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: *Prospective longitudinal cohort study of women and newborns exposed to Zika virus during the course of pregnancy* will be published as soon as the ethical review has been completed.

Standardized Protocol:

Prospective longitudinal cohort study of women and newborns exposed to Zika virus during the course of pregnancy

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Acknowledgements

This protocol outline is based on the pregnant women cohort study protocols generated by the Microcephaly Epidemic Research Group (MERG), the Centre d'Investigations Cliniques (CIC) Antilles-Guyane Inserm 1424 (i.e., Caribbean Cohort studies), the International Research Consortium of Dengue Risk Assessment, Management and Surveillance (IDAMS) and the ZIKA Cohort Jundiaí, São Paulo, Brazil.

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. Many individuals were involved in the content and revision of this protocol and are listed at the end of the protocol.

More information on CONSISE can be found on their website.

License

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.

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 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 prospective longitudinal cohort study, designed to collect data to estimate the risk of conditions related to a pathogen in a population. This standardized study protocol outlines methods to follow a cohort of pregnant women exposed to ZIKV during pregnancy in order to identify, describe and quantify the spectrum of abnormalities and/or outcomes, including microcephaly, in the fetuses and newborn infants born to women included in this cohort. The data collected from this standardized protocol will be used to refine and update recommendations for surveillance and case definitions for microcephaly, to help understand spread, severity and spectrum of the disease and to adapt public health measures, especially for pregnant women and couples planning a pregnancy.

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

characterization study). However, pregnant women should not be enrolled in multiple cohort studies of pregnant women and 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.

CONTENTS

LIST OF ABBREVIATIONS

BVDV	Bovine viral diarrhoea virus
CHIKV	Chikungunya virus
CIC	Centre d'Investigations Cliniques
CMV	Cytomegalovirus
CONSISE	Consortium for the Standardization of Influenza Seroepidemiology
CT	Computed tomography
DENV	Dengue virus
ELISA	Enzyme-linked immunosorbent assay
GPS	Global Positioning System
HELLP	Hemolysis, Elevated liver enzymes, Low platelet count
HIV	Human Immunodeficiency virus
HSV	Herpes Simplex virus
IDAMS	International Research Consortium of Dengue Risk Assessment, Management and Surveillance
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHR	International Health Regulations
IRB	Institutional Review Board
ISARIC	International Severe Acute Respiratory and Emerging Infection Consortium
LCMV	Lymphocytic choriomeningitis virus
MERG	Microcephaly Epidemic Research Group
MRN	Magnetic resonance neurography
NAb	Neutralizing antibody
NHS	National Health Service
NSAIDS	Nonsteroidal anti-inflammatory drugs
PCR	Polymerase chain reaction
PAHO	Pan American Health Organization
PRNT	Plaque-reduction neutralization test
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TORCHS	Toxoplasmosis, other (e.g. varicella), Rubella, Cytomegalovirus, Herpes, HIV, Syphilis
VZV	Varicella zoster virus
WHO	World Health Organization
WMA	World Medical Association
YFV	Yellow Fever virus
ZIKV	Zika virus

1.0 INTRODUCTION

In the months that have followed the WHO declaration of a Public Health Emergency of International Concern on 1st February 2016, increasing evidence of the association between exposure to ZIKV in pregnant women and microcephaly and other congenital defects in the fetus, has been published (Besnard, Eyrolle-Guignot et al. 2016, Brasil, Pereira et al. 2016, Broutet, Krauer et al. 2016, Cauchemez, Besnard et al. 2016, Driggers, Ho et al. 2016, Kleber de Oliveira, Cortez-Escalante et al. 2016, Mlakar, Korva et al. 2016, Musso and Gubler 2016, Schuler-Faccini, Ribeiro et al. 2016). ZIKV RNA has now been found in the brains of infants born with microcephaly as well as in the amniotic fluid of mothers exposed to ZIKV during pregnancy (Calvet, Aguiar et al. 2016, Oliveira Melo, Malinger et al. 2016). This implies that, as has been seen with other infections (i.e., TORCHS infections - toxoplasmosis, other (e.g., varicella, etc.), rubella, cytomegalovirus, herpes, HIV, syphilis) which can be transmitted mother-to-child and which cause congenital defects such as microcephaly, there is a range of possible effects, in addition to microcephaly, due to ZIKV exposure during pregnancy (Jones, Lopez et al. 2003, Naing, Scott et al. 2016, Yazigi, De Pecoulas et al. 2016).

Aside from microcephaly, various other complications in newborns, such as brainstem dysfunction and severe cerebral lesions, have been reported with ZIKV exposure *in utero* (Besnard, Eyrolle-Guignot et al. 2016, Brasil, Pereira et al. 2016, Broutet, Krauer et al. 2016, Driggers, Ho et al. 2016, Musso and Gubler 2016). However, the spectrum of associated adverse pregnancy outcomes and congenital defects has not yet been clearly described or quantified. Furthermore, limited evidence suggests the timing (trimester) of ZIKV infection in the mother has implications on the incidence of congenital defects (Cauchemez, Besnard et al. 2016). The timing of the appearance of congenital defects by gestational age has yet to be described.

The following standardized prospective cohort study protocol outlines methods to follow pregnant women who have had exposure, or potential exposure, to ZIKV during their pregnancy. This study will address the following public health questions:

1. What is the clinical presentation spectrum of ZIKV infection in pregnant women?
2. What is the absolute risk of microcephaly and other birth defects by gestational age, rash, viremia, and other co-factors?
3. What are the characteristics, grade of neurological impairment, evolution, complications, and mortality of newborns born to mothers with ZIKV infection?

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 study will be used to refine and update recommendations for surveillance and case definitions for microcephaly, to help understand spread, severity and spectrum of the disease and to adapt public health measures, especially for pregnant women and couples planning a pregnancy.

The **primary objectives** of this study are to:

- Measure the incidence of ZIKV infection in pregnant women
- Describe the clinical spectrum of ZIKV infection in pregnant women

- Identify, describe and quantify the spectrum of abnormalities and/or outcomes, including microcephaly, in the fetuses and newborn infants born to women included in this cohort:
    - Estimate the risk of abnormalities or outcomes in the fetuses/newborn of women infected with ZIKV compared to women not infected with ZIKV
    - Estimate the relative risk of complications or abnormalities in pregnant women infected with ZIKV compared to women not infected with ZIKV
- Measure the association between timing (trimester) of ZIKV infection in the mother and resulting frequency of abnormalities in the fetus
- Describe the timing of appearance of congenital abnormalities in the fetus

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

- Describe cohort of ZIKV infected women
- Quantify the proportion of asymptomatic and sub-clinical ZIKV infections and evaluate risk factors for infection.
- Compare the risk of abnormalities and/or outcomes between ZIKV infected women with symptoms and ZIKV infected women without symptoms

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

Comment: Technical, financial or capacity limitations in country may mean that including all pregnant women in the cohort may not be feasible. In this case, a cohort study of symptomatic pregnant women may be conducted. However, this study design may introduce selection bias, may face difficulties related to the definition of 'symptomatic ZIKV infection' and will be measuring different outcomes to that which is described in this protocol. This will exclude the revised study from inclusion in any aggregate/pooled analysis.

Equally, these technical, financial or capacity limitations may also mean that achieving the required sample size calculated below may not be feasible. However, this study design is designed so that smaller studies, each of which have followed the methodology described below, may be aggregated in order to achieve an overall pooled sample size with sufficient statistical power.

It is important to note that this protocol is designed to describe the core data variables 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: Although this cohort of pregnant women protocol is a standalone protocol, it is recommended that the implementation of this protocol be followed by the implementation of a cohort study of newborns born to women exposed to ZIKV.

2.0 STUDY PROCEDURES

Overview: This is a prospective longitudinal cohort study that follows pregnant women at risk of ZIKV infection during pregnancy from the time of enrollment until childbirth or end of pregnancy. Infants born to mothers enrolled in the cohort will be followed during the neonatal period.

The figure below presents the design of the cohort study, detailing the points of data collection from the pregnant women recruited into the study, including data about the outcome of the pregnancy:

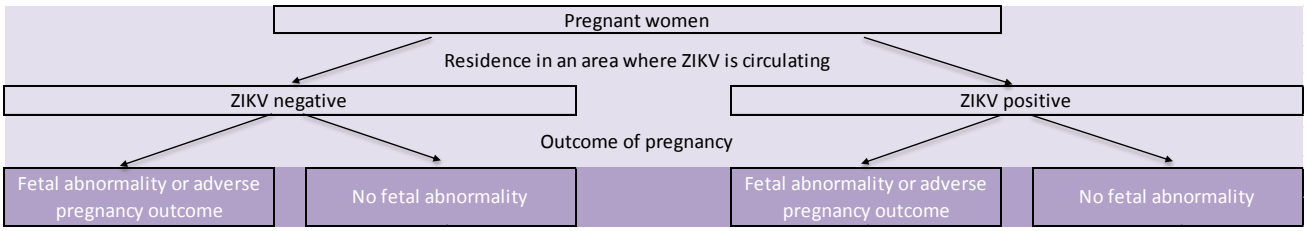

Figure 1: *Study design of prospective longitudinal cohort study of women and newborns exposed to Zika virus during the course of pregnancy*

Comment: Although this cohort of pregnant women protocol is a standalone standardized protocol, it is recommended that the implementation of this protocol be followed by the implementation of a cohort study of newborns born to women exposed to ZIKV.

2.1 STUDY SETTINGS

2.1.1 TIMING OF STUDY

Ideally, this study should be implemented in areas with an ongoing ZIKV outbreak. This will increase the chances of including pregnant women with ZIKV infection and identifying fetal abnormalities. However, this should not exclude this study from being conducted in areas in which the disease is well established.

2.1.2 STUDY AREA

The study design must clearly define the catchment area of the study population, the travel history of the participants and, if possible, any vector control interventions implemented in the catchment area.

2.2 SELECTION AND RECRUITMENT OF STUDY PARTICIPANTS

2.2.1 STUDY POPULATION

Selection of participants: This study will include women who have conceived in the study region, who have a pregnancy overlap with a ZIKV epidemic period. Women will be recruited at any stage of pregnancy, but, ideally, enrollment will occur as early as possible during the pregnancy. Such recruitment will depend on local surveillance capacities and health services.

Recruitment of pregnant women is most feasible in:

- Antenatal clinics or during antenatal visits at health facilities, or through community health workers attended by women from the beginning of pregnancy. At this moment, women could be sensitized to the study and enrolled.

Comment: Efforts should be made by the study group to recruit all pregnant women within the geographic scope of the study.

Comment: For recruitment, it is strongly recommended that women be enrolled early in their pregnancy, that is, during the first and second trimesters. However, enrollment of women in the third trimester should not be entirely excluded. If women are enrolled during their third trimester, although the sample size is increased, the study will not increase in statistical power, as women infected in third trimester are less likely to have fetal malformations.

2.2.2 PARTICIPANT FOLLOW-UP SCHEDULE

Recruitment and baseline visit: Recruitment of pregnant women should take place as soon as possible once the pregnancy has begun. Ideally, recruitment of pregnant women will occur at their first antenatal care visit in health care facilities in the study region.

Follow-up: Follow-up visits will occur at least once per trimester from the time of enrollment until the time of the pregnancy outcome (e.g. 3, 6, 9 months). As much as possible, visits will follow the local standard of care, during regular clinical antenatal visits and ultrasound examinations that follow enrollment.

Comment: Ideally, these follow-up visits will occur more frequently (e.g. once per month). If samples are collected more frequently (e.g. monthly, weekly), the study will be able to measure the association between gestational age at ZIKV infection in the mother and resulting frequency of abnormalities in the fetus

At birth: The outcome of the birth will be studied and newborns will be followed for a minimum of 28 days following birth.

Comment: Ideally, this cohort study of pregnant women will be followed by a cohort study of the newborn infants of the same pregnant women following children for at least two years. However, if the research group cannot implement this second cohort study, the minimum follow-up period to determine the presence of any abnormalities in the newborn, should be **28 days.**

2.2.3 ELIGIBILITY CRITERIA

- **Inclusion criteria:** Any woman, including minors, who becomes pregnant during, or has any overlap of pregnancy with the ZIKV epidemic period, in the defined study area, regardless of the presence of symptoms related to ZIKV infection.

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

Comment: Potential exclusion of any pregnant woman with confirmed ZIKV infection prior to pregnancy.

2.2.4 INFORMED CONSENT

Written informed consent will be collected from all study participants. Written informed assent from a parent or legal guardian will be collected for all minors participating in the study.

During the first interview with the pregnant woman, the purpose of the study will be explained and written informed consent will obtained from the participant upon enrollment into the study by a trained member of the investigation team. Each study participant will be informed that her participation is voluntary and that 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 differently by the withdrawing participant.

Informed consent will seek approval to collect samples from pregnant women and the outcome of the pregnancy 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 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. Informed consent will also seek approval to study the outcome of the pregnancy. This will address 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: This may need to be addressed in a second informed consent form, signed prior to delivery. This will likely depend on local IRB requirements.

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.

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.

Information for participant and informed consent form template for the pregnant woman and her newborn can be found in Appendix A.

2.2.5 INCENTIVES TO PARTICIPATE AND COMPENSATION

The primary benefit of this study is the extended medical care and intensified (i.e., beyond routine) follow-up of women with potential ZIKV infection, which will allow for timely detection of any abnormality or risk

and for appropriate decision-making. All study participants will also be provided by trained social and healthcare workers with additional information on means of protection against ZIKV vectors, on other potential modes of ZIKV transmission and on the risk of microcephaly.

The possibility to offer financial compensation (e.g., for expenses to attend medical visits) will depend on the context of the study and local policies and should be determined on a study-by-study 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.

2.2.6 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.

2.3 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 comment 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.

2.3.1 BENEFITS/RISKS FOR STUDY SUBJECTS

The primary benefit of this study is the extended medical care and intensified (i.e., beyond routine) follow-up of women with potential ZIKV infection, which will allow for timely detection of any abnormality or risk and for appropriate decision-making.

All biological specimens will be collected in accordance with routine medical procedures and will follow normal 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 and urine in order to test for ZIKV infection during each study visit poses minimal risk to the women participating in the study. Mothers will be informed 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 and should be provided to each participant as promptly as possible.

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):

"In the event of negative ZIKV test, information on protection from ZIKV infection will be provided.

In the event of positive ZIKV infection, the woman – and her partner, if she wishes – should receive accurate and evidence-based information on the potential impact of ZIKV infection on her pregnancy [likelihood of any abnormality related to ZIKV infection].

In the event of a suspected malformation in the fetus, the woman – and her partner, if she wishes – should receive individualized counseling and care. Depending on the severity and certainty of the fetal abnormalities and associated prognosis, this could range from specialized antenatal care and serial ultrasound follow-up to monitor any progression of the abnormalities to a discussion of the potential next steps in managing the pregnancy. It is important to ensure that an affected pregnant woman receives accurate and evidence-based information on the prognosis of the identified abnormalities. The woman – and her partner if she wishes – should be offered non-directive counseling so that she, in consultation with her health-care provider, can make a fully informed choice about the next steps in the management of her pregnancy.

Women who carry their pregnancy to term must receive appropriate care and support to manage anxiety, stress and the birth environment. Plans for care and management of the baby soon after birth should be discussed with the parents during the pregnancy, in consultation with a pediatrician or pediatric neurologist where available.

Women who wish to discontinue their pregnancy should receive accurate information about the options to the full extent of the law including harm reduction where the care desired is not readily available.

All women whatever their individual choices with respect to their pregnancies must be treated with respect and dignity."

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 women participating in this study.

2.4 DATA COLLECTION AND MANAGEMENT

After informed consent is obtained from eligible pregnant women, a standardized study questionnaire will be administered to all study participants. At enrollment, information to be collected from the **pregnant woman** includes:

- Background demographic information, including socioeconomic status, as indicated by wealth index
- Information on the pregnancy, including any ultrasound details collected up until the point of enrollment, if feasible and following national guidelines
- Known and potential risk factors (demographic, lifestyle, ecological factors, etc.) for congenital defects
- Background medical history, including previous pregnancy history and outcomes, background family medical history, and current medical condition, including vaccines received
- Signs and symptoms of ZIKV infection, including details and timing in relation to pregnancy/gestational age
- Laboratory evaluations, specifically, confirmation of ZIKV infection and other relevant infections such as arboviruses (e.g., Dengue) and TORCHS infections [toxoplasmosis, other (e.g., varicella, etc.), rubella, cytomegalovirus, herpes, HIV, syphilis]

At each of the follow-up visits throughout and until the end of pregnancy, the following information will be collected from the **pregnant woman**:

- Clinical data, including any treatments taken or given during pregnancy
- Signs and symptoms of ZIKV or other relevant infections (e.g., arboviruses, TORCHS [toxoplasmosis, other (e.g., varicella, etc.), rubella, cytomegalovirus, herpes, HIV, syphilis], including details and timing in relation to gestational age
- Laboratory evaluations, including testing for incidence of ZIKV and other relevant infections (e.g., arboviruses, TORCHS [toxoplasmosis, other (e.g., varicella, etc.), rubella, cytomegalovirus, herpes, HIV, syphilis]
- Basic (and advanced, when appropriate) ultrasounds conducted by a trained professional, if feasible and following national guidelines
- Details and timing of any pregnancy complications
- Details of any household or close contact with ZIKV infection
- Known and potential risk factors (demographic, lifestyle, ecological factors, etc.) for congenital defects

Comment: Conducting ultrasounds throughout the pregnancy enables the study group to measure the association between timing (trimester) of ZIKV infection in the mother and resulting frequency of abnormalities in the fetus. However, if ultrasounds are to be conducted as part of the study, they need to be standardized.

At the end of the pregnancy, the following information will be collected from the **pregnant woman**:

- Hair sample to screen for toxicants (~~ie~~ e.g. cocaine, lead, mercury or pesticide metabolites such as organophophates or carbamates)

At the end of the pregnancy, the following information will be collected about the **outcome of the pregnancy**:

- Outcome of the pregnancy (e.g., live birth, miscarriage, stillbirth, induced abortion)
- Basic (and advanced, when appropriate) ultrasounds conducted by a trained professional, according to national guidelines
- Signs and symptoms of ZIKV or other relevant infections (e.g., arboviruses, TORCHS~~-~~ [toxoplasmosis, other (e.g., varicella, etc.), rubella, cytomegalovirus, herpes, HIV, syphilis], including details and timing in relation to gestational age
- Laboratory evaluations, including testing for incidence of ZIKV and other relevant infections (e.g., arboviruses, TORCHS [toxoplasmosis, other (e.g., varicella, etc.), rubella, cytomegalovirus, herpes, HIV, syphilis]

Comment: A standardized questionnaire, specific to this protocol, can be found in Appendix B and has been developed by the Institut Pasteur, ISARIC, CONSISE, WHO and partners, adapted from:

- ISARIC (International Severe Acute Respiratory and Emerging Infection Consortium) Case Report Forms
- 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.'
- International Research Consortium of Dengue Risk Assessment, Management and Surveillance (IDAMS)

The questionnaire 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.

2.4.1 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.

2.5 SPECIMEN COLLECTION AND LABORATORY INVESTIGATIONS

2.5.1 SPECIMEN COLLECTION, STORAGE AND TRANSPORTATION

From **pregnant women**: up to 7.5 mL of blood and 1.0 mL of urine will be collected at enrollment in separate serum tubes according to standard procedures and labeled with a coded identification number that will also be recorded on the interview questionnaire.

From **outcome of pregnancy**: a range of biological samples will be collected according to the ISARIC/WHO/partners newborn algorithm found in Appendix C. This will also include a swab of amniotic fluid at delivery and a cranial ultrasound at birth.

Comment: Newborn urine sample is a bagged urine sample (rather than catheterized). This prevents unnecessary painful procedure for the newborn and increases the likelihood of sample collection.

Comment: In the case of a miscarriage, a stillbirth or an induced abortion, a post-mortem physical examination must be performed, and fetal and placental tissue samples should be collected and stored at -80°C for further analysis.

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

Specimen collection: All collection tubes will be labeled with a coded identification number that will also be recorded on the interview questionnaire. 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:

- Refrigerated (2-8° C) if it is to be processed (or sent to a reference laboratory) within 48 hours.
- Frozen (-10 to -20°C) if it is to be processed after the first 48 hours, but within 7 days.
- 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.

2.5.2 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 will be kept for future analysis. The principal tests described for ZIKV infection detection and differential diagnosis are listed in Table 1. Extended lab testing algorithms for mothers and newborns are included in Appendix C.

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 thus 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 tests to be performed on collected specimens*

Nature of specimen	Lab test	Targeted pathogen	Remarks
Blood/Serum at baseline and follow-up	Real-time RT-PCR Serology: IgM and IgG	ZIKV, chikungunya, Dengue, CMV, LCMV, VZV, HSV toxoplasmosis, rubella	If positive result, use same sample for plaque-reduction neutralization test (PRNT)
Urine at baseline and follow-up	Real-time RT-PCR	ZIKV (other pathogens may be tested)	
Amniotic fluid (taken at time of delivery)	Real-time RT-PCR	ZIKV, chikungunya, dengue, CMV, LCMV, VZV, HSV toxoplasmosis, rubella	

Comment: Extended lab testing algorithms for mothers and newborns are included in Appendix C.

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 shown 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.

Molecular methods: The method of choice to detect and quantify the presence of ZIKV particles in body fluids is real-time RT-PCR. Multiple primers specific for ZIKV have been designed by research teams and diagnostic laboratories (see Appendix D for examples of these primers). Commercial kits are also available, but for research use only (Musso & Gubler, 2016). Optimal standardization between laboratories has not yet been achieved. As the choice of primers may depend on the genetic diversity of currently circulating ZIKV strains, adaptation may be required on a study-by-study basis.

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

3.0 STUDY ENDPOINTS AND STATISTICAL ANALYSES

3.1 SAMPLE SIZE CONSIDERATIONS

Sample size calculations for this study are driven by the assumptions related to absolute risk and the assumptions related to seroconversion rate – a proxy for ZIKV exposure – during pregnancy. Assuming a moderate risk of microcephaly among women exposed to ZIKV (1-10%, based on estimates from Brazil and French Polynesia – Brasil, Pereira et al. 2016, Cauchemez, Besnard et al. 2016) and a low background risk of microcephaly (0.02-0.03%), a sample size of 12,000 pregnant women will need to be followed in this study.

This number accounts for loss to follow-up and is conservative considering that the risk for any congenital abnormality that may be associated with ZIKV infection is likely to be higher (e.g., 20-30%) (Brasil, Pereira et al. 2016). This number will therefore allow for significant detection of relative risks of 20 or higher with a power of 0.80 and a two-sided alpha of 0.05.

Table 2: *Suggested sample sizes of cohort, based on primary objectives, outcome and assumptions, embedded in the calculation*

Objective	**Outcome**	**Assumptions**	**Sample size**
1. Measure the incidence of ZIKV infection in pregnant women	Incidence of ZIKV in pregnant women	Incidence of ZIKV remains constant during the study period	500
2. Describe the clinical spectrum of ZIKV infection in pregnant women	Clinical signs of ZIKV infection in pregnant women		
3. Identify, describe and quantify the spectrum of abnormalities and/or outcomes, including microcephaly, in the fetuses and newborn infants born to women included in this cohort: • Estimate the risk of abnormalities or outcomes in the fetuses/newborn • Estimate the relative risk of abnormalities in pregnant women infected with ZIKV compared to women not infected with ZIKV	Abnormalities in newborn, outcome of pregnancy (low birth weight, neonatal mortality, preterm birth, stillbirth), maternal complications	Low background risk of microcephaly (0.02-0.03%)	12,000
4. Measure the association between timing (trimester) of ZIKV infection in the mother and resulting frequency of abnormalities in the fetus	Abnormalities in the fetus	Reliable and accurate dating of pregnancy	

5. Describe the timing of appearance of congenital abnormalities in the fetus	Congenital abnormalities in the fetus		

Comment: Ultimately, the final sample size will be determined by the local context and the choice of primary objectives. If multiple primary objectives are used, the largest sample size, as described in the table above, should be used.

Comment: The estimated sample size of 12,000 is estimated to achieve the primary objectives of the study. Researchers should not be discouraged by this large number. By using a standardized protocol, the researchers can address many research objectives (which require a smaller sample size) and will have the opportunity to collaborate with other research sites/countries conducting this same study and potentially 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.

3.2 STUDY OUTCOME MEASURES

The following primary outcomes 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.

WHO interim case definitions for ZIKV diseases (WHO 12 Feb 2016)

Suspected case:
A person presenting with rash and/or fever and at least one of the following signs or symptoms:

- arthralgia; or
- arthritis; or
- conjunctivitis (non-purulent/hyperaemic).

Probable case:
A suspected case with presence of IgM antibody against ZIKV [1], and an epidemiological link [2]
[1] With no evidence of infection with other flaviviruses
[2] Contact with a confirmed case, or a history of residing in or travelling to an area with local transmission of ZIKV within two weeks prior to onset of symptoms.

Confirmed case:

- A person with laboratory confirmation of recent ZIKV infection:
- Presence of ZIKV RNA or antigen in serum or other samples (e.g. saliva, tissues, urine, whole blood); or
- IgM antibody against ZIKV positive and PRNT90 for ZIKV with titer ≥20 and ZIKV PRNT90 titer ratio ≥ 4 compared to other flaviviruses; and exclusion of other flaviviruses

Comment: Additional classifications may be used, but they must be clearly defined.

Primary outcome 1: Number of infected women in cohort (lab and clinical confirmed case)/ total number of enrolled women in cohort

- Number of laboratory confirmed infections
- Number of probable infections

Comment: For reporting, follow WHO definition, which may be updated. Other case classifications may be used, but must be clearly defined in the protocol.

Primary outcome 2: Frequency of signs and symptoms of ZIKV infection

Comment: A sample table has been provided below to guide the user of this standardized protocol for reporting results related to primary outcome 2.

Signs and symptoms of ZIKV infection	Entire cohort	ZIKV positive (laboratory confirmed)	ZIKV probable	ZIKV negative
Rash				
Fever				
Conjunctivitis (non-purulent/hyperaemic)				
Arthralgia				
Myalgia				
Peri-articular edema …				

Primary outcome 3: Abnormalities in newborn, outcome of pregnancy (low birth weight, neonatal mortality, preterm birth, stillbirth), maternal complications

Comment: A sample table has been provided below to guide the user of this standardized protocol for reporting results related to primary outcome 3.

Fetal abnormality	Entire cohort	ZIKV positive (mother - laboratory confirmed)	ZIKV probable	ZIKV negative
Microcephaly				
Facial disproportion				
Hearing and visual impairments				
Dysphagia				
Calcifications				
Low birth weight				
…				

Pregnancy outcome	Entire cohort	ZIKV positive (mother - laboratory confirmed)	ZIKV probable	ZIKV negative
Normal birth				
Stillbirth				
Miscarriage				
Abortion				

Obstetric complications	Entire cohort	ZIKV positive (mother - laboratory confirmed)	ZIKV probable	ZIKV negative
Pre-eclampsia/ Eclampsia				
HELLP syndrome				
Gestational diabetes				
Intrauterine growth restriction				
Hemorrhaging				
Pregnancy-induced hypertension				
…				

Comment: Additional abnormalities, outcomes and/or complications can be added by the research group and should be added, as informed by ongoing studies on ZIKV infection and associated conditions

Primary outcomes 4 and 5: (Congenital) abnormalities in the fetus

Comment: A sample table has been provided below to guide the user of this standardized protocol for reporting results related to primary outcomes 4 and 5.

Appearance of abnormality in the fetus	Entire cohort	ZIKV positive (mother - laboratory confirmed)	ZIKV probable	ZIKV negative
Week 1 to week 13 + 6 days				
Week 14 to week 27 + 6 days				
Week 28 to week 40+				

Comment: This table allows for risks to be stratified by trimester, but equally, these risks may also be signs and symptoms (if sample size allows)

3.3 STATISTICAL ANALYSES

Statistical tests, as appropriate, will be used to test for statistical differences and describe 95% confidence intervals, as they relate to each of the primary objectives.

Objective	Outcome	Assumptions	Statistical analysis
1. Measure the incidence of ZIKV infection in pregnant women	Incidence of ZIKV in pregnant women	Incidence of ZIKV remains constant during the study period	Calculate incidence risk: Number of ZIKV cases/Total cohort Cumulative risk and incidence rates

2. Describe the clinical spectrum of ZIKV infection in pregnant women	Clinical signs of ZIKV infection in pregnant women		Mean, median, range for each clinical condition Chi-square tests for association
3. Identify, describe and quantify the spectrum of abnormalities and/or outcomes, including microcephaly, in the fetuses and newborn infants born to women included in this cohort: • Estimate the risk of abnormalities or outcomes in the fetuses/newborn • Estimate the relative risk of abnormalities in pregnant women infected with ZIKV compared to women not infected with ZIKV	Abnormalities in newborn, outcome of pregnancy (low birth weight, neonatal mortality, preterm birth, stillbirth), maternal complications		Odds ratio for abnormality/outcome: $\frac{OR-1}{OR} * Pe$ where *Pe* is the proportion of cases with ZIKV infection.
4. Measure the association between timing (trimester) of ZIKV infection in the mother and resulting frequency of abnormalities in the fetus.	Abnormalities in the fetus		Stratified analysis by trimester using logistic regression
5. Describe the timing of appearance of congenital abnormalities in the fetus	Congenital abnormalities in the fetus		Mean, median, range of timing of abnormality Non-linear regression modelling

4.0 REPORTING OF FINDINGS

Reports of the results of this study should follow the 'cohort 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 pregnant women recruited (2) baseline characteristics of the cohort and (3) 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 would be collected in a standardized format and anonymized data would be shared among multiple groups running similar protocols.

5.0 COMPLEMENTARY STUDIES

This protocol addresses specific questions relative to the potential association between ZIKV infection and congenital abnormalities in the fetus. However, additional aspects of ZIKV infection during pregnancy may be investigated depending on the study context. Complementary studies may therefore be considered in association with this protocol.

Additional standardized protocols for ZIKV are available and include:

- Case-control study to assess potential risk factors related to Guillain-Barré Syndrome including Zika virus infection
- Case-control study to assess potential risk factors related to microcephaly including Zika virus infection during 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
- Cross-sectional seroprevalence study of Zika virus infection in the general population
- Clinical characterization protocol for Zika virus infection in the context of co-circulating arboviruses

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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 C: Biological sampling algorithms

Appendix D: List of Published Primers for Detection and Quantification of Zika Virus by Real-time RT-PCR (Cao-Lormeau, Blake et al. 2016)

APPENDIX A: DESCRIPTION OF INVESTIGATION AND INFORMED CONSENT TEMPLATE

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 sheets and informed consent forms. The text will therefore need to be adapted based on the local setting and the IRB requirements.

Part I: to be completed prior to enrollment

INFORMATION FOR THE PARTICIPANT

Dear Mrs/Ms/Miss,

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

Prospective longitudinal cohort study of women and newborns exposed to ZIKV during the course of pregnancy

The study is being conducted by _____, the International Sponsor, _____, the 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. Please do not hesitate to ask us, the health-care professional taking care of you, any questions if anything is unclear or if you would like more information. You may take your time to think about and consider your participation in this research, and discuss with your doctor and your close family and friends. At the end of this document, if and when you accept to participate in the study, the health-care professional taking care of you will ask you to fill in, 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 [region of study] since [general time of ZIKV introduction into study region]. You are being asked to participate in a study which aims to understand the role of the infection with Zika virus during pregnancy on you and your unborn child. 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 or muscle pain, or red eyes.

It is believed that infection with Zika during pregnancy may harm the unborn child, sometimes causing microcephaly (a small head size in the fetus) or other congenital abnormalities. How often this occurs and

how the Zika virus affects the development of the fetus is not clearly understood. In this study, which we are asking for your consent to participate in, we are trying to understand these main questions.

The main objectives of this research study are to:

- Measure the incidence of Zika virus infection in pregnant women, that is, how many women enrolled in the study were infected with Zika
- Describe the clinical spectrum of Zika virus infection in pregnant women, that is, what symptoms or complications during pregnancy if any, did the women enrolled in the study experience
- Identify, describe and quantify the spectrum of abnormalities and/or outcomes, including microcephaly, in the fetuses and newborn infants born to women included in this cohort:
    - Estimate the risk of abnormalities or outcomes in the fetuses/newborn in pregnant women with Zika compared to pregnant women without Zika
    - Estimate the relative risk of complications or abnormalities in pregnant women infected with Zika compared to women not infected with Zika
- Measure the association between timing (trimester) of Zika infection in the mother and resulting frequency of abnormalities in the fetus, that is, is there a difference in the timing of infection and the outcome of the pregnancy
- Describe the timing of appearance of congenital abnormalities in the unborn baby
- Quantify the proportion of asymptomatic and sub-clinical ZIKV infections, that is, quantify how many infections in the pregnant women did not result in symptoms

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

RESEARCH PROCESS

If you agree to participate in this study, 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 will ask for access to your past and present medical records. We also would like to draw approximately 7.5ml of blood (less than two teaspoons) through a needle in your arm and collect a urine sample. This blood test will be repeated every three months, along with ultrasound scans and a clinical examination until the end of pregnancy. A medical doctor will inform you of any diagnosis of infection with Zika or other pathogen immediately and also council you on the results of your tests.

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.

At the time of delivery, we will ask to take a sample of amniotic fluid, obtained by swabbing the head of the baby. Following delivery, you will have approximately 7.5ml (less than 2 teaspoons) of blood drawn by a trained medical person and up to 3 ml (approximately ½ teaspoon) will also be collected from your baby. The samples collected from you and your baby will be tested for Zika virus and other pathogens known to cause congenital abnormalities if infection occurs during your pregnancy (for example, rubella, toxoplasmosis and cytomegalovirus) and other pathogens which are known to cause similar types of illness and to circulate in the area (Dengue virus, chikungunya virus, etc.)

Comment: Consent to collect blood samples from mother and the outcome of the pregnancy may need to be addressed in a second informed consent form, signed prior to delivery. This will likely depend on local IRB requirements.

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. The benefit of participating in this study is the extended medical care and intensified (i.e., beyond routine) follow-up of women with potential Zika infection, which will allow for timely detection of any abnormality or risk and for appropriate decision-making. You will also have access to information provided by trained social and healthcare workers on the means of protection against mosquitos and on other potential modes of Zika transmission.

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 and all study data will be confidential and only accessed by a small number of key study personnel 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

We will need to enter your data into an electronic database in order for us to analyse it and answer the medical questions of the study. Your medical data, and the data relating to your lifestyle and ethnic origins will be transmitted under strict protection only to your doctor or to persons working for the research group under strict protection and to key study personnel in [country of study] or overseas in other countries [name these individuals here].

If, during the course of the study, you no longer wish to participate, 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

We will also ask you if you would be willing to allow researchers to use any “left over” samples for other research 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 objectives. This can be done simply by contacting the health-care professional who is supervising your participation in this study.

Please now let us know if we can answer any questions about the information you have just read/been given or about the study in which we are seeking your participation.

INFORMED CONSENT OF MOTHER

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:

Prospective longitudinal cohort study of women and newborns exposed to ZIKV during the course of pregnancy

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 have had enough time to reflect on the implications of my participation in this medical research study.
- ☐ I agree to give access to the study investigators to my past and present medical records.
- ☐ I understand that my samples may need to undergo genetic testing, in the event that the role of genetics in determining the severity of Zika virus infection needs to be investigated.

CONSENT TO USE OF 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 TO THE USE OF BIOLOGICAL SAMPLES

I accept the use and storage of my biological samples as has been described by the study group.

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 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:

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:

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 by the Institut Pasteur, ISARIC, CONSISE, WHO, and partners and adapted from:

- ISARIC (International Severe Acute Respiratory and Emerging Infection Consortium) Case Report Forms
- 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.'
- International Research Consortium of Dengue Risk Assessment, Management and Surveillance (IDAMS)

Purpose of the standardized questionnaire and instructions for its use

This questionnaire has been designed to collect the minimum amount of data to address the primary objectives of the standardized cohort study of pregnant women. Additional questions may be added to the questionnaire, 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.

It is strongly recommended that this cohort study be followed by a cohort study of newborn and infants born to women exposed to Zika virus during pregnancy.

Comment: By using a standardized protocol, researchers can address many research objectives (which require a smaller sample size) and will have the opportunity to collaborate with other research sites/countries conducting this same study and potentially 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.

This questionnaire has four sections to be completed during the study period proposed in the standardized cohort study: at enrollment, first and second follow-up visit and at pregnancy completion.

Instructions for completing questionnaire

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

- The mother 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 for both mother / pregnant woman and neonate as per study protocol and guidelines. (Comment: These guidelines should be written into the protocol.)
- All information should be kept confidential at all times, and no identifiable information is to be recorded on the questionnaires.
- Patients' hospital ID and contact details are recorded on a separate contact list to allow later follow-up by a limited number of key/approved study personnel. 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 examinations, through parent/guardian/representative interview (for neonates), and the review of hospital charts.
- Patient ID codes should be filled in on all pages of the questionnaire (neonate and mother).
- Complete every line of every section, except for where the instructions say to skip a section based on certain responses.
- Selections with square boxes (☐) are single selection answers (choose one answer only). Selections with circles (○) are multiple selection answers (choose as many answers as are applicable).
- 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. Do not leave the question blank.
- 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 (Case Report Form) 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 or full stops, 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 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
Pregnancy during ZIKV epidemic period	☐	☐
EXCLUSION CRITERIA	**Yes**	**No**
Woman who is unable or unwilling to give informed consent	☐	☐
Contraindication to venipuncture	☐	☐

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

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

Part I: to be completed at the time of enrollment
(Ideally during the first trimester of pregnancy)

Part II: to be completed during the first follow-up visit
(Ideally during the second trimester of pregnancy)

Part III: to be completed during the second follow-up visit
(Ideally during the third trimester of pregnancy)

Part IV: to be completed following delivery/at discharge

Part V: to be completed 28 days following delivery

PART I: TO BE COMPLETED AT TIME OF ENROLLMENT

1) DEMOGRAPHIC INFORMATION

Date of birth (DD/MM/YYYY)	____ / ____ / ____
Area of residence during pregnancy (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	(Comment: add check boxes according to national guidelines)
Household income	(Comment: add check boxes for ranges appropriate to country in which the study is being conducted)
Socioeconomic status Comment: The following questions are commonly used in DHS surveys Type of flooring: Type of roofing: Wall material: Water supply: Sanitation facilities: Electricity: Radio: Television: Refrigerator: Watch: Type of vehicle: At least five items of furniture: –Table –Chair –Sofa –Bed –Armoire –Cabinet Persons per sleeping room: Ownership of agricultural land and size: Ownership of farm animals by type and number: Domestic servant: Telephone (fixed and mobile): Cooking fuel:	 _____ _____ _____ _____ ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown _____ ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown

Bank account: Windows –With shutters –With glass –With screens –With curtains	☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown

2) LIFESTYLE INFORMATION DURING PREGNANCY

The following questions aim to collect lifestyle information while the subject is/was pregnant

Comment: This is sensitive information and the study group may wish to collect this information at the end of the Part I section

Do you drink alcoholic beverages?	☐ Yes ☐ No ☐ Unknown
If yes, how frequently?	☐ Every day ☐ Less than every day, but at least weekly ☐ Less than weekly, but at least monthly ☐ On rare occasions
Do you currently smoke tobacco?	☐ Yes ☐ No ☐ Unknown
Have you smoked tobacco daily in the past?	☐ Yes ☐ No ☐ Unknown
In the past, have you smoked tobacco on a daily basis, less than daily, or not at all?	☐ Daily ☐ Less than every day, but at least weekly ☐ Not at all ☐ Unknown
Do you take recreational drugs?	☐ Yes ☐ No ☐ Unknown
If yes, which type?	☐ Crack ☐ Cannabis ☐ Cocaine ☐ Other: _____
If yes, how frequently?	☐ Every day ☐ Less than every day, but at least weekly ☐ Less than weekly, but at least monthly ☐ On rare occasions

Travel within your home country during pregnancy - If yes, list locations, including dates (DD/MM/YYYY – DD/MM/YYYY):	☐ Yes ☐ No
Travel outside of your home country during pregnancy - If yes, list locations, including dates (DD/MM/YYYY – DD/MM/YYYY):	☐ Yes ☐ No

3) EXPOSURE INFORMATION DURING PREGNANCY

The following questions aim to collect information on exposures while the subject is/was pregnant

Type of residence during pregnancy	☐ Apartment ☐ House ☐ Other (specify): _____
Location of residence during pregnancy	☐ City/urban ☐ Rural ☐ Other (specify): _____
Protection against mosquitoes during pregnancy	
- Do you wear/have you worn long trousers/long sleeves during your pregnancy?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
- During your pregnancy, have you used a mosquito net while you sleep during the day or at night?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
- Do you use/have you used essential oils to rid your home of mosquitos?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
- Do you use/have you used mosquito repellent spray during your pregnancy?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
- Do you use/have you used insecticides to remove mosquito larvae from your home?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
- Do you use/have you used other methods to rid your home of mosquitos during your pregnancy? If so, please indicate here which methods you've used:	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always

During your pregnancy, 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	_____

4) CLINICAL INFORMATION AND MEDICAL HISTORY

Current body weight:	_____ (kg)
Current gestational age:	_____ (weeks) _____ (days)
Body temperature:	_____ (°C)
Respiratory rate:	_____ (breaths/minute)
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 ☐

Please indicate if you have been diagnosed with any of the following conditions prior to or during your current pregnancy:

Chronic cardiovascular disease	☐Yes ☐ No ☐ Unknown
Chronic pulmonary disease	☐Yes ☐ No ☐ Unknown
Blood disorders - If yes, specify	☐Yes ☐ No ☐ Unknown
Chronic renal/kidney disease	☐Yes ☐ No ☐ Unknown
Chronic liver disease	☐Yes ☐ No ☐ Unknown
Chronic neurological disease - If yes, specify	☐Yes ☐ No ☐ Unknown
Paralysis - If yes, specify	☐Yes ☐ No ☐ Unknown

Type 1 or Type 2 diabetes	☐Yes ☐ No ☐ Unknown
Hypothyroidism	☐Yes ☐ No ☐ Unknown
Other endocrine disease	☐Yes ☐ No ☐ Unknown
Rheumatologic disease	☐Yes ☐ No ☐ Unknown
Epilepsy	☐Yes ☐ No ☐ Unknown
Immunosuppression	☐Yes ☐ No ☐ Unknown
HIV - If yes, antiretroviral therapy	☐Yes ☐ No ☐ Unknown ☐Yes ☐ No
Other chronic comorbidity - If yes, specify:	☐Yes ☐ No ☐Unknown
Dengue	☐Yes ☐ No ☐ Unknown
Chikungunya	☐Yes ☐ No ☐ Unknown
Toxoplasmosis	☐Yes ☐ No ☐ Unknown
Cytomegalovirus	☐Yes ☐ No ☐ Unknown
Rubella	☐Yes ☐ No ☐ Unknown

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Have you had any fever or pain treatment during this pregnancy? - Acetaminophen/paracetamol - NSAIDs - Other (specify)	☐Yes ☐ No ☐ Unknown ☐Yes ☐ No ☐ Unknown ☐Yes ☐ No ☐ Unknown _____
Have you had any anticonvulsants during your pregnancy? - If yes, name:	☐Yes ☐ No ☐Unknown
Have you used any anti-nausea drugs during this pregnancy? - If yes, name:	☐Yes ☐ No ☐Unknown
Have you used any prenatal vitamins? (folic acid etc.)	☐Yes ☐ No ☐Unknown
All other medications taken during this pregnancy, including antibiotics, antivirals and other regular medications, including herbal, and non-licensed remedies Please list generic names if possible	
Have you had a blood transfusion before or during your pregnancy? - If yes, indicate date (DD/MM/YYYY)	☐Yes ☐ No ☐Unknown _____

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Surgical history **Have you had any previous surgeries?** - If yes, specify and indicate date (DD/MM/YYYY)	☐Yes ☐ No

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Immunization history **Have you had any of the following immunizations?**	If yes, indicate date (DD/MM/YYYY)

Rubella	☐Yes ☐ No ☐Unknown	
Measles	☐Yes ☐ No ☐Unknown	
Mumps	☐Yes ☐ No ☐Unknown	
Acellular pertussis	☐Yes ☐ No ☐Unknown	
Varicella	☐Yes ☐ No ☐Unknown	
Tetanus	☐Yes ☐ No ☐Unknown	
Diphtheria	☐Yes ☐ No ☐Unknown	
Polio	☐Yes ☐ No ☐Unknown	
Seasonal influenza	☐Yes ☐ No ☐Unknown	
Yellow Fever	☐Yes ☐ No ☐Unknown	
Japanese encephalitis	☐Yes ☐ No ☐Unknown	
Tick-borne encephalitis	☐Yes ☐ No ☐Unknown	
Dengue virus	☐Yes ☐ No ☐Unknown	
Any other vaccination received during this pregnancy: - If yes, specify:	☐Yes ☐ No ☐Unknown _____	

Pregnant woman's head circumference	_____ (cm)	_____ (inches)
Known familial genetic conditions on maternal or paternal side - If yes, specify:	☐Yes ☐ No ☐ Unknown _____	
Known familial congenital disorders - If yes, specify:	☐Yes ☐ No ☐ Unknown _____	
Consanguinity - If yes, specify:	☐Yes ☐ No ☐ Unknown	

5) OBSTETRIC HISTORY

Excluding your current pregnancy, please indicate the number of:	
- Previous pregnancies	
- Spontaneous miscarriages	
- Voluntary abortions	

- Medical abortions	
- Fetal deaths *in utero*	
- Premature births	
- Microcephaly	
- Low birth weight Indicate weight: _____	
- Infants born with congenital abnormalities Description of abnormality:	

6) SIGNS AND SYMPTOMS OF ZIKA INFECTION

During your pregnancy, have you experienced any of the following?

Rash - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Oedema - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Other abnormal skin and/or subcutaneous tissue condition - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Fever - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Joint pain - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Joint swelling - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Muscle pain - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Eye redness/conjunctivitis - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown

Headache - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Vomiting/nausea - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Cough - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Sore throat - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Other condition - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown

7) LABORATORY EXAMINATION

		Date of test: (DD/MM/YYYY)
Zika virus - If positive, specify test:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____	
Dengue virus - If positive, specify test:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____	
Yellow Fever virus - If positive, specify test:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____	
West Nile virus - If positive, specify test:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____	
Chikungunya virus - If positive, specify test:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____	
Toxoplasmosis - If positive, specify test:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____	
Rubella - If positive, specify test:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____	
Cytomegalovirus - If positive, specify test:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____	
Herpes simplex virus - If positive, specify test:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____	
Syphilis - If positive, specify test:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____	

HIV - If positive, specify test:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____	
BVDV - If positive, specify test:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____	
Other (specify): _____ - If positive, specify test:	☐Positive ☐ Negative ☐ Unknown _____	

PART I COMPLETED BY

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

Date of interview: ____/____/_____

Interviewer: _____

PART II: TO BE COMPLETED DURING FIRST FOLLOW-UP VISIT

8) CLINICAL INFORMATION AND MEDICAL HISTORY

Current weight	_____ (kg)
Trimester of pregnancy	
Temperature	_____ (°C)
Heart rate	_____ (bpm)
Arterial blood pressure Systolic/ Diastolic	_____ (mmHg)

Since the first interview, have you taken:	
Fever or pain treatment - Acetaminophen/paracetamol - NSAIDs - Other (specify)	☐Yes ☐ No ☐ Unknown ☐Yes ☐ No ☐ Unknown ☐Yes ☐ No ☐ Unknown _____
Anticonvulsants - If yes, name:	☐Yes ☐ No ☐Unknown
Anti-nausea drugs - If yes, name:	☐Yes ☐ No ☐Unknown
Prenatal vitamins (folic acid etc.)	☐Yes ☐ No ☐Unknown
Any other medications, including antibiotics, antivirals and other regular medications, including herbal, and non-licensed remedies Please list generic names if possible	
Blood transfusion - If yes, indicate date (DD/MM/YYYY)	☐Yes ☐ No ☐Unknown _____

Any other vaccination received during this pregnancy since first interview - If yes, specify:	☐Yes ☐ No ☐Unknown _____

9) SIGNS AND SYMPTOMS OF ZIKA INFECTION

During your pregnancy, have you experienced any of the following?

Rash - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Oedema - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Other abnormal skin and/or subcutaneous tissue condition - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Fever - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Joint pain - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Joint swelling - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Muscle pain - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Eye redness/conjunctivitis - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Headache - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Vomiting/nausea - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Cough - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Sore throat - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Other condition - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown

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10) LABORATORY EXAMINATION

Zika virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Dengue virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Yellow Fever virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
West Nile virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Chikungunya virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Toxoplasmosis - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Rubella - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Cytomegalovirus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Herpes simplex virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
HIV - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
BVDV - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Other (specify): _____ - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown _____

PART II COMPLETED BY

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

Date of interview: ____/____/_____

Interviewer: _____

PART III: TO BE COMPLETED DURING SECOND FOLLOW-UP VISIT

11) CLINICAL INFORMATION AND MEDICAL HISTORY

Current weight	_____ (kg)
Trimester of pregnancy	
Temperature	_____ (°C)
Heart rate	_____ (bpm)
Arterial blood pressure Systolic/ Diastolic	_____ (mmHg)

Since the second interview, have you taken:	
Fever or pain treatment - Acetaminophen/paracetamol - NSAIDs - Other (specify)	☐Yes ☐ No ☐ Unknown ☐Yes ☐ No ☐ Unknown ☐Yes ☐ No ☐ Unknown _____
Anticonvulsants - If yes, name:	☐Yes ☐ No ☐Unknown
Anti-nausea drugs - If yes, name:	☐Yes ☐ No ☐Unknown
Prenatal vitamins (folic acid etc.)	☐Yes ☐ No ☐Unknown
Any other medications, including antibiotics, antivirals and other regular medications, including herbal, and non-licensed remedies Please list generic names if possible	
Blood transfusion - If yes, indicate date (DD/MM/YYYY)	☐Yes ☐ No ☐Unknown _____

Any other vaccination received during this pregnancy since first interview - If yes, specify:	☐Yes ☐ No ☐Unknown _____

12) SIGNS AND SYMPTOMS OF ZIKA INFECTION

During your pregnancy, have you experienced any of the following?

Rash - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Oedema - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Other abnormal skin and/or subcutaneous tissue condition - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Fever - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Joint pain - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Joint swelling - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Muscle pain - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Eye redness/conjunctivitis - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Headache - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Vomiting/nausea - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Cough - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Sore throat - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Other condition - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown

1151

13) LABORATORY EXAMINATION

Zika virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Dengue virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Yellow Fever virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
West Nile virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Chikungunya virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Toxoplasmosis - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Rubella - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Cytomegalovirus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Herpes simplex virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
HIV - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
BVDV - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Other (specify): _____ - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown _____

PART III COMPLETED BY

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

1159

1160

Date of interview: ____/____/_____

Interviewer: _____

PART IV: TO BE COMPLETED FOLLOWING DELIVERY/AT DISCHARGE

14) DELIVERY OUTCOME

Date of end of pregnancy (DD/MM/YYYY)	
Outcome of pregnancy	☐Live birth ☐ Stillbirth ☐Miscarriage ☐ Induced abortion
Pre-labour premature rupture of membranes	☐Yes ☐ No ☐ Unknown
Place of delivery **If applicable, name of health care facility**	☐Home ☐ Health facility ☐ Unknown _____
Type of delivery	☐Spontaneous ☐ Induced ☐ No labour ☐ Unknown
Mode of delivery	☐ Vaginal spontaneous ☐ Vaginal assisted (e.g. forceps, vacuum) ☐ Caesarean section ☐ Assisted breech or breech extraction
Fetal presentation at delivery	☐ Cephalic ☐ Breech ☐ Other (specify): _____
Intrapartum complications - If yes, specify type of complication:	☐Yes ☐ No ☐ Unknown _____
Postpartum complications (including postpartum haemorrhage) - If yes, specify complication:	☐Yes ☐ No ☐ Unknown _____
Maternal outcome at discharge - If discharged with sequelae, describe: - If deceased, specify likely cause of death:	☐Discharged without sequelae ☐ Discharged with sequelae ☐ Deceased _____

15) NEONATAL OUTCOME

Status at discharge	☐ Discharged home with no abnormalities ☐ Discharged home with abnormalities ☐ Referred to ICU in the same institution ☐ Referred to other institution ☐ Antepartum death ☐ Intrapartum death ☐ Postnatal death

Date of discharge (DD/MM/YYYY)	_____
If deceased, date of death (DD/MM/YYYY) Specify likely cause of death:	_____
Autopsy performed - If yes, indicate date (DD/MM/YYYY)	☐Yes ☐ No ☐ Unknown _____
Placenta analysed - If yes, placental weight - If yes, placental calcifications - If yes, other placental abnormalities Describe:	☐Yes ☐ No ☐ Unknown _____ (g) _____ (other unit: _____) ☐Yes ☐ No ☐ Unknown ☐Yes ☐ No ☐ Unknown _____

16) NEONATAL MEASUREMENTS AT BIRTH

Apgar scores	1 min: _____ 5 min: _____ 10 min: _____	
Birth weight (<12 hours after delivery)	_____ (gram)	☐ Unknown
Crown-to-heel length	_____ (cm)	☐ Unknown
Head circumference (Occipito-frontal after 24h following birth) (Ideally, average of 3 measurements)	_____ (cm)	☐ Unknown

17) NEONATAL PHYSICAL EXAMINATION AT BIRTH

Temperature	____.__°C or _____ Fahrenheit ☐Oral ☐Tympanic ☐Rectal ☐Axillary ☐Other (specify):	
Respiratory rate		breaths/minute
Heart rate		beats/minute
Capillary refill time (central)		seconds

Systolic blood pressure		mmHg
Diastolic blood pressure		mmHg
Peripheral O_2 saturation (SpO_2)		%

1172

1173

Cardiovascular system	☐Normal ☐Abnormal ☐Unknown	If abnormal, specify: ☐Murmur ☐Other:
Respiratory system	☐Normal ☐Abnormal ☐Unknown	If abnormal, describe:
Gastrointestinal system	☐Normal ☐Abnormal ☐Unknown	☐ Jaundice ☐ Abdominal tenderness ☐ Hepatomegaly ☐ Splenomegaly ☐ Hernia ☐ Omphaloceles ☐ Gastroschisis ☐Other (specify): _____

1174

Seizure(s) - If yes, describe:	☐Yes ☐No ☐Unknown ☐General ☐ Focal
Paralysis - If yes, describe:	☐Yes ☐No ☐Unknown ☐General ☐ Ascending
Hypotonia (floppiness)	☐Yes ☐No ☐Unknown
Stiffness or spasticity or increased tone of limbs - If yes, describe:	☐Yes ☐No ☐Unknown
Arthrogryposis - If yes, describe:	☐Yes ☐No ☐Unknown
Other neurological signs - If yes, describe:	☐Yes ☐No
Other abnormal movements *e.g writhing movements* - If yes, describe:	☐Yes ☐No

1175

Type of cry	☐Strong normal cry ☐Weak, high-pitched or continuous cry ☐Not crying ☐Other:
Tonic neck reflex	☐Present ☐Absent ☐Not Done
Moro reflex	☐Present ☐Absent ☐Not Done
Rooting reflex	☐Present ☐Absent ☐Not Done
Sucking reflex	☐Present ☐Absent ☐Not Done
Grasp reflex	☐Present ☐Absent ☐Not Done
Babinski reflex	☐Present ☐Absent ☐Not Done

1176

1177

	Abnormality	**Type**	**Localization**	**Description**

Head	☐Yes ☐ No			
Neck	☐Yes ☐ No			
Trunk	☐Yes ☐ No			
Upper limbs	☐Yes ☐ No			
Lower limbs	☐Yes ☐ No			

Rash - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Oedema - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Other abnormal skin and/or subcutaneous tissue condition - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Fever - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Eye redness/conjunctivitis - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Other condition - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown

18) COMPLEMENTARY DIAGNOSTIC TESTS

	Result	**Abnormality description**			**Images attached**	**Report attached**
		Image type	**Localization**	**Size**		
Cranial ultrasound scan	☐ Normal ☐ Abnormal ☐ Unknown ☐ Not done				☐Yes ☐ No	☐Yes ☐ No
CT Scan	☐ Normal ☐ Abnormal				☐Yes ☐ No	☐Yes ☐ No

MRN	☐ Normal ☐ Abnormal				☐Yes ☐ No	☐Yes ☐ No
Other (specify type of test):	☐ Normal ☐ Abnormal				☐Yes ☐ No	☐Yes ☐ No

1181

Fundoscopy	☐ Normal ☐ Abnormal ☐ Unknown ☐ Not done	If abnormal, describe:
Red reflex or chorioretinitis	☐ Present ☐ Absent ☐ Unknown ☐ Not done	If abnormal, describe:
Cataract	☐ Normal ☐ Abnormal ☐ Unknown ☐ Not done	If abnormal, describe:
Hearing test (specify test used): _____	☐ Normal ☐ Abnormal ☐ Unknown ☐ Not done	If abnormal, describe:
Newborn blood screening - Hypothyroidism - Phenylketonuria - Other (specify): _____	☐ Positive ☐ Negative ☐ Not done ☐ Positive ☐ Negative ☐ Not done ☐ Positive ☐ Negative	

1182

1183 ## 19) NEONATAL LABORATORY EXAMINATION

Zika virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Dengue virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Yellow Fever virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested

	_____
West Nile virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Chikungunya virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Toxoplasmosis - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Rubella - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Cytomegalovirus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Herpes simplex virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
HIV - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
BVDV - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Other (specify): _____ - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown _____

PART IV COMPLETED BY

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

Date of interview: ____/____/_____

Interviewer: _____

PART V: TO BE COMPLETED 28 DAYS FOLLOWING DELIVERY

20) NEONATAL MEASUREMENTS

Weight	_____ (gram)	☐ Unknown
Height	_____ (cm)	☐ Unknown
Head circumference	_____ (cm)	☐ Unknown

21) NEONATAL PHYSICAL EXAMINATION

Temperature	____.__°C or _____ Fahrenheit ☐Oral ☐Tympanic ☐Rectal ☐Axillary ☐Other (specify):	
Respiratory rate		breaths/minute
Heart rate		beats/minute
Capillary refill time (central)		seconds
Systolic blood pressure		mmHg
Diastolic blood pressure		mmHg
Peripheral O_2 saturation (SpO_2)		%

Cardiovascular system	☐Normal ☐Abnormal ☐Unknown	If abnormal, specify: ☐Murmur ☐Other:
Respiratory system	☐Normal ☐Abnormal ☐Unknown	If abnormal, describe:
Gastrointestinal system	☐Normal ☐Abnormal ☐Unknown	☐ Jaundice ☐ Abdominal tenderness ☐ Hepatomegaly ☐ Splenomegaly ☐ Hernia ☐ Omphaloceles

		☐ Gastroschisis ☐Other (specify): _____

Seizure(s) - If yes, describe:	☐Yes ☐No ☐Unknown ☐General ☐ Focal
Paralysis - If yes, describe:	☐Yes ☐No ☐Unknown ☐General ☐ Ascending
Hypotonia (floppiness)	☐Yes ☐No ☐Unknown
Stiffness or spasticity or increased tone of limbs - If yes, describe:	☐Yes ☐No ☐Unknown
Arthrogryposis - If yes, describe:	☐Yes ☐No ☐Unknown
Other neurological signs - If yes, describe:	☐Yes ☐No
Other abnormal movements *e.g. writhing movements* - If yes, describe:	☐Yes ☐No

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1200

	Abnormality	Type	Localization	Description
Head	☐Yes ☐ No			
Neck	☐Yes ☐ No			
Trunk	☐Yes ☐ No			
Upper limbs	☐Yes ☐ No			
Lower limbs	☐Yes ☐ No			

1201

Rash	☐Yes ☐ No ☐ Unknown

- If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	
Oedema - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Other abnormal skin and/or subcutaneous tissue condition - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Fever - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Eye redness/conjunctivitis - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown
Other condition - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐ No ☐ Unknown

22) COMPLEMENTARY DIAGNOSTIC TESTS

	Result	**Abnormality description**			**Images attached**	**Report attached**
		Image type	**Localization**	**Size**		
Cranial ultrasound scan	☐ Normal ☐ Abnormal ☐ Unknown ☐ Not done				☐Yes ☐ No	☐Yes ☐ No
Other (specify type of test):	☐ Normal ☐ Abnormal				☐Yes ☐ No	☐Yes ☐ No

Fundoscopy	☐ Normal ☐ Abnormal ☐ Unknown ☐ Not done	If abnormal, describe:
Red reflex or chorioretinitis	☐ Present ☐ Absent ☐ Unknown ☐ Not done	If abnormal, describe:
Cataract	☐ Normal ☐ Abnormal ☐ Unknown ☐ Not done	If abnormal, describe:

Hearing test (specify test used): _____	☐ Normal ☐ Abnormal ☐ Unknown ☐ Not done	If abnormal, describe:
Newborn blood screening - Hypothyroidism - Phenylketonuria - Other (specify): _____	☐ Positive ☐ Negative ☐ Not done ☐ Positive ☐ Negative ☐ Not done ☐ Positive ☐ Negative	

1205

1206 ## 23) NEONATAL LABORATORY EXAMINATION

Zika virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Dengue virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Yellow Fever virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
West Nile virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Chikungunya virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Toxoplasmosis - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Rubella - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Cytomegalovirus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
Herpes simplex virus - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
HIV - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____
BVDV - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown ☐ Not tested _____

Other (specify): _____ - If positive, specify test and result:	☐Positive ☐ Negative ☐ Unknown _____

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1209 ## PART V COMPLETED BY

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

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APPENDIX C: BIOLOGICAL SAMPLING ALGORITHM (ISARIC/WHO/PAHO DRAFT DOCUMENTS)

Comment: Updated versions of the biological sampling algorithms are under development. The study group will need to use the most recent version when implementing the protocol.

DRAFT
Version 5
6/MAY/2016

Pregnant woman living in outbreak area with No symptoms consistent with ZIKV infection

Sampling algorithm for research purposes

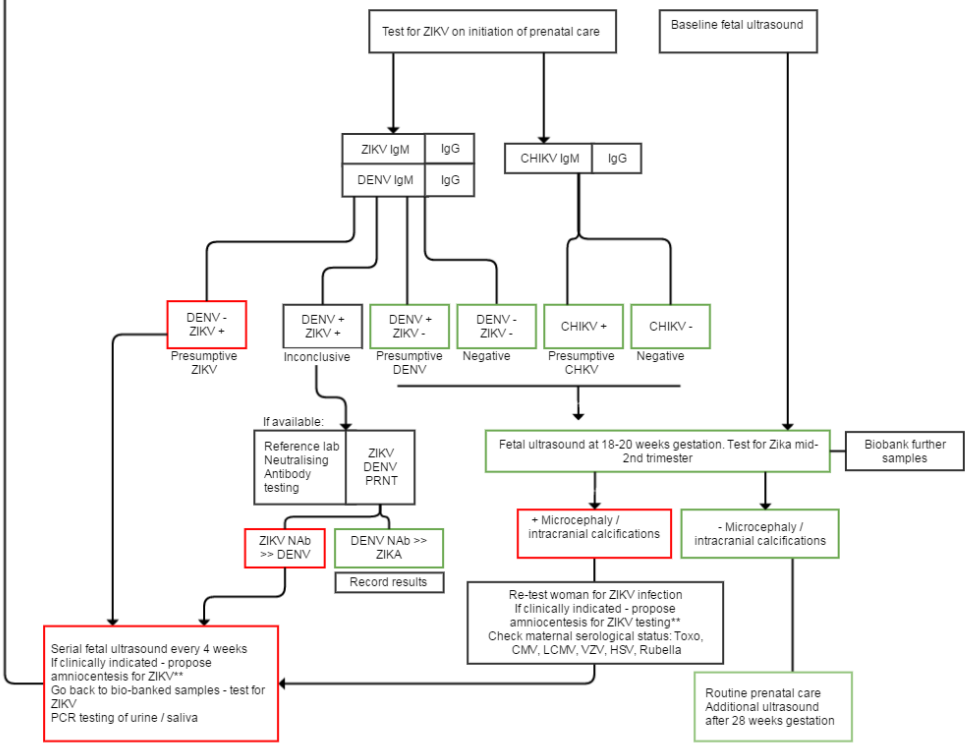
Tier Sampling approach

Tier 1: minimum sample set - single sample set taken at recruitment
Tier 2: comprehensive sampling set for research study
Tier 3-4: samples to be taken dependent on resources. Tier 4 may be most resource intensive

Tier	Sample	Tests	Initial test	Follow up	Storage*	Minimum volume
1	Serum/Plasma	IgM ELISA	Sample at enrollment	Each trimester and at delivery	2-8'C if <48h; -20'C if 48h-7days; -80'C if >7days	0.5ml
2	Serum/Plasma	IgG ELISA	Biobank sample(s)	Biobank sample(s)	-80'C	0.5ml
3	Serum/Plasma	PRNT	Biobank sample(s)	Biobank sample(s)	-80'C	use IgM sample
1	Urine	PCR	Biobank sample(s)	Biobank sample(s)	-80'C	1ml
1	Saliva	PCR	Biobank sample(s)	Biobank sample(s)	-80'C	Swab; 2mL lysis buffer / Point of care test^
2	Oral fluid	IgM ELISA	Biobank sample(s)	Biobank sample(s)	-80'C	0.5ml
2	Oral fluid	IgG ELISA	Biobank sample(s)	Biobank sample(s)	-80'C	0.5ml
2	Partner's semen	PCR	Biobank sample(s)	Test if woman positive	-80'C	0.5ml
3	Amniotic fluid	PCR	If collected for clinical purposes		-80'C	0.5ml

* Freeze at -80'C where possible, -20'C otherwise.

^ If point of care test available. Serology & PCR test if possible.



**Amniocentesis should be done after 6 weeks from exposure and not before 21 weeks gestation

Serology
Extensive cross-reactivity would be expected in samples from DENV/ZIKV circulation areas. A positive IgM or IgG assay with either antigen should be confirmed by using PRNT against both ZIKV and DENV as well as any other flavivirus (e.g. SLEV, WNV etc.) that might be found in that geographic area (including travel areas)

DRAFT
Version 5
6/MAY/2016

Pregnant woman living in outbreak area with symptoms consistent with ZIKV infection

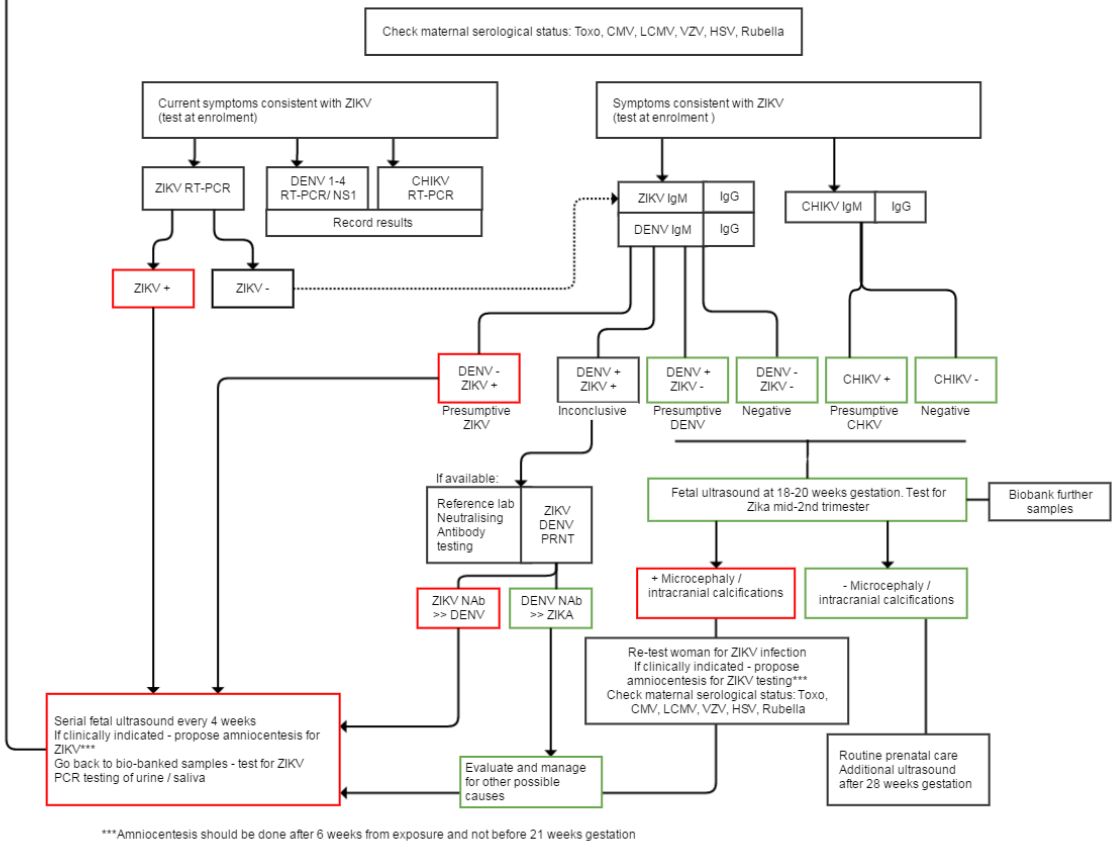
Sampling algorithm for research purposes

Tier	Sampling approach
Tier 1	minimum sample set - single sample set taken at recruitment
Tier 2	comprehensive sampling set for research study
Tier 3-4	samples to be taken dependent on resources. Tier 4 may be most resource intensive

Tier	Sample	Tests	Initial test	Follow up	Storage**	Minimum volume
1	Serum/Plasma	PCR	At enrolment	Serial PCR if positive* Test at convalescent visit	-20'C ≤ 7 days; -80'C if > 7 days	0.5ml
1	Serum/Plasma	IgM ELISA	At enrolment Paired samples - ideally sample ≥ 5 days post onset	2-3 weeks post initial sample Test at convalescent visit	2-8'C if <48h; -20'C if 48h-7days; -80'C if >7days	0.5ml
2	Serum/Plasma	IgG ELISA	> 1 week post onset	Serial ELISA if positive* Test at convalescent visit	2-8'C if <48h; -20'C if 48h-7days; -80'C if >7days	0.5ml
3	Serum/Plasma	PRNT	≥ 7 days post onset		-20'C ≤ 7 days; -80'C if > 7 days	use IgM sample
1	Urine	PCR	Within 30 days post onset	Daily follow-up during acute illness, then at convalescent visit	-20'C ≤ 7 days; -80'C if > 7 days	1ml
1	Saliva	PCR	Within 30 days post onset	Serial PCR if positive*	-20'C ≤ 7 days; -80'C if > 7 days	Swab; 2ml lysis buffer / Point of care test^
2	Oral fluid	IgM ELISA	At enrolment	Serial ELISA if positive	2-8'C if <48h; -20'C if 48h-7days; -80'C if >7days	0.5ml
2	Oral fluid	IgG ELISA	> 1 week post onset	Serial ELISA if positive	2-8'C if <48h; -20'C if 48h-7days; -80'C if >7days	0.5ml
2	Partner's semen	PCR	Biobank	Test if woman positive	-20'C ≤ 7 days; -80'C if > 7 days	0.5ml
1	Amniotic fluid	PCR	If collected for clinical purposes		-80'C	0.5ml

*Serial PCR if positive - for viral dynamics studies; resource dependent
** freeze at -80'C where possible, -20'C otherwise.

^ If point of care test available. Serology & PCR test if possible.



***Amniocentesis should be done after 6 weeks from exposure and not before 21 weeks gestation

Serology
Extensive cross-reactivity would be expected in samples from DENV/ZIKV circulation areas. A positive IgM assay with either antigen should be confirmed by using PRNT against both ZIKV and DENV as well as any other flavivirus (e.g. SLEV, WNV etc.) that might be found in that geographic area (including travel areas)

1219

DRAFT

Version 5
6/MAY/2016

Sampling Protocol Newborns without microcephaly or other congenital central nervous system malformations related to Zika

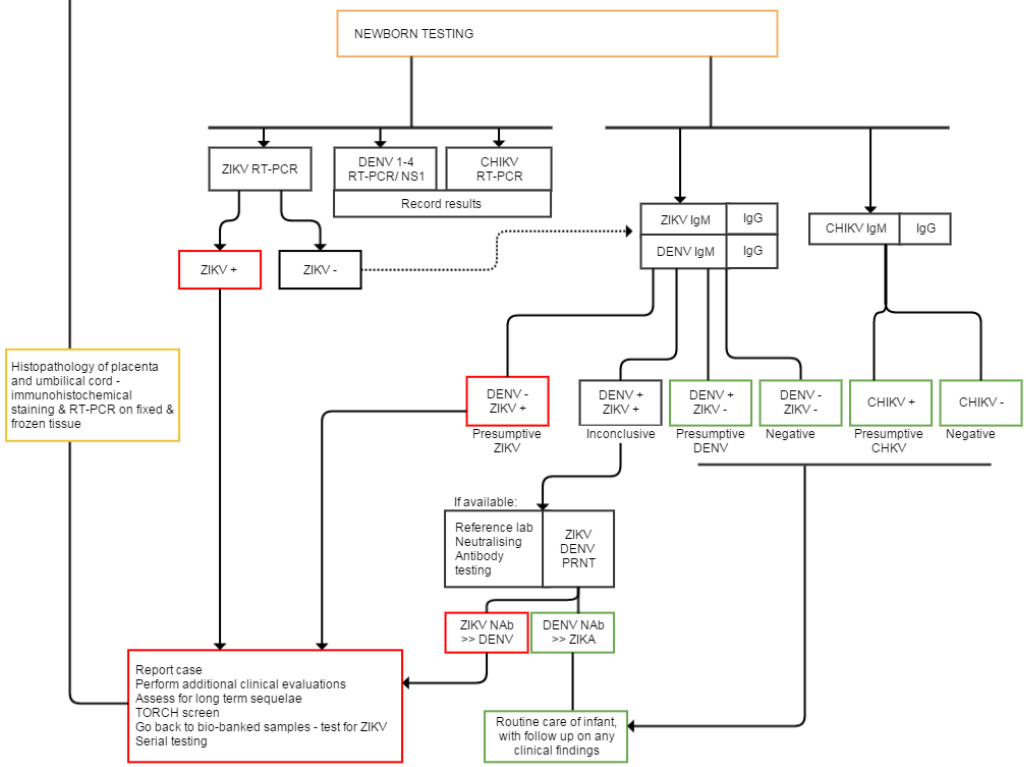
Sampling algorithm for research purposes

Tier Sampling approach
Tier 1: minimum sample set - single sample set taken at recruitment
Tier 2: comprehensive sampling set for research study
Tier 3-4: samples to be taken dependent on resources. Tier 4 may be most resource intensive

Tier	Sample	Tests	Initial test	Follow up	Storage***	Minimum volume
1	Serum/Plasma*	PCR	Within 2 days of birth	Serial PCR if positive**	-80'C	0.5ml
1	Serum/Plasma*	IgM ELISA	Within 2 days of birth	Serial ELISA if positive	2-8'C if <48h; -20'C if 48h-7days; -80'C if >7days	0.5ml
2	Serum/Plasma*	IgG ELISA	Within 2 days of birth To detect maternal antibodies	Serial ELISA if positive	2-8'C if <48h; -20'C if 48h-7days; -80'C if >7days	0.5ml
3	Serum/Plasma*	PRNT	Within 2 days of birth		-80'C	use IgM sample
1	Cord blood*	PCR	At birth		-80'C	4.5ml
1	Cord blood*	IgM ELISA	At birth		2-8'C if <48h; -20'C if 48h-7days; -80'C if >7days	4.5ml
3	Cord blood*	PRNT	At birth		-80'C	use IgM sample
1	Saliva	PCR	At birth	Serial PCR if positive**	-80'C	Swab; 2mL lysis buffer / Point of care test^
1	Urine	PCR	At birth	Serial PCR if positive**	-80'C	1ml
	CSF	PCR	If sampled for other reasons		-80'C	0.5ml
	CSF	IgM ELISA	If sampled for other reasons		2-8'C if <48h; -20'C if 48h-7days; -80'C if >7days	0.5ml
	CSF	PRNT	If sampled for other reasons		-80'C	use IgM sample
1	Placenta	PCR	At birth		-80'C	1cm3
1	Placenta	Histopathology	At birth		-80'C	1cm3
3	Umbilical cord	PCR	At birth		-80'C	1cm3
4	Umbilical cord	Histopathology	At birth		-80'C	1cm3
2	Partner's semen	PCR	Biobank	Test if woman positive	-80'C	0.5ml

*Either serum testing directly from baby **or** testing from cord blood
**Serial PCR if positive - for viral dynamics studies; resource dependent
*** Freeze at -80'C where possible, -20'C otherwise.

^ If point of care test available. Serology & PCR test if possible.



Serology
Extensive cross-reactivity would be expected in samples from DENV/ZIKV circulation areas. A positive IgM or IgG assay with either antigen should be confirmed by using PRNT against both ZIKV and DENV as well as any other flavivirus (e.g. SLEV, WNV etc.) that might be found in that geographic area (including travel areas)

1220

DRAFT
Version 5
6/MAY/2016

Sampling Protocol for Newborns with microcephaly and other congenital central nervous system malformations related to Zika

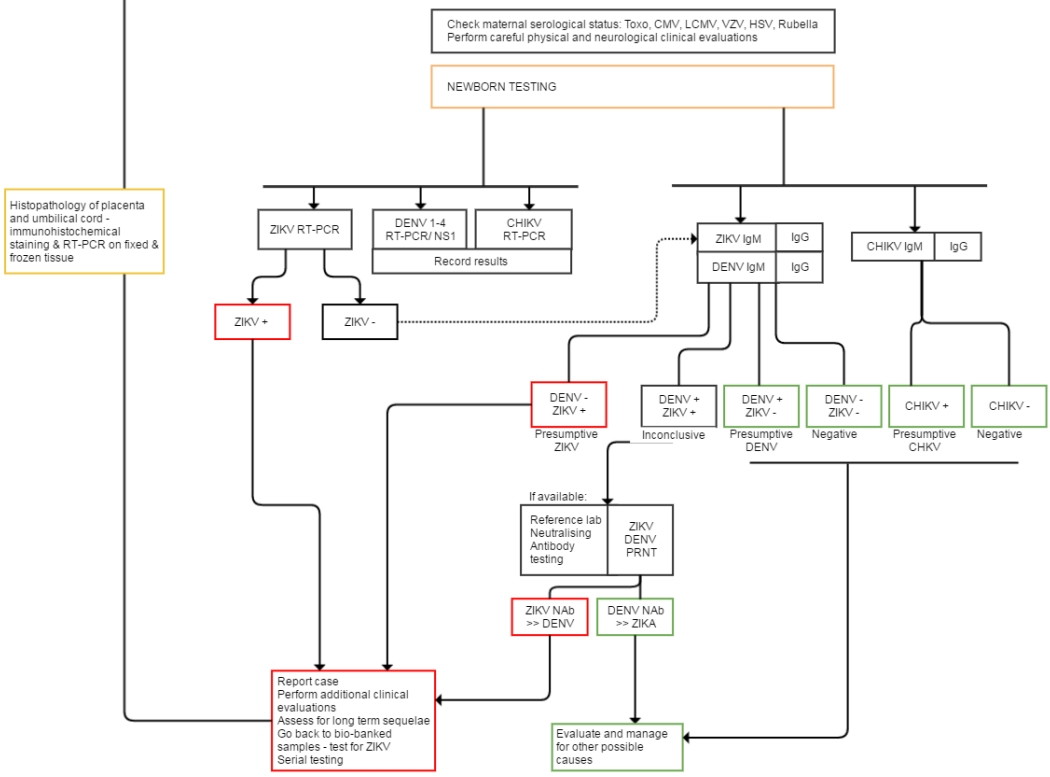
Sampling algorithm for research purposes

Tier Sampling approach
Tier 1: minimum sample set - single sample set taken at recruitment
Tier 2: comprehensive sampling set for research study
Tier 3-4: samples to be taken dependent on resources. Tier 4 may be most resource intensive

Tier	Sample	Tests	Initial test	Follow up	Storage***	Minimum volume
1	Serum/Plasma*	PCR	Within 2 days of birth	Serial PCR if positive**	-80'C	0.5ml
1	Serum/Plasma*	IgM ELISA	Within 2 days of birth	Serial ELISA if positive	2-8'C if <48h; -20'C if 48h-7days; -80'C if >7days	0.5ml
2	Serum/Plasma*	IgG ELISA	Within 2 days of birth To detect maternal antibodies	Serial ELISA if positive	2-8'C if <48h; -20'C if 48h-7days; -80'C if >7days	0.5ml
3	Serum/Plasma*	PRNT	Within 2 days of birth		-80'C	use IgM sample
1	Cord blood*	PCR	At birth		-80'C	4.5ml
1	Cord blood*	IgM ELISA	At birth		2-8'C if <48h; -20'C if 48h-7days; -80'C if >7days	4.5ml
3	Cord blood*	PRNT	At birth		-80'C	use IgM sample
1	Saliva	PCR	At birth	Serial PCR if positive**	-80'C	Swab; 2mL lysis buffer / Point of care test^
1	Urine	PCR	At birth	Serial PCR if positive**	-80'C	1ml
	CSF	PCR	If sampled for other reasons		-80'C	0.5ml
	CSF	IgM ELISA	If sampled for other reasons		2-8'C if <48h; -20'C if 48h-7days; -80'C if >7days	0.5ml
	CSF	PRNT	If sampled for other reasons		-80'C	use IgM sample
1	Placenta	PCR	At birth		-80'C	1cm3
1	Placenta	Histopathology	At birth		-80'C	1cm3
3	Umbilical cord	PCR	At birth		-80'C	1cm3
4	Umbilical cord	Histopathology	At birth		-80'C	1cm3
2	Partner's semen	PCR	Biobank	Test if woman positive	-80'C	0.5ml

*Either serum testing directly from baby **or** testing from cord blood
**Serial PCR if positive - for viral dynamics studies; resource dependent
*** Freeze at -80'C where possible, -20'C otherwise.

^ If point of care test available. Serology & PCR test if possible.



Serology
Extensive cross-reactivity would be expected in samples from DENV/ZIKV circulation areas. A positive IgM or IgG assay with either antigen should be confirmed by using PRNT against both ZIKV and DENV as well as any other flavivirus (e.g. SLEV, WNV etc.) that might be found in that geographic area (including travel areas)

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APPENDIX D: LIST OF PUBLISHED PRIMERS FOR DETECTION AND QUANTIFICATION OF ZIKA VIRUS BY REAL-TIME RT-PCR (CAO-LORMEAU, BLAKE ET AL. 2016)

ZIKV target	Primer/Probe name	Primer sequence	Primer position	Reference
M/E	ZIKV835	TTGGTCATGATACTGCTGATTGC	835-857	(Lanciotti, Kosoy et al. 2008)
	ZIKV911c	CCTTCCACAAAGTCCCTATTGC	911-890	
	ZIKV860F FAM	CGGCATACAGCATCAGGTGCATAGGAG	860-886	
pE	ZIKV1086	CCGCTGCCCAACACAAG	1086-1102	(Lanciotti, Kosoy et al. 2008)
	ZIKV1162c	CCACTAACGTTCTTTTGCAGACAT	1162-1139	
	ZIKV1107FAM	AGCCTACCTTGACAAGCAGTCAGACACTCAA	1107-1137	
E	ZIKVENVF	GCTGGDGCRGACACHGGRACT	1538-1558	(Faye, Faye et al. 2008)
	ZIKVENVR	RTCYACYGCCATYTGGRCTG	1902-1883	
NS5	ZIKVF9027a	CCTTGGATTCTTGAACGAGGA	9121-9141	(Balm, Lee et al. 2012)
	ZIKVR9197ca	AGAGCTTCATTCTCCAGATCAA	9312-9290	
NS5	Forward	AARTACACATACCARAACAAAGTGGT	9271-9297	(Faye, Faye et al. 2013)
	Reverse	TCCRCTCCCYCTYTGGTCTTG	9352-9373	
	ProbeFAM	CTYAGACCAGCTGAAR	9304-9320	