Disclaimer

This document is a draft and the information contained herein is subject to change as this document is currently undergoing review by the World Health Organization Ethical Review Committee.

The final version of this standardized protocol: *Cross-sectional seroprevalence study of Zika virus infection in the general population* will be published as soon as the ethical review has been completed.

Standardized Protocol:

Cross-sectional seroprevalence study of Zika virus infection in the general population

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This document was adapted from a protocol developed by the Consortium for the Standardization for Influenza Seroepidemiology (CONSISE), a global partnership aiming to develop influenza investigation protocols and standardize seroepidemiology to inform public health policy for pandemic, zoonotic and seasonal influenza. This international partnership was created out of a need, identified during the 2009 H1N1 pandemic, for better (standardized, validated) seroepidemiological data to estimate infection attack rates and severity of the pandemic virus and to inform policy decisions. More information on CONSISE can be found on their website.

Collaborators from Institut Pasteur, the World Health Organization (WHO), and members of the Consortium for the Standardization of Influenza Seroepidemiology (CONSISE) adapted this protocol as a generic tool for research of Zika virus (ZIKV) infection. A large number of individuals were involved in the content and revision of this protocol and are listed at the end of the protocol.

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PROTOCOL SUMMARY

The World Health Organization (WHO) and Pan American Health Organization (PAHO), Institut Pasteur, the networks of Fiocruz, the Consortium for the Standardization of Influenza Seroepidemiology (CONSISE), the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) and many other international research groups have generated standardized clinical and epidemiological research protocols and questionnaires to address key public health questions for Zika virus (ZIKV).

The geographic scope of the current ZIKV outbreak is vast, extending throughout the Americas and the Caribbean and into parts of Africa. The use of standardized research protocols will ensure that results from these studies can be compared across regions and countries and can potentially improve the quality of observational studies by identifying and minimizing biases. Furthermore, data collected using the standardized protocols will be used to refine and update recommendations for prevention of ZIKV spread, surveillance and case definitions for microcephaly, to help understand the spread, severity, spectrum and impact on the community of ZIKV and to guide public health measures, particularly for pregnant women and couples planning a pregnancy.

Each standardized protocol, including the protocol described below, has been designed to maximize the likelihood that epidemiological, clinical and exposure data and biological samples are systematically collected and shared rapidly in a format that can be easily aggregated, tabulated and analyzed across many different settings globally. We encourage any and all study centers to contribute to this effort regardless of resource availability or patient volume, but the ownership of the primary data remains firmly with the individual countries and study sites.

The protocol described below is a representative serologic study, designed to collect data to estimate the prevalence of antibodies to a new pathogen in a population. This information is critical to better understand the extent of infection in a population and the severity of the new virus. This study protocol outlines methods to collect data to measure the seroprevalence of cross-reactive antibodies to ZIKV in the general population, and may also evaluate risk factors for infection among those with evidence of infection (seropositive subjects) compared to those without infection (seronegative subjects).

Other protocols currently under development include:

- Case-control study to assess potential risk factors related to microcephaly including Zika virus infection
- Case-control study to assess potential risk factors related to Guillain-Barré Syndrome including Zika virus infection
- Prospective longitudinal cohort study of women and newborns exposed to Zika virus during the course of pregnancy
- Prospective longitudinal cohort study of newborns and infants born to mothers exposed to Zika virus during pregnancy

- Prospective longitudinal cohort study of Zika-infected patients to measure the persistence of Zika virus in body fluids
- Clinical characterization protocol for Zika virus infection in the context of co-circulating arboviruses

Study groups may decide to implement several protocols during a ZIKV epidemic. In this case, participants may be enrolled in several studies (e.g. cohort of pregnant women and cohort of newborns or cross sectional seroprevalence). However, each study group needs to consider carefully the burden on each participant.

Comments for the user's consideration are provided in purple text throughout the document, as the user may need to modify methods slightly as a result of the local context in which this study will be carried out.

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LIST OF ABBREVIATIONS

CONSISE	Consortium for the Standardization of Influenza Seroepidemiology
HIV	Human Immunodeficiency virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHR	International Health Regulations
IRB	Institutional Review Board
ISARIC	International Severe Acute Respiratory and Emerging Infection Consortium
PAHO	Pan American Health Organization
PRNT	Plaque-reduction neutralization test
SD	Standard deviation
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TORCHS	Toxoplasmosis, other (e.g. varicella), Rubella, Cytomegalovirus, Herpes, HIV, Syphilis
WHO	World Health Organization
WMA	World Medical Association
YFV	Yellow Fever virus
ZIKV	Zika virus

1.0 INTRODUCTION

Since 1947, Zika virus (ZIKV) was only reported to circulate sporadically in Africa and South-East Asia (Faye et al, 2014). However in 2007, ZIKV was isolated for the first time in the Pacific, on the Micronesian island of Yap (Duffy et al, 2009), followed by the largest Zika outbreak ever previously reported in French Polynesia from October 2013 to April 2014 (Cao-Lormeau et al, 2013). In March 2015, Brazil reported autochthonous transmission of ZIKV (Zanluca et al, 2015) and an outbreak was declared 6 months later (WHO, 2016). As of February 2016, ZIKV had been reported in 28 countries in South/Central America and on 1 February 2016, the World Health Organization declared that the cases of microcephaly and neurologic conditions following the outbreak of Zika constituted a Public Health Emergency of International Concern (WHO, 2016).

It is thought that current surveillance systems, limited by available specific and sensitive diagnostic assays for ZIKV, detect only a small proportion of ZIKV infections and, as a result, the exact number of people who have been infected is unknown (Stoler-Poria et al, 2010). The gold standard for estimating cumulative incidence in a population is the collection of individually paired acute and convalescent sera with a longitudinal study design. However, these are rarely available during an outbreak situation particularly in the early stages of the event. An alternative method to estimate cumulative incidence at a population level is the comparison of sera from a representative cross-section of the population drawn before and after the event. This method uses pre-event seroprevalence of antibodies that react with the novel virus as a baseline value for comparison against the seroprevalence of antibodies in the same population after the event. A large number of countries used this type of cross-sectional study design to estimate cumulative incidence of the pandemic influenza H1N1 virus (H1N1pdm09) (Kelly et al, 2011; Van Kerkhove et al, 2013). This can also be achieved by comparing seroprevalence in virus exposed and virus non-exposed areas.

The following prospective cohort study protocol outlines methods for a cross-sectional seroepidemiological study of populations in ZIKV-exposed and non ZIKV-exposed areas to estimate seroprevalence of ZIKV at one point in time.

Comment: A serial cross-sectional study design (collection of sera at two or more time periods) could follow the completion of this cross sectional study in order to determine cumulative incidence. This could be done by repeating this study either on the same study participants at a different point in time, or on a random sample of people living within the same geographic area as the first cross sectional study at a different point in time.

This study will address the following public health question:

What is the extent of ZIKV seroprevalence in the general population?

Comment: It is important to note that this protocol is designed to describe the core data variables to be collected in order to answer the public health questions and primary objectives. As such, the implementation of this study may include additional objectives or study components, as determined by the financial and technical capacity of the study group implementing this protocol and by the outbreak context.

Comment: By using a standardized protocol, researchers can address many research objectives and will have the opportunity to collaborate with other research sites/countries conducting this same

study. This may enable researchers to pool data to address the primary research question(s) of this protocol. However, to be able to pool data across studies, the adapted protocols and corresponding questionnaires must be aligned.

Comment: Before submission to a local/national Institutional Review Board (IRB), the introduction will need to be updated with the most recent research findings and further description of the epidemiology of the outbreak in the country conducting this study.

1.1 OBJECTIVES

The data collected from this standardized study will be used to refine and update recommendations for surveillance of Zika virus, to characterize the key epidemiological transmission features of Zika virus, to help understand spread, severity, spectrum of disease and to inform public health measures.

The **primary objective** of this study is to:

- Estimate the frequency of ZIKV infection among the general population in both ZIKV-exposed areas and non ZIKV-exposed areas
- Identify risk factors and modifiable risk factors (exposures, behaviors, practices) for ZIKV infection

Cross-sectional studies, such as the one described here, provide the opportunity to assess several **secondary objectives** including, but not limited, to:

- Describe the spectrum of illness with ZIKV infection
- Quantify the proportion of asymptomatic and sub-clinical ZIKV infections

Comment: Additional secondary objectives can be included in the protocol and will be informed by the outbreak characteristics and by the local context.

2.0 STUDY PROCEDURES

Overview: This study uses a cross-sectional design, i.e., measuring the seroprevalence of ZIKV in the study of a population at one point in time.

Comment: A serial cross-sectional study design (collection of sera at two or more time periods) could follow the completion of this cross sectional study in order to determine cumulative incidence. This could be done by repeating this study either on the same study participants at a different point in time, or on a random sample of people living within the same geographic area as the first cross sectional study at a different point in time.

2.1 STUDY SETTINGS

2.2.1 TIMING OF THE STUDY

Ideally, this study should be implemented in an exposed area in which the disease and/or the vector is well established.

Comment: It is important that the research group to clearly define the timing of the sample collection in relation to the epidemic. Ideally, the timing of data collection will be plotted on an epidemic curve.

2.2.2 STUDY AREA

Comment: Below are examples of areas from which sampling may be conducted. The specific methods for selection and recruitment of the study population from these areas will depend on the geographic areas chosen for the cross-sectional survey. Identification and classification of each area will need information from surveillance in the vector population to determine if ZIKV is currently circulating and information from human surveillance activities to determine if any ZIKV cases are reported. A non-exposed area can also include an area where the vector is not present.

ZIKV exposed area:

- An area with laboratory confirmed ZIKV cases in humans and evidence of ZIKV in the vector, or:
- An area with confirmed ZIKV circulation in the vector, without laboratory confirmed human ZIKV cases

ZIKV non-exposed area:

- An area with no laboratory confirmed ZIKV cases and with no reports of ZIKV in possible vectors.

Comment: The study design must clearly define the catchment area of each of the study populations, and include information on the travel history of the participants from each area and, if possible, any vector control interventions implemented in the study areas.

2.3 SELECTION AND RECRUITMENT OF STUDY PARTICIPANTS

2.3.1 STUDY POPULATION

From each defined study area, a random sample of eligible residents will be recruited for the study.

Comment: The research group needs to clearly define and describe the sampling procedure. When defined study area is large such as a country or province/state/district, stratified sampling or cluster sampling may be more appropriate over simple random sampling.

To determine the rate of infection in the general population within an area in which ZIKV is currently circulating (as measured by ZIKV in the vector, or as indicated by human surveillance activities), it is necessary to identify individuals of all ages, from a range of geographic locations with a mix of gender and racial composition.

Ideally, participants will be recruited from each of the following age groups: <1 month, 1-23 months, 2 years to <5 years, 5-9 years, 10-19 years, 20-29 years, 30-39 years, 40-49 years, 50-59 years, 60+ years.

Within each area, all eligible study participants will first be asked whether he/she is over the age of consent [modify to be specific to the local age of consent]. If the respondent is not over the age of consent, the trained interviewer will ask to speak with an individual who is over the local age of consent. The interviewer will ask the potential adult study participant how many individuals are living at the location who are over the age of consent, who are minors, who are present and to name them. A random name will be chosen from those present and the study will be briefly described to the responding participant or parent/guardian. It may be possible to collect the blood sample at this time. Alternatively, a time can be arranged to return to take the blood sample.

Recruitment within the defined study area should be ongoing until the sample size is met.

Comment: The age of consent and assent may vary between countries – this needs to be verified by local IRB requirements.

Comment: An alternative for sample collection is the use of stored sera from registered blood banks. If this option is chosen, there may be limited information available about individual samples (e.g. sex, residence, medical history) available. At a minimum, it is important to collect information about age, timing of sample collection and residence/location information for each sample.

2.3.2 ELIGIBILITY CRITERIA

- **Inclusion criteria:** Any individual 18 years old or above [modify to be specific to the local age of consent], who gives informed consent; any child under the age of consent for whom a

parent or guardian provides written informed consent; any minor under the local age of consent who can provide assent in the presence of a witnessing adult.

- **Exclusion criteria**: Any individual who is unable or unwilling to give informed consent, or with any contraindication to venipuncture.

2.3.3 INFORMED CONSENT

Written informed consent will be collected from all study participants. Written informed consent from a parent or legal guardian will be collected for all children, and assent will be collected from minors old enough to understand the proposed study and sample collection procedures.

During an initial interview with an eligible participant with a trained member of the research study team, the purpose of the study will be explained and consent will obtained from the participant upon enrollment into the study. Each study participant must be informed that his or her participation in voluntary and that he or she will be free, without justification, to withdraw from the study at any time without consequences. Data contributed to the study up until the point of withdrawal will remain with the study group, unless stated by the withdrawing participant.

Informed consent will seek approval to collect samples for the intended purpose of the study, the possibility that samples may be shipped outside of the home country for additional testing and/or analysis and that samples may be used for future research purposes.

Comment: The transfer of samples outside the home country may not be legal in some countries – this needs to be verified by local IRB requirements.

Comment: The study group will need to define the parameters of data sharing with partners outside the country and of future research for which the samples may be used.

Informed consent will also indicate that any suspected or confirmed ZIKV infection may be notified to national authorities under the International Health Regulations (IHR) requirements.

Information for participant and informed consent form templates can be found in Appendix A.

If the study participant agrees, the consent form must be completed legibly, with both surname and first name, dated and signed by the participant and the member of the investigation team, before any procedure can be performed as part of the current study. The member of the investigation team is responsible for obtaining the written consent of the participant and parent/guardian for children and assent for minors.

Once the informed consent form has been signed, one copy will be made and given to the study participant. The original version of the consent form for each participant will be retained by the investigation team and kept in a secure place for a period of time determined by national/local IRB requirements.

2.3.4 INCENTIVES TO PARTICIPATE AND COMPENSATION

All study participants will be provided with additional information on means of protection against ZIKV vectors, on other potential modes of ZIKV transmission (e.g., sexual transmission) and on the risk of microcephaly and other ZIKV-related conditions by trained social and healthcare workers.

The possibility to offer financial compensation (e.g., to compensate costs to visit a medical center for blood sampling, if necessary) will depend on the context of the study and local policies and should be determined on a case-by-case basis. This will need to be detailed in the information provided to the participant and in the informed consent.

Comment: The clinical management of patients is not a part of this research protocol. It will be at the discretion of the medical consultant and carried out according to standard of care at the site at which recruitment occurred.

2.3.5 POLICY ON INCIDENTAL FINDINGS

Unexpected incidental findings not related to ZIKV may be identified during the course of the study. In this context, the study participant and/or parent/guardian will be informed and, with their consent, a referral will be made to an appropriate clinic or health facility for further investigation or longer term follow-up. Patient confidentiality will be maintained throughout the study.

2.4 ETHICAL CONSIDERATIONS

Ethical approval will be sought in accordance with local, regional and national regulations. The sponsor and the investigators will be committed to conducting this research in accordance with the World Medical Association (WMA) Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects) adopted by the 64th WMA General Assembly, Fortaleza, October 2013.

Comment: The seven standardized protocols are being submitted for approval to the Ethics Review Committee of the World Health Organization.

Comment: The study group will need to indicate which IRB has approved the adapted protocol, including the date of ethical approval.

2.4.1 BENEFITS/RISKS FOR STUDY PARTICIPANTS

The primary benefit of this study is indirect in that the data collected in this study will help improve and guide efforts to prevent the spread of the virus and inform vaccination plans should a vaccine become available in the coming years.

All biological specimens will be collected in accordance with routine medical procedures and standards of practice. All risks associated with biological specimen collection will be explained in accordance with normal practice for the health care facility.

The collection of a small amount of venous blood in order to test for ZIKV exposure poses minimal risk to those participating in the study. All participants will be informed promptly of their individual results (e.g., if they have evidence of infection with ZIKV or any other relevant infection). Results of any testing are the property of each participant.

Comment: The implemented protocol and accompanying informed consent must explain the tests that will be performed on any samples collected, how the results of these tests will be used and how they will be delivered to the participants. This will likely depend on local IRB requirements.

Prevention of ZIKV infection and treatment following ZIKV testing will follow national/WHO guidelines, which may be updated. **World Health Organization Interim guidance** - Pregnancy management in the context of Zika virus infection (13 May 2016).

Comment: The study group will need to provide more information to study participants based on the local context and legal setting, as well as details of the counselling services that will be made available to study participants.

2.5 DATA COLLECTION AND MANAGEMENT

After informed consent is obtained from eligible study participants, a standardized study questionnaire will be administered to all study participants. Information to be collected from the **each study participant** (or parent/guardian in the case of children) includes:

- Background demographic information, including socioeconomic status, as indicated by wealth index
- Background medical history, background family medical history, and current medical condition, including vaccines received
- Known and potential risk factors (demographic, lifestyle, ecological factors, previous infection etc.) for ZIKV infection, including travel history, vector exposure and vector protection measures
- Basic physical examination by a trained health care worker
- Signs and symptoms of ZIKV infection
- Laboratory evaluations, specifically, confirmation of any ZIKV exposure and other relevant infections such as arboviruses (e.g., Dengue) and TORCHS infections [toxoplasmosis, other (e.g., varicella, etc.), rubella, cytomegalovirus, herpes, HIV, syphilis]

Comment: A standardized questionnaire has been developed specifically for this protocol in collaboration with ISARIC (International Severe Acute Respiratory and Emerging Infection Consortium). It contains the core data variables that should be collected from the study participants to address the objectives of this study. Further questions may be added at the discretion of the research group. The questionnaire is designed to be implemented by trained study personnel, without advanced or specialized medical degrees and can be found in Appendix B.

2.5.1 DATA MANAGEMENT

All data collected will be stored in password-protected databases. The password-protected databases will have patient-identifiable information attached such as name and address, and each patient will have an anonymized study ID. The database's location and responsibility will depend on national regulations and thus decided on a case-by-case basis. A password-protected copy of the de-identified/anonymized database (without name, address) will be sent for data analysis to the designated data manager(s).

Diagnostic test results will be securely transmitted to the center in charge of data centralization and analysis, which will then be responsible for making the tests results available to the study participants. Testing results will be conveyed to participants or to their primary care provider.

Patient identity will be protected and only aggregate summary data released publically (e.g., in the form of a peer-reviewed publication). Original data collection forms will be kept in locked storage in accordance with national regulations. An identification log will be implemented and will be kept in a secure, locked facility within the study country.

Comment: The study group will need to detail procedures for data management, protection and storage in the adaptation of the protocol.

2.6 SPECIMEN COLLECTION AND LABORATORY INVESTIGATIONS

2.6.1 SPECIMEN COLLECTION, STORAGE AND TRANSPORTATION

From **each study participant,** 7.5 mL of blood will be collected during enrollment according to standard procedures and labeled with a coded identification number that will also be recorded on the interview questionnaire.

All biological sampling collection will follow WHO guidelines in relation to treatment following ZIKV testing.

Any leftover samples will be stored after infectious testing is complete at [name of testing facility] with an identification number for possible additional testing for other infectious pathogens.

Informed consent (see Appendix A) will seek approval to store any residual specimens. If a participant does not provide consent to store the specimens, all specimens for that participant will be destroyed once testing for infectious disease pathogens has been completed.

Specimen collection: All collection tubes will be labeled with a coded identification number that will also be recorded on the interview questionnaire. Date and time of collection, location, and name of person collecting the specimen will be recorded.

Specimen storage and preservation: Specimen tubes will be stored temporarily on ice carried by the study teams until they can be transported to the laboratory.

- Keep refrigerated (2-8° C) if it is to be processed (or sent to a reference laboratory) within 48 hours.
- Keep frozen (-10 to -20°C) if it is to be processed after the first 48 hours or within 7 days.
- Keep frozen (-70 °C) if it is to be processed after a week. The sample can be preserved for extended periods.

If air transportation is needed, ship (insofar as possible) using triple packaging with dry ice within 48 hours. Or, at the very least, maintain the cold chain with cooling gels.

Specimen transportation: Transport of specimens within national borders should comply with applicable national regulations and international transport should comply with applicable international regulations. The original samples will be packed, labeled and marked (if dry ice is used), and documented as Category B.

Comment: At least two aliquots of sample should be made and at least one should be kept for future analysis. As such, specimens may remain in country and only aliquots may be sent to a reference lab, if necessary. This will depend on local IRB requirements.

2.6.2 LABORATORY PROCEDURES

Laboratory testing will be carried out in the country of the research institution collecting biological samples or in collaboration with an external laboratory partner as needed. At least two aliquots of sample will be made and at least one should be kept for future analysis. The principal tests described for ZIKV infection detection and differential diagnosis are listed in Table 1.

Comment: The list of the laboratory tests and the targeted pathogens provided below may be subject to modifications depending on the local laboratory capacities and circulating pathogens, and thereby needs to be considered on a study-by-study basis.

Comment: Yellow Fever virus (YFV) may be included in the list of pathogens to investigate in regions in which YFV is currently circulating.

Table 1: *List of the different biological testing to be performed on collected specimens*

Nature of specimen	Lab test	Targeted pathogens	Remarks
Serum	Serology: IgM and IgG	ZIKV, Chikungunya virus, Dengue virus, Yellow fever virus, West Nile virus	If positive result, use same sample for plaque-reduction neutralization test (PRNT)

Serological methods: Multiple serological assays may be needed to confirm seropositivity. Indeed, even if antibodies cross-reaction with other genetically related viruses is minimal during primary infection, sera of individuals with a previous history of infection from other flaviviruses (especially Dengue, Yellow Fever and West Nile) may cause cross-reactivity. Although neutralization by plaque reduction (PRNT) offers greater specificity in the detection of neutralizing antibodies (IgG), cross-reactions have also been documented. In fact, some patients with a previous history of infection by other flaviviruses have demonstrated up to a fourfold increase in neutralizing antibody titers when infected with ZIKV. Thus, primary screening should be performed by enzyme-linked immunosorbent assays, immunoassays or immunofluorescence assays and confirmation will need to include virus neutralization assay. Please see the latest WHO laboratory guidance for serologic assays for ZIKV.

Comment: These recommendations are subject to further updates whenever new, reliable diagnostic tests become available for clinical use.

3.0 SAMPLE SIZE, STUDY ENDPOINTS AND STATISTICAL ANALYSES

3.1 SAMPLE SIZE CONSIDERATIONS

Sample size calculations for this study are driven by assumptions related to seroprevalence. Knowledge on seroprevalence during past outbreaks is limited, but has been estimated for French Polynesia to be between 50-66% (Cauchemez et al, 2016) and 73% during the Yap outbreak (Duffy et al, 2009).

Table 2. *Suggested sample size of cohort, based on primary objectives*

Objective	**Outcome**	**Assumptions**	**Min. sample size**
Estimate the frequency of ZIKV seroprevalence among the general population in ZIKV-exposed areas and non ZIKV-exposed areas	Seroprevalence of antibodies to ZIKV in ZIKV-exposed areas and non ZIKV-exposed areas	Estimated seroprevalence in exposed populations of 50%; estimated seroprevalence in non-exposed populations of 10%; two sided significance level of 95; 80% power	500 individuals from ZIKV-exposed areas and 500 individuals from non ZIKV-exposed areas

Comment: The research group should aim to recruit more than the minimum sample size described above to account for overestimation of prevalence, absent data, damaged specimens etc. Ultimately, the final sample size will be determined by the local context and the choice of primary objectives. If additional primary objectives are used, the largest sample size should be used.

Comment: A serial cross-sectional study design (collection of sera at two time periods) could follow the completion of this cross sectional study. This would enable the cumulative incidence and/or the role of natural immunity to be determined.

3.2 STUDY OUTCOME MEASURES

Primary outcomes need to correspond to the primary objectives described above. Any secondary outcomes will need to be defined by the research group, as determined by the selection of secondary objectives.

Seropositivity will be based on the serologic assay used and the timing of the sera collection with respect to the circulation of ZIKV. Please see the latest WHO laboratory guidance for serologic assays for ZIKV.

Comment: A sample table has been provided below to guide the reporting of results related to primary outcomes.

	Zika exposed area (%)	ZIKV non-exposed area (%)	Entire cohort (%)
ZIKV antibodies + clinical signs of illness			
ZIKV antibodies, no clinical signs of illness			
No evidence of ZIKV antibodies			

According to WHO laboratory guidance for serologic assays for ZIKV.

3.3 STATISTICAL ANALYSES

Statistical tests, as appropriate, will be used to test for statistical differences, as they relate to the primary objectives.

Table 3. *Statistical analysis tests to be performed, based on primary objectives*

Objective	**Outcome**	**Statistical analysis**
Estimate the frequency of ZIKV seroprevalence among the general population in both ZIKV-exposed areas and non ZIKV-exposed areas	Seroprevalence of antibodies to ZIKV in ZIKV-exposed areas and non ZIKV-exposed areas	Comparison of seroprevalence in multiple populations, with possible stratification by study population characteristics if sample size permits
Identify risk factors and modifiable risk factors for ZIKV infection	Participant demographics, exposures to different risk factors	Descriptive epidemiology for each characteristics or exposure (mean, SD, median, interquartile range percentages for categorical variables)

Comment: Analysis involving antibody levels may require transformation (e.g. log transformation) before analysis, as the distribution of antibody data is usually positively skewed.

The seroprevalence of antibodies to the ZIKV virus can be determined for the overall study population and individual populations using ZIKV serologic results, as follows:

$$\textit{Seroprevalence}_{\textit{Population exp}} = \frac{\textit{Number of seropositive individuals in population 1 at a given time}}{\textit{Total number of subjects in population 1 at that same time}}$$

$$_{\textit{Population non-exp}} = \frac{\textit{Number of seropositive individuals in population 2 at a given time}}{\textit{Total number of subjects in population 2 at that same time}}$$

All numerical values (e.g. age) will be described using frequency distribution and summarized by presenting the median and interquartile range of the variable for each study population. If the distribution is normal and there is an adequate sample size, a parametric approach will be used for comparisons. Comparisons of means between exposed and non-exposed groups may also be conducted. Age, will also be categorized into age groups: <1 month, 1-23 months, 2 years to <5 years, 5-9 years, 10-19 years, 20-29 years, 30-39 years, 40-49 years, 50-59 years, 60+ years.

All risk factor variables (e.g. vector exposure) will be asked as a binary (yes/no) questions. Descriptive information in the form of numbers and percentages will be presented for individual level risk factor for each study population. All analyses of exposure variables will be stratified by age and gender. The risk factors will be analyzed by age and gender using t-tests or Wilcoxon rank-sum tests, as appropriate. For categorical variables, chi-square tests will be used to test whether these characteristics were distributed differently between age groups and study populations. Linear/multiple regression, and binary/multivariate logistic regressions can also be used to adjust for multiple risk factors, potential confounders, and test for interaction.

Comment: At the stage of data analysis, adjustments or analysis for biases may be required if the non-response rates are higher than adjusted at the time of estimating sample size.

4.0 REPORTING OF FINDINGS

Reports of the results of this study should follow the 'cross-sectional studies' checklist of the STROBE statement, and include sufficient information to permit pooling of data with similar studies.

Important information to report include (1) the number of participants recruited and (2) the number of confirmed ZIKV infections or the number of cases with serologic evidence of ZIKV infection.

It is also important to fully document the study design, including recruitment methods, the approach to determining ZIKV infection, the laboratory methods used and the outcome measurements.

Ideally, information should be collected in a standardized format and anonymized data should be shared among multiple groups running similar protocols.

5.0 COMPLEMENTARY STUDIES

This study protocol outlines methods to collect data to measure the seroprevalence of cross-reactive antibodies to ZIKV in the general population, and may also evaluate risk factors for infection among those with evidence of infection (seropositive subjects) compared to those without infection (seronegative subjects).

A serial cross-sectional study (collection of sera at two time periods) could follow the completion of this cross sectional study in order to determine cumulative incidence and/or the role of natural immunity.

Other protocols currently under development include:

- Case-control study to assess potential risk factors related to microcephaly including Zika virus infection
- Case-control study to assess potential risk factors related to Guillain-Barré Syndrome including Zika virus infection
- Prospective longitudinal cohort study of women and newborns exposed to Zika virus during the course of pregnancy
- Prospective longitudinal cohort study of newborns and infants born to mothers exposed to Zika virus during pregnancy
- Prospective longitudinal cohort study of Zika-infected patients to measure the persistence of Zika virus in body fluids
- Clinical characterization protocol for Zika virus infection in the context of co-circulating arboviruses

6.0 ACKNOWLEDGEMENTS

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Additional review has been provided by: Eric Ohuma (University of Oxford), Nathalie Jolly (Institut Pasteur), Samira Ouchhi (Institut Pasteur) Virginie Pirard (Institut Pasteur), and reviewers from World Health Organization Research Panel 2.

Comment: This list needs to reviewed, adding individuals and affiliations as appropriate.

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APPENDICES

Appendix A: Description of investigation and informed consent template

Appendix B: Standardized questionnaire/DRAFT undergoing review

APPENDIX A: PROPOSITION FORM FOR THE DESCRIPTION OF THE INVESTIGATION AND THE INFORMED CONSENT

This informed consent form was adapted from a study protocol developed by Centre for Clinical Investigation Antilles-Guyane Inserm 1424: 'Observational studies on the consequences of being infected by Zika virus while pregnant during the epidemic period in the French Overseas Departments in 2016.'

Comment: The language of this document is more technical than information for participants and informed consent forms. The text may therefore need to be adapted based on the local setting and the IRB requirements.

INFORMATION FOR THE PARTICIPANT

Dear Mr/Mrs/Ms/Miss,

We are inviting you to participate in the research study entitled:

Cross-sectional seroprevalence study of Zika virus infection in the general population

The study is being conducted by [_____ International sponsor], [_____ local investigator] and several international collaborators including [_____]

INFORMATION

This document is meant to provide you with the written information necessary to make a decision regarding your participation in the study. We ask that you read this document carefully. Do not hesitate to ask us if anything is unclear or if would like more information. You may take your time to think about your participation in this research. At the end of this document, if and when you accept to participate in the study, please sign and date the consent form in the indicated spaces.

CONSENT PROCESS

Your participation in this study is completely voluntary: you are free to accept or refuse to participate. If you decide to participate, you can withdraw your consent at any time, without any consequences, ill-feeling or prejudice.

GENERAL BACKGROUND AND RESEARCH OBJECTIVES

As you may be aware, the Zika virus has been circulating in the [region/country of study] since [general time of ZIKV introduction into study region]. You are being asked to participate in a study which aims to determine the frequency of Zika virus infection in the general population. Zika virus is usually transmitted to people by mosquitoes. Most people who are infected with Zika virus do not get sick, but some will have mild symptoms, including rash, headache, fever, joint pain, and red eyes.

The main objectives of this study are to:

- Estimate the frequency of Zika virus infection among the general population in both Zika-exposed areas and non Zika-exposed areas
- Identify risk factors and modifiable risk factors (exposures, behaviors, practices) for Zika infection
- Describe the spectrum of illness with Zika infection
- Quantify the proportion of asymptomatic and sub-clinical Zika infections

Comment: Describe in 1-2 sentences specific details about the location of the study, the number of participants, the other locations within your study that are conducting this research etc.

RESEARCH PROCESS

If you agree to participate in this study then you will be asked to answer questions about your health and daily life, such as the type of protection measures you use against mosquitoes. We also would like to draw blood through a needle in your arm. We would like approximately 7.5ml of blood (around than two teaspoons).

The samples collected from you will be tested for Zika virus and other pathogens known to cause the same type of illness and are also circulating in the area, such as Dengue virus, Chikungunya virus, etc. A medical doctor will council you on the results of your tests and inform you of any diagnosis of Zika or other pathogen immediately.

There is a risk that you experience some discomfort when we take your blood. A small bruise may also appear. Some people might feel lightheaded when they have their blood drawn. However, this is transient and does not require treatment or medical consultation.

RISKS AND BENEFITS OF YOUR PARTICIPATION

This research does not present any foreseeable risk for you; no procedure will be done on you that is not designed for the purpose of this study. You will receive no direct benefit from participating in this study. However, you will have access to information on the means of protection against mosquitoes and on other potential modes of Zika transmission by trained social and healthcare workers.

RESEARCH RESULTS

The main results of this research will be shared with national and international authorities, such as the World Health Organization. The results of this research may be presented in scientific conferences and publications. However, your personal data will not be identifiable in any way. All study data will be accessed by a small number of researchers within the study group and will be confidential through use of a specific coding system that will remove your first and last name and any other identifying information.

Comment: If the results of the study will be made available online and/or if there are specific details on how the participants can access this information, this should be added in this section.

GENETIC TESTING

Comment: in the event that the role of genetics in determining the severity of Zika virus infection needs to be investigated, a paragraph explaining the purpose of genetic testing, which samples will undergo genetic testing, and how the results of this testing will be used will need to be added.

CONFIDENTIALITY AND TREATMENT OF COMPUTERIZED DATA

Your data will need to be entered into an electronic database in order for us to analyze it and answer the questions of this study. Your medical data, and the data relating to your lifestyle and ethnic origins will be transmitted to your doctor or to persons working for the research group under strict protection to a small number of researchers within the study group, in [country of study] or overseas in other countries [insert other countries].

If, during the course of the study, you no longer wish to participate or you no longer wish your baby participates, the study group will seek your permission to keep the data contributed up to the point at which you withdraw from the study, or to destroy all data.

INFORMATION ON YOUR SAMPLES DURING AND AFTER THIS STUDY

If there are any 'left-over' samples, we would like to ask you to allow researchers to use these for other studies. What we mean is, if your samples are not completely used upon completion of this study, they could be stored and used for other research studies that are looking at Zika, or other viral infections that are transmitted by mosquitoes. In any future studies, your identity would remain confidential. The remaining samples will be stored at [name of national/designated laboratory] and could be given, without cost, to other teams doing private or public research, national or international.

At any time, and without consequence to your participation in the present study or to your medical care, you may withdraw your consent for the use of your samples for these other research studies that are looking at Zika, or other viral infections that are transmitted by mosquitoes. This can be done simply by contacting the health care professional who is supervising your participation in this study

Please let us know if we can answer any questions about the information above or about the study for which we are seeking your participation.

INFORMED CONSENT OF PARITICIPANTS

I, undersigned, _____ confirm that I have read and understood all the information presented to me, relative to my participation in this study which is entitled:

Cross-sectional seroprevalence study of Zika virus infection in the general population

This study has been described to me and the document 'Information for the participant' has been read to me by _____ and I have received answers for all the questions that I asked.

- ☐ I have read or orally received all the necessary information to understand the topic and enrollment process of the study.
- ☐ I was able to ask questions and received clear and adequate responses.
- ☐ I confirm my participation in this study, which includes responding to a questionnaire and allowing the taking of biological samples from me.
- ☐ I acknowledge that these samples may need to be shipped overseas.
- ☐ I understand that there are no predicted risks of my participation in this study.
- ☐ I have been advised that there is no financial incentive foreseen in this study.
- ☐ I agree to the storage of my samples for potential future studies on circulating pathogens or exposure to poisonous substances in the region.
- ☐ I am willing to be contacted at a later date, at which time further samples or questions may be requested. At this point, I am able to refuse or agree to participation.
- ☐ I understand that I can withdraw, at any moment, my consent to participate in this study, for whatever reason and without having to justify myself, and without incurring any consequence or prejudice. I must simply inform the health care professional in charge of this study.

Comment: Additional statements may be added to the informed consent checklist, such as:

- ☐ I agree to give access to the study investigators to my past and present medical records.

CONSENT RELATED TO PERSONAL DATA

I accept that my personal data will be recorded and computerised by a data manager for the purpose of this study.

I accept that my medical files may be looked at by appropriate persons implicated in this research study, all of whom will keep my identity confidential.

CONSENT RELATIVE TO THE USE OF MY BIOLOGICAL SAMPLES

I accept the use and storage of my biological samples as has been described by this research protocol.

I have been informed that my biological samples may be stored even after the end of the study period, in order to conduct further research on Zika virus infection or on other infections transmitted by mosquitos. Other research teams, private or public, national or international, may carry out this research. This authorisation will no longer be valid if I withdraw my consent during the study.

SIGNATURES

Study participant I freely and voluntarily accept to participate in the study that has been described to me.	
LAST NAME, First name:	Date: Signature:
Researcher I have accurately read or witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.	
LAST NAME, First name: Contact number:	Date: Signature:

Study participant (minor) I freely and voluntarily accept to participate in the study that has been described to me.	
LAST NAME, First name:	Date: Signature:
Witnessing adult I have witnessed the accurate reading of the assent form to the minor, and the minor has had the opportunity to ask questions. I confirm that the minor has given consent freely.	

LAST NAME, First name:	Date: Signature:
Researcher I have accurately read or witnessed the accurate reading of the assent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given assent freely.	
LAST NAME, First name: Contact number:	Date: Signature:

Parent/legal guardian of child participant I freely and voluntarily accept for my child to participate in the study that has been described to me.	
LAST NAME, First name (child):	
LAST NAME, First name (parent/legal guardian):	Date: Signature:
Researcher I have accurately read or witnessed the accurate reading of the assent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given assent freely.	
LAST NAME, First name: Contact number:	Date: Signature:

Comment: The last page of this document must have the signatures of the researcher and of the person being solicited and must be dated by the hand of the person who has consented in the spaces where indicated.

This information and consent document must be made in two original copies: one copy is to be given to the participant and one is to be kept for the required legal duration for research documents by the

health care professional in charge of the research, in the research locations at each regional site of the study.

APPENDIX B: STANDARDIZED QUESTIONNAIRE/DRAFT UNDERGOING REVIEW

Development of the draft questionnaire

This questionnaire has been designed specifically for the standardized cross-sectional seroprevalence study of ZIKV in the general population adapted from:

- Clinical report form of the case-control study protocol 'Assessment of the association of Zika virus infection and microcephaly' (Brazil Ministry of Health & US CDC)
- Clinical report form of a cohort study of pregnant women protocol 'Observational studies on the effects of having Zika virus infection while pregnant during the 2016 epidemic in the French Overseas Departments' (CIC Antilles Guyane, INSERM & Institut Pasteur)
- Clinical report form for the clinical characterization of newborns in the context of Zika (WHO/PAHO)

Purpose of the standardized questionnaire and instructions for its use

This questionnaire has been designed to collect the minimum amount of data from the study participants to address the objectives of this study. Further questions may be added at the discretion of the research group as determined by the financial and technical capacity of the study group and by the outbreak characteristics. The questionnaire is designed to be implemented by trained study personnel, without advanced or specialized medical degrees.

Comment: By using a standardized protocol, researchers can address many research objectives and will have the opportunity to collaborate with other research sites/countries conducting this same study. This may enable researchers to pool data to address the primary research question(s) of this protocol. However, to be able to pool data across studies, the adapted protocols and corresponding questionnaires must be aligned.

Comment: A serial cross-sectional study design (collection of sera at two or more time periods) could follow the completion of this cross sectional study in order to determine cumulative incidence and/or the role of natural immunity. In this case, this questionnaire can be repeated.

Instructions for completing questionnaire

When completing the sections of the questionnaire, please make sure that:

- The participant or consultee/guardian/representative has been given information about the study and the informed consent form has been completed and signed.
- The study ID codes have been assigned to the participant as per study protocol and guidelines.
- All information should be kept confidential at all times, and no identifiable information is recorded on the questionnaires.
- Participant's study ID and contact details are recorded on a separate contact list to allow later follow up. The contact forms must be kept separate from the questionnaires at all times and kept in a secure location.

General guidance

- The questionnaire is designed to collect data obtained through patient examination, through parent/guardian/representative interview (for minors), and review of hospital charts.
- Patient ID codes should be filled in on all pages of the questionnaire.
- Complete every line of every section, except for where the instructions say to skip a section based on certain responses.
- It is important to indicate when the answer to a particular question is not known. Please mark the 'Unknown' box if this is the case.

- Some sections have open areas where you can write additional information. To permit standardized data entry, please avoid writing additional information outside of these areas.
- Place an (X) when you choose the corresponding answer. To make corrections, strike through (----) the data you wish to delete and write the correct data above it. Please initial and date all corrections.
- Please keep all of the sheets for each study participant together e.g. with a staple or in a folder that is unique to the patient.
- Please contact us if we can help with any CRF completion questions, if you have comments, and to let us know that you are using the forms.
- We recommend writing clearly in black or blue ink, using BLOCK-CAPITAL LETTERS.
- Do not use abbreviations; write out each letter.
- Complete the heading on each page.
- Use standard medical language.
- Write only one character per box (|__|)
- Numerical values :
  - Align numerical values to the right
  - Do not add commas, they will already be present in the field if appropriate
  - Do no leave any space empty, enter a zero if necessary

Incorrect: |_2_|_1_|_| Correct: |_0_|_2_|_1_|

- If the response must be entered into closed tick-boxes, mark the box as follows:

For example: Yes ☐ No ☒

- Dates: enter the dates in the format Day-Month-Year (DD/MM/YYYY).
- In the case that data is missing or unknown, leave tick-boxes or other spaces empty and enter the codes that follow, as appropriate:

  - NA: Not applicable
  - ND: Not done
  - NK: Not known. Each error must be crossed-out with a single line (the original incorrect value must still be readable), then corrected to the side of the page, including the date and the initials of the person correcting the value, with a black pen. Do not use any 'white-out' or other correcting tool.

For the Primary Investigators for this the study, please contact us if we can help with any questionnaire completion questions, if you have comments, and to let us know that you are using the forms. Please contact Dr Maria Van Kerkhove (maria.van-kerkhove@pasteur.fr).

Disclaimer: This questionnaire is intended for use as a standardized document for the collection of clinical data in studies investigating the Zika virus. Responsibility for use of these questionnaires rests with the study investigators. The authors of the questionnaire accept no responsibility for the use of the questionnaire in an amended format nor for the use of the questionnaire outside its intended purpose.

Date of interview (DD/MM/YYYY): ____/____/_____

Interviewer: _____

IDENTIFICATION: STUDY PARTICIPANT

Study code	Center code	Participant code	Participant initials (surname/first name)
I___I	I___I	I___I___I___I___I	I___I___I

VERIFICATION OF ELIGIBILITY

INCLUSION CRITERIA	Yes	No
Any individual 18 years old or above, who gives informed consent	☐	☐
Any minor whose parents, legal representative or guardian can provide written informed consent.	☐	☐
EXCLUSION CRITERIA	**Yes**	**No**
Any individual who is unable or unwilling to give informed consent	☐	☐
Contraindication to venipuncture	☐	☐

If the eligibility criteria have been confirmed, the individual can be enrolled in the study

Date of inclusion (DD/MM/YYYY):	____ / ____ / _____
Name of site/clinic hospital:	
City/town:	
State:	
Country:	

Date of interview (DD/MM/YYYY): ____/____/_____

Interviewer: _____

5) DEMOGRAPHICS

Date of birth:	____ / ____ / _____
Area of residence: (Or, enter GPS coordinates):	_____ _ _ . _ _ _ _ _ S, _ _ _ . _ _ _ _ _ E
Maternal language	(Add check boxes here)
Social-professional category Comment: Add occupation/professional categories that are appropriate for the country implementing the study	☐ Student ☐ Farmer ☐ Artisan, merchant, business owner ☐ Highly qualified professional (management) ☐ Employee ☐ Labourer/factory worker ☐ Without profession ☐ Retired ☐ Does not wish to respond ☐ Other (specify): _____
Ethnicity	(Add check boxes according to national guidelines)

2) MEDICAL HISTORY

The following questions aim to collect information on any past and/or current medical conditions.

History of cardiovascular disease: - If yes, specify condition and duration:	☐ Yes ☐ No ☐ Unknown
High blood pressure: - If yes, specify duration:	☐ Yes ☐ No ☐ Unknown
High cholesterol: - If yes, specify type and duration**:**	☐ Yes ☐ No ☐ Unknown
History of stroke:	☐ Yes ☐ No ☐ Unknown

- If yes, specify date:	____ / ____ / _____
Diabetes: - If yes, specify type and duration**:**	☐ Yes ☐ No ☐ Unknown
Obesity:	☐ Yes ☐ No ☐ Unknown
Asthma:	☐ Yes ☐ No ☐ Unknown
COPD:	☐ Yes ☐ No ☐ Unknown
Kidney disease:	☐ Yes ☐ No ☐ Unknown
Liver disease:	☐ Yes ☐ No ☐ Unknown
Rheumatologic disease:	☐ Yes ☐ No ☐ Unknown
HIV infection:	☐ Yes ☐ No ☐ Unknown
Cancer: - If yes, specify type: - If yes, specify nature of treatment: If yes, specify duration of treatment:	☐ Yes ☐ No ☐ Unknown ____ / ____ /_____ - ____ / ____ /_____
Other medical history: If yes, specify condition and duration:	☐ Yes ☐ No ☐ Unknown
Surgical history: If yes, specify:	☐ Yes ☐ No ☐ Unknown
Blood transfusion: If yes, specify date:	☐ Yes ☐ No ☐ Unknown ____ / ____ /_____

3) EXPOSURES

Travel within your home country during the last six weeks - If yes, list locations, including dates (DD/MM/YYYY – DD/MM/YYYY):	☐ Yes ☐ No
Travel outside of your home country within the last six weeks? - If yes, list countries, including dates (DD/MM/YYYY – DD/MM/YYYY):	☐ Yes ☐ No
Type of residence during the last six weeks:	☐ Apartment ☐ House ☐ Other, specify: _____
Location of residence during the last six weeks:	☐ City/Urban ☐ Rural/Country-side ☐ Other, specify: _____
Air conditioning in residence during the last six weeks: (tick all that apply)	☐ Local air conditioning (at least 1 room) ☐ Fans ☐ None

Usual means of protection against mosquitoes	
Do you wear long trousers/long sleeves?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
Do you use a mosquito net while you sleep during the day or at night?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
Do you use essential oils to rid your home of mosquitos?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
Do you use window or door screens to keep mosquitos out of your home?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
Do you use mosquito repellent spray?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
Do you use insecticides to remove mosquito larvae from your home?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
Do you use other methods to rid you home of mosquitos?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always

- If yes, indicate here which methods you've used:	

Has anyone you know had a Zika virus infection?		**If yes, did this individual go to a health care clinic?**	**(DD/MM/YYYY)**
Husband/partner	☐Yes ☐ No	☐Yes ☐ No	_____
Children	☐Yes ☐ No	☐Yes ☐ No	_____
Neighbours	☐Yes ☐ No	☐Yes ☐ No	_____
Close friends/relative	☐Yes ☐ No	☐Yes ☐ No	_____
Other (specify): _____		☐Yes ☐ No	_____
In the last six weeks, has anyone in your household been sick at all?		**If yes, did this individual go to a health care clinic?**	**(DD/MM/YYYY)**
Spouse/partner	☐Yes ☐ No	☐Yes ☐ No	_____
Children	☐Yes ☐ No	☐Yes ☐ No	_____
Other (specify): _____		☐Yes ☐ No	_____
If yes, indicate symptoms : (tick all that apply)	☐ Fever ☐ Chills ☐ Nausea or vomiting ☐ Diarrhoea ☐ Muscle pains ☐ Joint pains ☐ Skin rash ☐ Abnormally red eyes ☐ Headache ☐ Pain behind eyes ☐Stiff neck ☐ Confusion ☐ Abdominal pain ☐ Coughing ☐ Runny nose ☐ Sore throat ☐ Calf pain ☐ Pruritus ☐ Other: Specify _____		

4) PHYSICAL EXAMINATION

Body weight:	_____ (kg)
Height:	_____ (cm)
Body temperature:	_____ (°C)
Respiratory rate:	_____ (kg)
Heart rate:	_____ (bpm)
Arterial blood pressure: Systolic/ Diastolic	_____ (mmHg)
Pulse:	_____ (bpm)
Pulse oximetry:	_____ (%)
Clinical characteristics indicative of infectious illness: - If yes, indicate symptoms: (tick all that apply)	☐ Yes ☐ No ☐ Unknown ☐ Fever ☐ Chills ☐ Nausea or vomiting ☐ Diarrhoea ☐ Muscle pains ☐ Joint pains ☐ Skin rash ☐ Headache ☐ Pain behind eyes ☐ Stiff neck ☐ Confusion ☐ Abdominal pain ☐ Coughing ☐ Runny nose ☐ Sore throat ☐ Calf pain ☐ Pruritus ☐ Bleeding ☐ Conjunctival hyperaemia ☐ Petechiae ☐ Limb swelling ☐ Other: specify _____
Other clinical symptoms: - If yes, specify:	☐ Yes ☐ No
In the past two weeks, have you experienced any clinical signs suggestive of infectious illness? - If yes, indicate symptoms : (tick all that apply) Date of onset of symptoms (DD/MM/YYYY):	☐ Yes ☐ No ☐ Unknown ☐ Fever ☐ Chills ☐ Nausea or vomiting ☐ Diarrhoea ☐ Muscle pains ☐ Joint pains ☐ Skin rash ☐ Headache ☐ Pain behind eyes ☐ Stiff neck ☐ Confusion ☐ Abdominal pain ☐ Coughing ☐ Runny nose ☐ Sore throat ☐ Calf pain ☐ Pruritus ☐ Bleeding ☐ Conjunctival hyperaemia ☐ Petechiae ☐ Limb swelling ☐ Other: specify _____ ____ / ____ /_____

5) LABORATORY EVALUATION FROM BLOOD SAMPLE

Pathogen	Date of collection	Type of test	Result
Zika virus	___ / ___ /____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Dengue virus	___ / ___ /____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Chikungunya virus	___ / ___ /____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Yellow Fever virus	___ / ___ /____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
West Nile virus	___ / ___ /____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
BVDV	___ / ___ /____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Other (specify): _____	___ / ___ /____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml

INTERVIEW COMPLETED BY

Name and role:			
Signature:		**Date** (DD/MM/YYYY)	**___ / ___ / ___**