (enrolment visit) will be tabulated by study site and overall on the ATP cohort.

General medical history, number of subjects with at least 2 consecutive days of fever within one month before Visit 1, dengue history and yellow fever vaccination history at Visit 1 will be summarized on the ATP cohort. Changes in general medical history and vaccination against yellow fever occurring during study conduct will be summarized at each scheduled study Visits at Month 6, Month 12 and Month 24.

8.2. Description of ad-hoc visits

The analyses will be performed on the ATP cohort.

The proportion of SDC reported without fever will be tabulated by study site and overall.

SDC without fever will be excluded from all analyses described below up to Section 8.4 included.

The distribution of ad-hoc visits with suspicion of dengue (stratified by laboratory outcome, i.e. laboratory-confirmed, probable, negative and indeterminate) and ad-hoc visits without suspicion of dengue will be tabulated by study site, age category and calendar month.

A summary of characteristics (age category, year quarter, gender, diagnosis) of ad-hoc visits without suspicion of dengue will be tabulated by study site and overall. A summary of characteristics (age category, year quarter, gender, laboratory outcome) of ad-hoc visits with suspicion of dengue will be tabulated by study site and overall.

The proportion of episodes of dengue suspicion having return visits, the number of visits (i.e. first, returned and follow-up visits), the time interval between the first visit and the first return visit and the time interval between the first visit and the follow-up visits will be tabulated. The number of blood samples taken at first and follow-up visits, the time interval between the visit and the blood sample, the time interval between fever onset and blood sample taken at first visit and the time interval between blood samples will be tabulated.

8.3. Characteristics of the suspected dengue cases

The analyses will be performed on the ATP cohort.

SDC will be listed with the following characteristics: subject number, study site, date of first visit for SDC, dengue episode number, household number, age at first symptom onset, gender, date of first symptoms onset, date of fever onset, the first symptom(s), time interval between first symptom onset and first visit for SDC (in days), time interval between fever onset and first visit for SDC (in days), early/late classification, date of follow-up visit for SDC, date of blood samples, dengue classification (lab-confirmed, probable, indeterminate or negative), case confirmed by RT-qPCR at least (Yes/no), case confirmed by NS1 at least (Yes/no), case confirmed by seroconversion of IgM between first and follow-up visit at least (Yes/no), laboratory results at first and follow-up visits and IgG result at scheduled visit before and after the episode.

The RT-qPCR (overall and by DENV type), NS1, IgM and IgG laboratory results for SDC will be summarized by presenter status (early versus late) and overall.

The distribution of SDC in the following categories will be computed for primary and secondary infections, and by calendar year:

- Cases confirmed by RT-qPCR only
- Cases confirmed by NS1 positivity only
- Cases confirmed by IgM seroconversion only
- Cases confirmed by both RT-qPCR and NS1 positivity, without IgM seroconversion
- Cases confirmed by both RT-qPCR and IgM seroconversion, without NS1 positivity

- Cases confirmed by both NS1 positivity and IgM seroconversion, without RT-qPCR positivity
- All laboratory confirmed cases
- All cases confirmed by RT-qPCR at least
- All probable cases
- All confirmed and probable cases
- Negative
- Indeterminate

The following characteristics will be summarized for each category of dengue cases (laboratory-confirmed, probable, negatives and indeterminate):

- Fever at onset: proportion of cases with fever being part of first symptoms
- Age at first symptoms onset, study site, IgG results at the preceding scheduled visit, surveillance source leading to Visit 1 (active/passive/scheduled visit), study hospital where the visit was performed, time interval between onset of fever and first visit for SDC, time interval between onset of fever (if fever is part of the first symptom) and first visit for SDC, time interval between onset of first symptom (if fever is not the first symptom) and first visit for SDC and time interval between first visit and follow-up visit for SDC
- Temperature at first visit: <37.5, ≥ 37.5, >38, >38.5, >39, >39.5, >40
- Clinical symptoms at onset, from first and returned visits, since onset of symptoms: frequency and proportion of cases with each symptom
- Vital signs at first visit (heart rate, systolic and diastolic blood pressure): number and proportion of subjects below, within and above the reference ranges of values (see 12.4.5 for reference ranges)
- Hematology/Biochemistry at first visit: number and proportion of subjects below, within and above the reference ranges of each test provided by the laboratory. Summary statistics of continuous variables for haemoglobin, haematocrit, platelets, white blood cells, neutrophils, monocytes, albumin, ASAT and ALAT.
- General medical history at scheduled visit before SDC
- Follow-up visits (‘yes’, ‘phone contact’, ‘no’)

– Final classification of the episode (duration of fever, most likely diagnosis according to investigator opinion (dengue, chikungunya, influenza, malaria rotavirus or other enteric infection, other infection disease or non-infectious disease), hospitalisation (yes/no, reason, duration), outcome, moderate to severe dengue episode (yes/no)).

– Severity criteria of SDC (each warning sign (yes/no), each criteria for severe dengue (yes/no))

8.4. Analysis of incidences

The analyses will be performed on the ATP cohort.

The following analyses will be performed overall and by DENV type:

- Incidence rate (expressed as number of cases/1000 person-years) of laboratory-confirmed symptomatic dengue infection with 95% CI (refer to Section 12.1 for the methodology for computing 95%CI) for each dengue season separately: the numerator will be the total number of laboratory-confirmed symptomatic dengue infection during the specific season (first of such event per subject during the specific season, using the date of first symptom onset). The denominator will be the total person-years at risk during the specific season, i.e. from the start of the specific season* until the first laboratory-confirmed symptomatic dengue infection during the specific season, the end of the specific season or the subject's withdrawal, whichever comes first. Subjects withdrawn before the start of the specific season will not be included in the denominator.

  *From Visit 1 for subjects enrolled during the specific season. Subjects diagnosed with dengue between the start of the specific season up to before Visit 1 will not be included in the denominator for the specific season.

- Incidence rate (expressed as number of cases/1000 person-years) of laboratory-confirmed symptomatic dengue infection with 95% CI for all dengue seasons combined: the numerator and denominator will be respectively the sum of each numerator and denominator used in the computation of incidence rates by season.

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

- Incidence rate (expressed as number of cases/1000 person-years) of laboratory-confirmed and probable symptomatic dengue infection with 95% CI for each dengue season separately: the numerator will be the total number of laboratory-confirmed and probable symptomatic dengue infection during the specific season (first of such event per subject during the specific season, using the date of first symptom onset). The denominator will be the total person-years at risk during the specific season, i.e.

from the start of the specific season* until the first laboratory-confirmed or probable symptomatic dengue infection during the specific season, the end of the specific season or the subject's withdrawal, whichever comes first. Subjects withdrawn before the start of the specific season will not be included in the denominator.

- Incidence rate (expressed as number of cases/1000 person-years) of laboratory-confirmed and probable symptomatic dengue infection with 95% CI for all seasons combined: the numerator and denominator will be respectively the sum of each numerator and denominator used in the computation of incidence rate by season.

For calculation of incidence by age category, the time at risk will end at the day before the18th birthday for strata ≥9-17 years, and begin at the 18th birthday for strata ≥18-49 years. Same rule will applied for the other age categories.

The same analysis as describe above will also be generated by calendar year. For the analyses by calendar years the analysis periods will begin on 01-Jan and end on 31-Dec of the considered year.

In addition, the number of subjects at risk and the number of households with at least one event (i.e. laboratory-confirmed symptomatic dengue cases, laboratory-confirmed and probable symptomatic dengue cases) will also be tabulated along with the incidence rates.

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

- The seroprevalence of dengue infection with 95% CI (refer to Section 12.1 for the methodology for computing 95%CI) will be calculated at Visit 1 (i.e. the number of subjects tested positive for dengue IgG at Visit 1 divided by the total number of subjects with dengue IgG results at Visit 1).
- The incidence proportion of subjects with primary inapparent dengue infection with 95% CI (refer to Section 12.1 for the methodology for computing 95%CI) will be estimated for each dengue season and overall. It will be estimated as the number of documented seroconversion (anti-dengue IgG antibodies) without clinical suspicion of dengue (identified during the time period in which seroconversion occurred, using the blood sampling dates) per total number of subjects at risk. The total number of subjects at risk will be calculated using dengue IgG results at the scheduled visits. Only subjects with available test results for two consecutive scheduled visits will be considered in the denominator by season. Only subjects negative for anti-dengue IgG antibodies at one visit will contribute for the following period of analysis. For overall seasons, only subjects with negative result for anti-dengue IgG antibodies at the scheduled visit planned before their first season follow-up and with either available anti-dengue IgG results for the scheduled visit planned after the last season or with positive result for anti-dengue IgG antibodies at any scheduled visits planned in between will be included in the denominator.

8.5. Analysis of tertiary objectives

- Tertiary objectives are not addressed in this SAP.

8.6. Analysis of serious adverse events

Serious adverse events related to study procedures reported during the study period will be described.

9. ANALYSIS INTERPRETATION

All analyses are descriptive.

10. CONDUCT OF ANALYSES

10.1. Sequence of analyses

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

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

Note that a study progress report is generated one week prior to each Study Core Team Meeting (SCTM) to follow study progress using specified tables and using the study database as source data, reflecting the data encoded a few days before each SCTM.

Description	Analysis ID (SDD sub-folder)
Final analysis	E1_01
Study progress reports	E1_02
Interim analysis	E1_03

10.2. Statistical considerations for interim analyses

The interim analysis on primary and secondary objectives on available data as cleaned as possible will be performed on the following study sites using data up to the specified study visit:

Study sites number	Study sites name	Study visits
PPD	Rio	Visit 1 to Visit 4 Replacement: Visit 1 to Visit 2
PPD	Manaus	Visit 1 to Visit 4 Replacement: not included in the interim analysis
PPD	Salvador	Visit 1 to Visit 3 Replacement: not included in the interim analysis

The following laboratory tests will be available for the interim analysis in case of SDC:

	Early presenters		Late presenters	
Laboratory test	**Acute sample (first visit)**	**Convalescent sample (follow-up visit)**	**Acute sample (first visit)**	**Convalescent sample (follow-up visit)**
RT-qPCR	Yes	TNP	TNP	TNP
NS1 ELISA	Yes	TNP	Yes up to PA3 TNP for PA4	TNP
Dengue IgM ELISA	Yes	Yes	Yes	Yes
Dengue capture IgG ELISA	TNP	Yes	Yes	Yes

TNP = test not performed on the sample as per protocol
PA3 = protocol amendment 3
PA4 = protocol amendment 4

The following laboratory tests will be available for the interim analysis at the scheduled visits:

- Dengue IgG ELISA will be available at Visit 1
- For Visit 2 onwards, Dengue IgG ELISA will be available for subjects with IgG negative at the previous visit as planned in the protocol.

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

No study report will be written for the interim analyses.

11. CHANGES FROM PLANNED ANALYSES

The cohorts were clarified.

Some tables for the analysis of demographics will also be performed on ATP cohort.

The computation of incidence rates was clarified. Incidences rates will also be performed on calendar year.

CIs for incidence rates and proportions will not account for stratification by centre for the overall analyses in the interim analysis.

12. STATISTICAL METHODS AND DATA DERIVATION RULE

12.1. Methodology for computing CI

All confidence intervals (CIs) will be two sided 95% CI.

This study will use cluster (household) sampling, inducing a potential clustering effect of the 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.

Therefore, CIs for incidence rates and proportions will account for clustering of observations within households.

12.1.1. Confidence interval for proportion / prevalence

The 95% CIs will be based on logistic generalized estimating equations models (GEE) with robust variance estimate [Liang, 1986].

12.1.2. Confidence interval for incidence rate

Because of the potential clustering effect of the households, such data often present overdispersion, defined as the variance of the incidence rate exceeding its mean which is expected to be equal under the Poisson distribution assumption. If ten events or more are observed for the estimation of the incidence rate, a normal approximation will be used and the variance will be based on Taylor series (PROC SURVEYMEANS in SAS with CLUSTER statement). If less than ten events are observed, the variance will be corrected

for overdispersion with Pearson's X^2 for goodness of fit divided by its degrees of freedom (PROC GENMOD with SCALE=PEARSON) [McCullagh,1989].

12.2. Number of decimals

The following decimal description will be used for the analyses:

Display Table	Parameters	Number of decimal digits
Dengue cases	% of count, including LL & UL of CI	1
Dengue cases	Minimum, maximum, range	Number of decimals in the raw data
Dengue cases	Mean, median	Number of decimals in the raw data +1
Dengue cases	SD	Number of decimals in the raw data +2
Dengue cases	Incidence (in 1000 person-years) with LL & UL	1
Demographic characteristics	Mean, median age, standard deviation	1

CI = confidence interval
LL = lower limit of the confidence interval
SD = standard deviation
UL = upper limit of the confidence interval

12.3. Handling missing data

No data handling will be performed in case of missing data.

12.4. Derived and transformed data

12.4.1. Date derivation

SAS date derived from a character date: in case day is missing, 15 is used. In case day and month are missing, 30 June is used.

12.4.2. Age

- Age: age at the reference activity is computed as the number of units between the date of birth and the reference activity. The units will be either month or year (refer to Tables Figures and Listing (TFL) template annexed to SAP).

12.4.3. Time interval

Time interval between 2 visits is expressed in days. It is the number of days between the 2 visit dates. Therefore the time interval is of 0 day if the 2 visit dates are the same.

12.4.4. Duration

Duration of an event is expressed in days. It is the number of days between the start and the stop dates + 1. Therefore duration is 1 day for an event starting and ending on the same day.

12.4.5. Reference values for vital signs

Reference* range of values for heart rate:

Age	Heart rate (beats/min)
0 - 6 months	110-160
7 months - < 1 year	90-160
1 - 3 years	80-150
4 - 6 years	70-120
7 - 12 years	60-110
>12 years	60-100

*PEDIATRIC ADVANCED LIFE SUPPORT from American Heart Association

Reference* range of values for blood pressure:

Age	Systolic (mmHg)		Diastolic (mmHg)	
	Female	Male	Female	Male
6months - <1 year	82-102	87-105	46-66	48-68
1 year	86-104	85-103	40-58	37-56
2 years	88-105	88-106	45-63	42-61
3 years	89-107	91-109	49-67	46-65
4 years	91-108	93-111	52-70	50-69
5 years	93-110	95-112	54-72	53-72
6 years	94-111	96-114	56-74	55-74
7 years	96-113	97-115	57-75	57-76
8 years	98-115	99-116	58-76	59-78
9 years	100-117	100-118	59-77	60-79
10 years	102-119	102-119	60-78	61-80
11 years	103-121	104-121	61-79	61-80
12 years	105-123	106-123	62-80	62-81
13 years	107-124	108-126	63-81	62-81
14 years	109-126	111-128	64-82	63-82
>15 years	110-127	113-131	65-83	64-83

*Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents, U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

12.4.6. Hematology and Biochemistry

The following unit conversions will be applied:

Laboratory test	Local laboratory unit	Reference unit (international unit)
Albumin	1 G/100ML	10 G/L
	1 UMOL/L	0.069010 G/L
Hemoglobin	1 G/100ML	10 G/L
	1 MG/100ML	0.01 G/L
	1 MG/DL	0.01 G/L
Monocytes / Neutrophils / Platelets / White Blood Cells	1 /MM3	0.001 10E9/L
	1 CELL/MM3	0.001 10E9/L
	1 10E3/MM3	1 10E9/L

12.4.7. Moderate to severe dengue cases

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

12.4.8. Fever at the visit

The status of fever at the visit for suspicion of dengue will be derived from the fever tick box of the eCRF from the list of main signs.

12.4.9. Safety

For analysis of serious adverse events related to study procedures, all enrolled subjects will be considered. Subjects who did not report the event will be considered as subjects without the event.

13. REFERENCES

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