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: *Case-control study to assess potential risk factors related to microcephaly including Zika virus infection during pregnancy* will be published as soon as the ethical review has been completed.

Standardized Protocol:

Case-control study to assess potential risk factors related to microcephaly including Zika virus infection during pregnancy

Contact:

Maria Van Kerkhove
Center for Global Health
Institut Pasteur
maria.van-kerkhove@pasteur.fr

Nathalie Broutet
Department of Reproductive Health and Research
World Health Organization
broutetn@who.int

Ludovic Reveiz
Knowledge Management, Bioethics and Research
Pan American Health Organization
reveizl@paho.org

Version: 1.13
Date: 26 January 2017

Acknowledgements

This document was adapted from a protocol developed by the Brazilian Ministry of Health and the United States Centers for Disease Control and Prevention, "Brazil Guillain-Barré Syndrome Case Control Investigation. Pernambuco and Bahia states, Brazil", a WHO protocol developed for MERS-CoV, "Case-control study to assess potential risk factors related to human illness caused by MERS-CoV" and the WHO Protocol "Identification and management of Guillain-Barré syndrome in the context of Zika virus" (Interim Guidance, February 2016).

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 infection (ZIKV).) A large number of 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 case-control study, designed to examine the differences in types of exposures between cases and controls. This standardized study protocol outlines methods to guide data collection in order to evaluate *in utero* exposures and risk factors for microcephaly between newborns or fetuses with microcephaly and newborns or fetuses without microcephaly and to quantify the strength of these associations with ZIKV infection. The data collected from this standardized protocol will help characterize pregnancy data demographic features or exposures associated with the development of microcephaly and guide the public health response.

Other standardized protocols available include:

- Case-control study to assess potential risk factors related to Guillain-Barré Syndrome including Zika virus infection
- Prospective longitudinal cohort study of women and newborns exposed to Zika virus during the course of pregnancy
- Prospective longitudinal cohort study of newborns and infants born to mothers exposed to Zika virus during pregnancy
- Prospective longitudinal cohort study of Zika-infected patients to measure the persistence of Zika virus in body fluids
- 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. case-control microcephaly and cohort of newborns). However, each study group needs to consider carefully the burden on each participant.

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

CONTENTS

LIST OF ABBREVIATIONS

ABR	Auditory brainstem response
CHIKV	Chikungunya virus
CIC	Centre d'Investigations Cliniques
CMV	Cytomegalovirus
CT	Computed tomography
CNS	Central nervous system
CONSISE	Consortium for the Standardization of Influenza Seroepidemiology
CSF	Cerebrospinal fluid
DENV	Dengue virus
HIV	Human Immunodeficiency virus
HSV	Herpes Simplex virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHR	International Health Regulations
IRB	Institutional Review Board
ISARIC	International Severe Acute Respiratory and Emerging Infection Consortium
IUGR	Intrauterine growth restriction
LCMV	Lymphocytic choriomeningitis virus
MERG	Microcephaly Epidemic Research Group
MRI	Magnetic resonance imaging
OAE	Otoacoustic emissions
OR	Odds ratio
PAHO	Pan American Health Organization
PRNT	Plaque-reduction neutralization test
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
SD	Standard deviation
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
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 Zika virus (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 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).

Despite the strong spatiotemporal correlation between the ZIKV outbreak in the Americas and the unusual increase in incidence of microcephaly cases, the potential causal association existing between ZIKV infection during pregnancy and congenital disorders, such as microcephaly, in the newborn or fetus has yet to be demonstrated.

The following standardized research protocol outlines methods to identify risk factors for microcephaly, and potential risk factors for the larger spectrum of congenital disorders (currently referred to as congenital Zika syndrome) presumably caused by ZIKV infection. This study will address the following public health questions:

1. What is the risk of microcephaly associated with ZIKV infection
2. What are the risk factors for microcephaly?

Comment: Evidence is accumulating that ZIKV infection in mothers causes additional abnormalities beyond microcephaly, but as a clear definition of congenital Zika syndrome is not yet available, this standardized protocol focuses on microcephaly at birth.

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

1.1 OBJECTIVES

The data collected from this standardized study will be used to refine and update recommendations for surveillance and case definitions for microcephaly, to help understand the spread, severity, spectrum and impact on the community of ZIKV and to guide public health measures, particularly for pregnant women and couples planning a pregnancy.

The primary objectives of this study are to:

- Estimate the risk of microcephaly at birth associated with ZIKV infection
- Identify and quantify risk factors for microcephaly at birth

The secondary objectives of this study are to:

- Identify effect modifiers and interactions
- Estimate the attributable risk of microcephaly associated with ZIKV infection
- Describe and quantify the clinical, laboratory and imaging characteristics and outcome of newborns with microcephaly at birth that is associated with ZIKV infection

2.0 STUDY PROCEDURES

2.1 STUDY SETTINGS

The study uses a case-control design that examines the differences in types of *in utero* exposures between newborns or fetuses with microcephaly, determined by head circumference measured at the end of pregnancy, and newborns or fetuses without microcephaly.

The study may be prospective (only incidental cases are included) or retrospective (cases are enrolled retrospectively using a list of declared microcephaly cases in the area of interest), depending on its geographical and epidemiological context. In the case of a prospective study, all newborns delivered at the study site should

2.2 SELECTION AND RECRUITMENT OF STUDY PARTICIPANTS

2.2.1 STUDY POPULATION

Study participants are to be selected from areas in which ZIKV has been reported to circulate (i.e., exposed populations). In case of a prospective study design, cases will be recruited at the time of diagnosis in health care facilities located in or within an immediate proximity to the defined study area(s) of interest. To identify cases and controls, all newborns will have a head circumference measurement taken at birth at study sites identified by the study group. For live newborns, head circumference will need to be confirmed after 24 hours following birth.

Comment: Head circumference measurement needs to be a standardized procedure. Please see WHO Screening, assessment and management of neonates and infants with complications associated with Zika virus exposure in utero (August 2016).

The mother of each newborn/fetus included in the study (or the appropriate legal representative in case the mother is under the age of 18 years old) will be asked for her voluntary informed consent for her and her newborn/fetus to participate in the study.

2.2.2 CASE AND CONTROL DEFINITION

- **Case**: fetus or newborn (dead or alive) with a head circumference below -2 SD (standard deviation) according to INTERGROWTH-21 standards for gestational age and sex. For live newborns, head circumference is to be measured after 24 hours following birth.
- **Control**: fetus or newborn (dead or alive) without microcephaly, matched by sex and gestational age (± 7 days), birth in the same maternity ward /hospital and mother's area of residence. For each case, 2-3 controls will be recruited.
  Gestational age for the fetus/newborn will be obtained by consulting medical notes of the mother, or by record of the date of the last menstrual period.

Comment: Any changes to the case or control definitions as described above need to be clearly described in the protocol. It should be noted that in this situation, the results of the research

protocol are unlikely to be comparable to other case-control studies, and will not be able to be pooled. It is therefore not recommended to change the case or control definitions.

2.2.3 ELIGIBILITY CRITERIA

- **Inclusion criteria:** Any newborn or fetus diagnosed with microcephaly by a medical professional between 24-48 hours after birth and for whom the mother given voluntary informed consent. For infants whose mother is under the age of consent or unable to provide voluntary informed consent, her legal representative or guardian will be asked to provide written voluntary informed consent.
- **Exclusion criteria:** Any newborn or fetus with macrocephaly. Any newborn or fetus whose mother (or her legal representative or guardian) is unable to provide written informed consent. Any individual (mother or child) with contraindication to venipuncture.

2.2.4 INFORMED CONSENT

Written informed consent from a parent or legal guardian will be collected for all fetuses and newborns.

During the first interview with the eligible fetus or newborn's mother or parent/guardian, the purpose, nature and constraints of the study will be explained and written consent will be obtained from the parent/guardian by a trained member of the investigation team. Each parent/guardian must be informed that the participation of his/her fetus or newborn is voluntary and that he/she will be free, without needing to justify himself/herself, to withdraw at any time without consequences. Data contributed to the study up until the point of withdrawal will remain with the study group, unless stated by the withdrawing parent/guardian.

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

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

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

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

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

If the parent/guardian agrees for his/her fetus or newborn to participate in the study, the consent form must be completed legibly, with both surname and first name, dated and signed by the

parent/guardian 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 parent/guardian of the fetus or newborn.

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, as determined by national/local IRB requirements.

2.2.5 INCENTIVES TO PARTICIPATE AND COMPENSATION

The primary incentive to participate will be the access to extended medical investigations and care for cases and standard medical examinations and care if needed for the controls. All mothers/guardians will also be provided with additional information on the means of protection against ZIKV vectors, on other potential modes of ZIKV transmission and on microcephaly by trained social and healthcare workers.

The possibility to propose a financial compensation (e.g., to offset costs to attend an interview) will depend on the context of the study and local policies and should be decided 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 approval to the Ethics Review Committee of the World Health Organization.

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

2.3.1 BENEFITS/RISKS FOR STUDY PARTICIPANTS

Infants, and potentially their mothers, will benefit by knowing whether they were infected with ZIKV. In the case of prospective studies, the cases are likely to benefit from additional testing (ultrasounds, cerebral imaging and neurological tests, if available) and extended medical care. The cases' mothers will be provided with extensive medical information about microcephaly as soon as the condition has been diagnosed.

In addition, the data collected will help improve and guide efforts to prevent the spread of ZIKV, improve public health measures towards pregnant women and inform ZIKV vaccination strategies should a vaccine become available in the coming years. Participants will be informed of their individual results (e.g., if they have evidence of past infection with ZIKV) as well as their infant's results (e.g., if there is evidence of *in utero* ZIKV infection).

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.

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

The collection of a small amount of blood and urine poses minimal risk to participants. Cerebrospinal fluid (CSF) analysis may be indicated in newborns with microcephaly as part of the differential diagnostic investigation and is required to investigate an infection of the central nervous system. Lumbar puncture for CSF is a procedure with potential risk of complications including minor headaches, bleeding, rare infections of the central nervous system and, even more rarely, transtentorial herniation. However, this risk is low when the procedure is performed in a hospital setting by a trained professional. In some cases, a volume of CSF or serum larger than what may normally be collected as part of routine care may need to be collected from patients for the specific purposes of this study. For cases of intrauterine diagnosis of microcephaly, the amniotic fluid collection will be performed exclusively in situations where there is clear clinical indication as determined by the attending physician.

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

2.4 DATA COLLECTION AND MANAGEMENT

After informed consent is obtained, a standardized study questionnaire will be administered to all study participants. This will include demographic information, date of symptom onset in the mother,

and a series of detailed questions about behaviors, practices and exposures during pregnancy and underlying medical conditions.

Following the end of the pregnancy, the following information will be collected from **cases**:

- Vital status, head circumference measured after 24 hours following delivery
- Laboratory evaluations, including blood tests, cord blood, liver function, confirmation of any ZIKV exposure and other relevant infections such as arboviruses (e.g., Dengue) and TORCHS infections [toxoplasmosis, other (e.g., varicella, etc.), rubella, cytomegalovirus, herpes, HIV, syphilis]
- For live newborns: clinical evaluation, including laboratory evaluations, neurological signs, ophthalmology, auditory screening, reflexes

Following the end of the pregnancy, the following information will be collected from **controls**:

- Vital status, head circumference measured after 24 hours following delivery
- Laboratory evaluations, including blood tests, cord blood, liver function, confirmation of any ZIKV exposure and other relevant infections such as arboviruses (e.g., Dengue) and TORCHS infections [toxoplasmosis, other (e.g., varicella, etc.), rubella, cytomegalovirus, herpes, HIV, syphilis]
- For live newborns: clinical evaluation, including laboratory evaluations, neurological signs, ophthalmology, auditory screening, reflexes

Comment: Please note that this protocol focuses only on microcephaly. Once a clear definition of congenital Zika syndrome becomes available, the protocol could be expanded to focus on congenital Zika syndrome. In this case, information regarding data collection from cases and controls will need to be altered accordingly.

The following information will also be collected from the **mothers of cases and controls**:

- Information on the outcome of the pregnancy and delivery, including ultrasound details if available
- Known and potential risk factors (demographic, lifestyle, ecological factors, etc.) for congenital defects
- Laboratory evaluations, specifically, confirmation of any 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]

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

- Clinical report form of the case-control study protocol 'Assessment of the association of Zika virus infection and microcephaly' (Brazil Ministry of Health & US CDC)

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

The questionnaire can be found in Appendix B and contains the core data variables that should be collected from the study participants to address the objectives of this study. The questionnaire is designed to be implemented by trained study personnel, without advanced or specialized medical degrees.

2.4.1 PHYSICAL AND NEUROLOGICAL INVESTIGATIONS ON NEWBORNS

Different physical, neurological and biological tests will be performed in order to confirm the diagnosis of microcephaly and characterize the clinical spectrum observed among enrolled cases:

- Full physical examination including the evaluation of vital signs, neurological reflexes, spasticity and tone
- Evaluation of hearing and vision using two tests recommended by WHO: Otoacoustic Emissions (OAE) or Auditory Brainstem Response (ABR)
- Neonatal measurements including head circumference after 24 hours following birth
- Evaluation of the presence of seizures and epilepsy
- Transcranial echography and echocardiography when possible
- MRI or CT scan when possible
- Ocular exam, including funduscopy

Comment: The implemented protocol and accompanying informed consent must explain all physical, neurological and biological tests that will be performed at the follow-up visits, 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.

2.4.2 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

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

Table 1 lists the different biological samples to be collected from the newborn/ fetus and the mother, at time of birth, within the first days of birth or at the time of a induced abortion/fetal death. Appendix C provides a more extensive list of samples to be collected and may be subject to further updates.

Comment: The list of specimens to collect provided here may be subject to adaptation to local context. Oversampling of newborns should be avoided, and in case of limited possibilities to collect all types of specimens mentioned here, sampling of cord blood should be prioritized. More information on the minimal and maximal volumes of biological specimens to be collected from newborn and adults are available here.

Comment: Amniotic fluid collection may be performed by swabbing the newborn's entire body at time of birth, and may be used for testing for pathogens (PCR or RT-PCR), like urine and saliva.

Comment: A CSF sample is only to be collected from newborns/fetuses with microcephaly as part of the differential diagnostic investigation and is required to investigate an infection of the central nervous system.

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. In the event that neither cord blood nor urine samples are available, saliva could be used for pathogen testing.

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.

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

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

Table 1: *List of biological samples to be collected from the study participants*

		Specimen	Volume*	Container	Timing	Remarks
Mother	**Case**	Blood	7.5 mL	Dry tube (serum)	Within 2 days of birth	Minimal volume needed: 0.5 mL of whole blood.
		Amniotic fluid	0.5 mL	Sterile collection tube	At time of amniocentesis	Only in pregnant women with fetuses with microcephaly diagnosed *in utero* who are subject to amniocentesis by medical indication. For newborns, amniotic fluid can be collected by swabbing the newborn at birth.
	Control	Blood	7.5 mL	Dry tube (serum)	Within 2 days of birth	Minimal volume needed: 0.5 mL of whole blood.
Newborn / Fetus	**Case**	Blood	• Peripheral blood: 3 mL • Cord blood: 10 mL	Dry tube (serum)	• Peripheral blood: within 2 days of birth • Cord blood: at birth	When feasible, collecting cord blood should be prioritized over peripheral blood. Minimal volume needed: 0.5 mL of whole blood.
		CSF	1 mL	Sterile collection tube	Within 2 days of birth	Minimal volume needed: 0.5 mL of CSF.
		Urine (bagged not catheterized)	1 mL	Sterile collection tube	At birth	Minimal volume needed: 0.5 mL of urine.
		Fetal tissue / placenta**	N/A	Sterile collection tube	At time of stillbirth / induced abortion	Fetal tissue and placenta should be sampled and stored at -80°C for further analysis.
	Control	Blood	• Peripheral blood: 3 mL • Cord blood: 10 mL	Dry tube (serum)	• Peripheral blood: within 2 days of birth • Cord blood: at birth	When feasible, collecting cord blood should be prioritized over peripheral blood. Minimal volume needed: 0.5 mL of whole blood.

** Suggested volume to collect, may be increased if clinically acceptable. ** In case of stillbirth, miscarriage or fetal extraction because of induced or spontaneous abortion.*

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

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

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

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

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

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

2.5.2 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 should be kept for future analysis. The principal tests described for ZIKV infection detection and differential diagnosis are listed in Table 2.

The serum will be tested for antibodies against suspected infectious pathogens, such as Dengue virus, Zika virus. Urine, CSF, saliva and amniotic fluid will be tested using molecular diagnostics for Zika virus and other pathogens nucleic acid.

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

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

Table 2: *List of the different biological testing to be performed on collected specimens*

		Specimen	Type of test	Targeted pathogen
Mother	**Case**	Serum	• Real-time RT-PCR • Serology (IgM / IgG) • Biochemical and hematological analysis	• **RT-PCR & serology**: ZIKV* • **Serology only:** CHIKV, DENV, HSV, VZV, CMV, LCMV, HIV, HTLV, rubella, *Treponema pallidum, Toxoplasma sp.*
		Amniotic fluid	Real-time RT-PCR	ZIKV, CMV
	Control	Serum	• Real-time RT-PCR • Serology (IgM / IgG) • Biochemical and hematological analysis	• **RT-PCR & serology**: ZIKV* • **Serology only:** CHIKV, DENV, HSV, VZV, CMV, LCMV, HIV, HTLV, rubella, *Treponema pallidum, Toxoplasma sp.*
Newborn / Fetus	**Case**	Serum	• Real-time RT-PCR • Serology (IgM / IgG) • Biochemical and hematological analysis	• **RT-PCR & serology**: CMV, ZIKV* • **Serology only:** CHIKV, DENV, HSV, VZV, CMV, LCMV, HIV, HTLV, rubella, *Treponema pallidum, Toxoplasma sp.*
		CSF	• Real-time RT-PCR • Serology (IgM only) • Biochemical & cytological analysis (proteins, glucose, cells)	• **RT-PCR & serology**: ZIKV* • **Serology only:** CHIKV, DENV, HSV, VZV, CMV, LCMV, HIV, HTLV, rubella, *Treponema pallidum, Toxoplasma sp.*
		Urine (bagged not catheterized)	Real-time RT-PCR	ZIKV, CMV
		Placenta	Real-time RT-PCR	ZIKV
	Control	Serum	• Real-time RT-PCR • Serology (IgM / IgG) • Biochemical and hematological analysis	• **RT-PCR & serology**: CMV, ZIKV* • **Serology only:** CHIKV, DENV HSV, VZV, CMV, LCMV, HIV, HTLV, rubella, *Treponema pallidum, Toxoplasma sp.*

		Urine (bagged not catheterized)	Real-time RT-PCR	ZIKV, CMV
		Placenta	Real-time RT-PCR	ZIKV

** In case of a positive result for ZIKV with serology, use the same sample for confirmation with a plaque-reduction neutralization test.*

Biological methods: The biochemical and hematological analyses to be performed on both cases and controls correspond to routine laboratory work of standard medical care, including complete blood count, ionogram, liver function rests, renal markers, C-reactive protein, etc.

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. Please see the latest WHO laboratory guidance for ZIKV.

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.

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 calculation for determining the association between ZIKV infection and the occurrence of microcephaly will depend on: the prevalence of ZIKV infection among cases and the expected strength of the association between ZIKV infection and microcephaly. These factors are both either highly susceptible to variation due to geographical location of the study, or are still undetermined due to the lack of published case-control studies.

Comment: Sample size calculations should be performed as two-tailed statistical tests, with 80% of statistical power and 5% significance level. Table 4 shows different scenarios of sample size in case and control groups, depending on the proportion of exposure to ZIKV infection among cases and on the number of controls that will be matched to each case. The calculation was performed using Stata 13 software, with an odds ratio superior or equal to 2.

Table 4: *Different sample sizes calculated using different scenarios of exposure of microcephaly cases to ZIKV infection*

Proportion of ZIKV infection among cases	No. of controls per case	No. of cases	No. of controls	Minimum detectable odds ratio	Precision (%)
70%	2	107	214	1.48	27.7
	3	95	285	1.40	25.7
50%	2	102	204	1.50	27.9
	3	90	270	1.42	26.0
30%	2	135	270	1.52	28.3
	3	118	236	1.52	30.0

3.2 STUDY OUTCOME MEASURES

The following will be assessed as study endpoints corresponding to the primary and secondary objectives:

- Provide descriptive epidemiology: demographic characteristics of cases and controls (number of cases and controls; median age, sex, area of residence)
- Description of the clinical characteristics among enrolled cases:
    - Microcephaly
    - Head circumference
    - Cranial facial disproportions and abnormalities

- Morbidity/Mortality
- Birth weight and height
- Vital signs
- Neurological reflexes
- Spasticity and tone
- Hearing disability
- Vision problems
- Epilepsy and seizures
- Cerebral palsy
- Contractures
- Intrauterine Growth Restriction (IUGR)
- Hospitalization
- Laboratory analysis results and central nervous system (CNS) imaging characteristics

- Assessment of risk factors for microcephaly by calculating the odds of different exposures between newborns with microcephaly and controls, followed by logistic regression, adjusting for potential confounders. This will include assessment of the frequency of ZIKV infection among cases and controls. ZIKV infection will be assessed by a positive test for anti-ZIKV antibody and/or detection of ZIKV nucleic acid using RT-PCR as defined by the standards of the laboratory. Please see the latest WHO laboratory guidance for ZIKV.
- Calculating the attributable risk of microcephaly among laboratory confirmed ZIKV cases.
- Assessing the frequency of infection with other pathogens than ZIKV among cases and controls.

3.3 STATISTICAL ANALYSIS

Statistical tests, as appropriate, will be used to test for statistical differences and describe 95% confidence intervals between case and controls and are described in Table 5.

Table 5: *Statistical analysis recommended for each study objective*

Objective	Outcomes	Statistical analysis

1. Describe and quantify the clinical, laboratory and imaging characteristics and outcome of infants with microcephaly that is associated with Zika virus infection	Demographic characteristics of cases and controls as well as of their mothers (number of cases and controls; median age, sex, area of residence) Clinical spectrum of microcephaly among enrolled cases: microcephaly, cranial facial disproportions , morbidity/mortality, birth weight, vital signs, neurological reflexes, spasticity and tone, hearing disability / vision , epilepsy and seizures, cerebral palsy, contractures, Intrauterine Growth Restriction (IUGR), hospitalization, laboratory analysis results and central nervous system (CNS) imaging characteristics, etc.	Provide descriptive epidemiology for each outcome (mean/median, SD, percentages etc.)
2. Identify and quantify risk factors for microcephaly Identify effect modifiers and interactions	Participant demographics, medical characteristics and exposures to different potential risk factors will be collected using a questionnaire (CRF) and laboratory tests	The risk factors for microcephaly will be determined by calculating the odds of different exposures between microcephaly cases and controls, followed by logistic regression adjusting for potential confounders
3. Estimate the risk of microcephaly associated with ZIKV infection	ZIKV infection will be assessed by a positive test for anti-ZIKV antibody and/or detection of ZIKV nucleic acid using RT-PCR as defined by the standards of the laboratory	The increase of risk of microcephaly if infected with ZIKV that is associated with ZIKV will be estimated by the odds-ratio (OR) of the association between mother infection with ZIKV and microcephaly
4. Estimate the attributable risk of microcephaly associated with infection with ZIKV	ZIKV infection will be assessed by a positive test for anti-ZIKV antibody and/or detection of ZIKV nucleic acid using RT-PCR as defined by the standards of the laboratory	The fraction of microcephaly that is attributable to ZIKV infection will be calculated as follows: $\frac{OR-1}{OR} * Pe$, where *Pe* is the proportion of cases with ZIKV infection

4.0 REPORTING OF FINDINGS

Reports of the results of this study should follow the checklist for case-control studies 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 recruited case and control mother/child pairs and (2) the number of confirmed ZIKV infections or the number of cases with serologic evidence of ZIKV infection among each group.

It is also important to fully document the study design, including the definition of case and control, the approach to ascertainment of ZIKV infection of the mother and the fetus/infant, the laboratory methods used and the outcome measurements.

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

5.0 COMPLEMENTARY STUDIES

We have drafted here a protocol that addresses specific questions relative to the potential association between ZIKV infection and microcephaly. However, additional aspects of microcephaly etiology might be investigated, depending on the study context. Therefore, complementary studies might be considered in association with this protocol, including genetic or toxicology studies.

Furthermore, there is increasing evidence that ZIKV infection in pregnant women may be associated with other congenital disorders in the newborn or fetus than microcephaly, including brainstem dysfunction and other severe cerebral lesions, leading the scientific community to consider the existence of a so-called “Congenital Zika Syndrome” (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). Further studies, especially cohort studies on newborns from mothers that were exposed to ZIKV infection during their pregnancy should provide a reliable clinical definition of this congenital Zika syndrome.

Meanwhile, other groups may be interested in expanding the inclusion criteria for cases, in order to broaden the clinical spectrum of studied congenital disorders. In such cases, researchers might want to consider either adapting several methodological points of the current protocol (especially case definition, inclusion criteria, study objectives and endpoints, outcome measurements and questionnaire), or addressing these major public health question by a complementary study.

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

- Prospective longitudinal cohort study of newborns and infants born to mothers exposed to Zika virus during pregnancy
- Prospective longitudinal cohort study of Zika-infected patients to measure the persistence of Zika virus in body fluids
- 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

6.0 ACKNOWLEDGEMENTS

A large number of individuals were involved in the creation and revision of this protocol. These include: Maria Van Kerkhove (Institut Pasteur), Rebecca Grant (Institut Pasteur), Anna Funk (Institut Pasteur), Sibylle Bernard Stoecklin (Institut Pasteur), Ludovic Reveiz (Pan American Health Organization), Vanessa Elias (Pan American Health Organization), Nathalie Broutet (World Health Organization), Joao Paulo Souza (World Health Organization), Gail Carson (International Severe Acute Respiratory & Emerging Infection Consortium).

Additional review has been provided by: Eric Ohuma (University of Oxford), Nathalie Jolly (Institut Pasteur), Samira Ouchhi (Institut Pasteur), Virginie Pirard (Institut Pasteur), Maggie Brewinski Issacs (NIH National Institute of Child Health and Human Development), Cristina Cassetti (NIH National Institute of Allergy and Infectious Diseases), Hilary Marston (NIH National Institute of Allergy and Infectious Diseases), Anne Yu (US Department of Health and Human Services), Tiffany Locus (US Department of Health and Human Services), and reviewers from World Health Organization Research Project Review Panel (RP2).

A meeting, organized by the Pan American Health Organization and the World Health Organization, was held in Mexico City in June 2016. During this meeting, this protocol was reviewed and discussed. We would like to acknowledge the following participants for their input and expertise during this meeting:

Tarun Dua (World Health Organization), Pablo Duran (Pan American Health Organization/World Health Organization), Democrito de Barros Miranda Filho (Pernambuco, Brazil), Nahida Chaktoura (National Institutes of Health/National Institute of Child Health and Human Development), Devika Dixit (World Health Organization), Thomas Janisch (Heidelberg University Hospital), Gregory Lindenberg (Suriname), Cynthia Moore (US Centers for Disease Control and Prevention), Christina Nelson (US Centers for Disease Control and Prevention), Constanza Vallenas (World Health Organization), Zaida E. Yadon (Pan American Health Organization/World Health Organization).

The biological sampling algorithms, included in Appendix C, were developed by Gail Carson (International Severe Acute Respiratory & Emerging Infection Consortium), Raúl Pardíñaz-Solís (International Severe Acute Respiratory & Emerging Infection Consortium), Jake Dunning (Imperial College London), Marion Koopmans (Erasmus MC, University Medical Center, The Netherland), Van-Mai Lormeau-Cao (Institut Louis Malardé, French Polynesia), Bethan McDonald (Oxford University Hospitals NHS Foundation Trust), Catrin Moore (University of Oxford), Ken Mutton (Public Health England), Nikki Shindo (World Health Organization), Jessica Vanhomwegen (Institut Pasteur, France).

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

7.0 SELECTED REFERENCES

Balm, M. N., C. K. Lee, H. K. Lee, L. Chiu, E. S. Koay and J. W. Tang (2012). "A diagnostic polymerase chain reaction assay for Zika virus." J Med Virol **84**(9): 1501-1505.

Besnard, M., D. Eyrolle-Guignot, P. Guillemette-Artur, S. Lastere, F. Bost-Bezeaud, L. Marcelis, V. Abadie, C. Garel, M. L. Moutard, J. M. Jouannic, F. Rozenberg, I. Leparc-Goffart and H. P. Mallet (2016). "Congenital cerebral malformations and dysfunction in fetuses and newborns following the 2013 to 2014 Zika virus epidemic in French Polynesia." Euro Surveill **21**(13).

Brasil, P., J. P. Pereira, Jr., C. Raja Gabaglia, L. Damasceno, M. Wakimoto, R. M. Ribeiro Nogueira, P. Carvalho de Sequeira, A. Machado Siqueira, L. M. Abreu de Carvalho, D. Cotrim da Cunha, G. A. Calvet, E. S. Neves, M. E. Moreira, A. E. Rodrigues Baiao, P. R. Nassar de Carvalho, C. Janzen, S. G. Valderramos, J. D. Cherry, A. M. Bispo de Filippis and K. Nielsen-Saines (2016). "Zika Virus Infection in Pregnant Women in Rio de Janeiro - Preliminary Report." N Engl J Med.

Broutet, N., F. Krauer, M. Riesen, A. Khalakdina, M. Almiron, S. Aldighieri, M. Espinal, N. Low and C. Dye (2016). "Zika Virus as a Cause of Neurologic Disorders." N Engl J Med **374**(16): 1506-1509.

Calvet G. et al. (2016). "Detection and sequencing of Zika virus from amniotic fluid of foetuses with microcephaly in Brazil: a case study." Lancet Infect Dis **16**: 653-660.

Cao-Lormeau, V. M., A. Blake, S. Mons, S. Lastere, C. Roche, J. Vanhomwegen, T. Dub, L. Baudouin, A. Teissier, P. Larre, A. L. Vial, C. Decam, V. Choumet, S. K. Halstead, H. J. Willison, L. Musset, J. C. Manuguerra, P. Despres, E. Fournier, H. P. Mallet, D. Musso, A. Fontanet, J. Neil and F. Ghawche (2016). "Guillain-Barre Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study." Lancet **387**(10027): 1531-1539.

Cauchemez, S., M. Besnard, P. Bompard, T. Dub, P. Guillemette-Artur, D. Eyrolle-Guignot, H. Salje, M. D. Van Kerkhove, V. Abadie, C. Garel, A. Fontanet and H. P. Mallet (2016). "Association between Zika virus and microcephaly in French Polynesia, 2013-15: a retrospective study." Lancet.

Driggers, R. W., C. Y. Ho, E. M. Korhonen, S. Kuivanen, A. J. Jaaskelainen, T. Smura, A. Rosenberg, D. A. Hill, R. L. DeBiasi, G. Vezina, J. Timofeev, F. J. Rodriguez, L. Levanov, J. Razak, P. Iyengar, A. Hennenfent, R. Kennedy, R. Lanciotti, A. du Plessis and O. Vapalahti (2016). "Zika Virus Infection with Prolonged Maternal Viremia and Fetal Brain Abnormalities." N Engl J Med.

Faye, O., O. Faye, D. Diallo, M. Diallo, M. Weidmann and A. A. Sall (2013). "Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes." Virol J **10**: 311.

Faye, O., O. Faye, A. Dupressoir, M. Weidmann, M. Ndiaye and A. Alpha Sall (2008). "One-step RT-PCR for detection of Zika virus." J Clin Virol **43**(1): 96-101.

Kleber de Oliveira, W., J. Cortez-Escalante, W. T. De Oliveira, G. M. do Carmo, C. M. Henriques, G. E. Coelho and G. V. Araujo de Franca (2016). "Increase in Reported Prevalence of Microcephaly in Infants Born to Women Living in Areas with Confirmed Zika Virus Transmission During the First Trimester of Pregnancy - Brazil, 2015." MMWR Morb Mortal Wkly Rep **65**(9): 242-247.

Lanciotti, R. S., O. L. Kosoy, J. J. Laven, J. O. Velez, A. J. Lambert, A. J. Johnson, S. M. Stanfield and M. R. Duffy (2008). "Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007." Emerg Infect Dis **14**(8): 1232-1239.

MERG. (2016). "Microcephaly in Infants, Pernambuco State, Brazil." Emerg Infect Dis. **22**(6).

Mlakar, J., M. Korva, N. Tul, M. Popovic, M. Poljsak-Prijatelj, J. Mraz, M. Kolenc, K. Resman Rus, T. Vesnaver Vipotnik, V. Fabjan Vodusek, A. Vizjak, J. Pizem, M. Petrovec and T. Avsic Zupanc (2016). "Zika Virus Associated with Microcephaly." N Engl J Med **374**(10): 951-958.

Musso, D. and D. J. Gubler (2016). "Zika Virus." Clin Microbiol Rev **29**(3): 487-524.

Schuler-Faccini, L., E. M. Ribeiro, I. M. Feitosa, D. D. Horovitz, D. P. Cavalcanti, A. Pessoa, M. J. Doriqui, J. I. Neri, J. M. Neto, H. Y. Wanderley, M. Cernach, A. S. El-Husny, M. V. Pone, C. L. Serao, M. T. Sanseverino and F. Brazilian Medical Genetics Society-Zika Embryopathy Task (2016). "Possible Association Between Zika Virus Infection and Microcephaly - Brazil, 2015." MMWR Morb Mortal Wkly Rep **65**(3): 59-62.

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

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

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

INFORMATION FOR THE PARTICIPANT

Dear Mrs/Ms/Miss,

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

Case-control study to assess potential risk factors related to microcephaly including Zika virus infection during pregnancy

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

INFORMATION

This document is meant to provide you with the written information necessary to make a decision regarding your participation and that of your newborn 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, if anything is unclear, or if you would like more information. Please take your time to think about 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 understood that infection with Zika during pregnancy may harm the unborn child, sometimes causing microcephaly (small head size in the fetus) or other congenital abnormalities. The role of Zika virus in the development of these abnormalities is not yet clearly understood. We are asking you and your baby to participate in this study to understand these main questions.

The main objectives of this study are to:

- Estimate the risk of microcephaly associated with ZIKV infection according gestational age at infection
- Identify and quantify risk factors for microcephaly
- Identify effect modifiers and interactions
- Estimate the attributable risk of microcephaly associated with ZIKV infection, and
- Describe and quantify the clinical, laboratory and imaging characteristics and outcome of infants with microcephaly that is associated with ZIKV infection

Comment: Describe in 1-2 sentences specific details about the location of the study, the number of participants, etc.

RESEARCH PROCESS

Comment: The implemented informed consent must explain all physical, neurological and biological tests that will be performed throughout the study, 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.

If you agree to participate in this study then you will be asked to answer questions about your health and daily life, such as the type of protection measures you use against mosquitoes. Following delivery, you will have approximately 7.5mL of blood drawn (less than 2 teaspoons) by a trained medical person. At this time, a blood sample will be collected from your baby (up to 3 mL/ approximately ½ teaspoon) as well as non-invasive urine sample. If recommended by the clinician, your baby may also have a sample of cerebrospinal fluid (up to 1 mL/approximately 1/5 teaspoon) taken. The samples collected from you and your baby will be tested for Zika virus and other pathogens known to cause congenital abnormalities if the infection occurs during pregnancy (for example, rubella, toxoplasmosis and cytomegalovirus). A medical doctor will inform you of the results of your tests and those of your baby.

There is a risk that you or your baby may 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. Babies often get upset when they have their blood of cerebrospinal fluid drawn, but this will only be collected if part of the normal care recommended by your doctor.

In the case of a loss of pregnancy (miscarriage, induced abortion, stillbirth etc.), we will ask to collect a blood sample and a sample from the placenta to test for Zika virus and other pathogens. A medical doctor will inform you of the results of this testing.

RISKS AND BENEFITS OF YOUR PARTICIPATION

This research does not present any foreseeable risk for you or your baby; no procedure will be done on you that is not designed for the purpose of this study. Furthermore, all procedures that are done will follow the current standard of care for infants born to mothers who were exposed to Zika virus during pregnancy. The primary benefit of this study is the intensified assessment and extended medical care (i.e., beyond routine) for your infant- who may have had exposure to Zika virus. This will allow for timely detection in the event of the detection of any abnormality.

RESEARCH RESULTS

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

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

GENETIC TESTING

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

CONFIDENTIALITY AND TREATMENT OF COMPUTERIZED DATA

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

If, during the course of the study, you no longer wish to participate or you no longer wish for your baby to continue 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

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

At any time, and without consequence to your participation in the present study or to your medical care, you may withdraw your consent for the use of your samples for these other research objectives. This can be done simply by contacting the health care professional who is supervising your participation in this study.

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

INFORMED CONSENT OF 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:

Case-control study to assess potential risk factors related to microcephaly including Zika virus infection during 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 enrolment 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 and the outcome of my pregnancy.
- ☐ 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 computerized 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 authorization 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:

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

Purpose of the standardized questionnaire and instructions for its use

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

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 fetus/newborn 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 newborns), and the review of hospital charts.
- Patient ID codes should be filled in on all pages of the questionnaire (newborn 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 fullstops, 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 of 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: ____/____/_____

Interviewer: _____

IDENTIFICATION: STUDY PARTICIPANT

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

CASE/CONTROL STATUS

CASE	Yes	No
Aborted fetus with signs of microcephaly as a result of a regular ultrasound examination	☐	☐
Live infant diagnosed with microcephaly after 24 hours following birth	☐	☐
CONTROL	**Yes**	**No**
Aborted fetus with no signs of microcephaly as a result of a regular ultrasound examination	☐	☐
Live infant without evidence of microcephaly	☐	☐

VERIFICATION OF ELIGIBILITY

INCLUSION CRITERIA	Yes	No
Any newborn/fetus whose mother or legal representative or guardian provides written informed consent	☐	☐
EXCLUSION CRITERIA	**Yes**	**No**
Newborn/infant's mother (or her legal representative or guardian) is unable to provide written informed consent	☐	☐
Contraindication to venipuncture	☐	☐

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

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

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

Interviewer: _____

PART I - NEWBORN

1) DEMOGRAPHICS

Sex:	☐Male ☐ Female ☐ Unknown
Date of birth (DD/MM/YYYY):	____ / ____ / 20 ____
Time of birth (HH:MM 24 hr):	____ : ____
Gestational age at birth:	____ weeks ____ days
Basis of gestational age estimation at birth:	☐ Last menstrual period ☐ Ultrasound ☐ Assisted reproduction ☐ Other (specify): _____
Birth order	☐ Singleton ☐ Twin I ☐ Twin II ☐ Triplet I ☐ Triplet II ☐ Triplet III ☐ Other:_____
Ethnicity	(Add check boxes according to national guidelines)

2) NEWBORN 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

3) PHYSICAL EXAMINATION AT BIRTH

VITAL SIGNS OF NEWBORN

Maximum 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

Oedema - If yes, describe affected parts:	☐Yes ☐No ☐Unknown
Rash - If yes, describe type of rash - If yes, describe body distribution of rash - If yes, date of rash onset (DD/MM/YYY):	☐Yes ☐No ☐Unknown __ __ / __ __ / 20 __ __
Other abnormal skin and/or subcutaneous tissue condition - If yes, describe: - If yes, indicate date of onset (DD/MM/YYYY):	☐Yes ☐No ☐Unknown __ __ / __ __ / 20 __ __

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

BIRTH ABNORMALITIES PRESENT AT BIRTH

	Abnormality				
	Yes	**No**	**Type**	**Localization**	**Description**
Head	☐	☐			
Neck	☐	☐			
Trunk	☐	☐			
Chest/abdomen	☐	☐			
Upper limb	☐	☐			
Lower limb	☐	☐			

4) COMPLEMENTARY DIAGNOSTIC TESTS

IMAGING

If abnormal, please describe abnormality and enclose images if possible.

Neuroimaging	**Results**	**If abnormal, please summarize key results from report:**		**Images attached**	**Report attached**
		Localization	**Findings**		
Cranial ultrasound scan	☐ Normal ☐ Abnormal			☐ Yes ☐ No	☐ Yes ☐ No

	☐ Not done				
CT Scan	☐ Normal ☐ Abnormal ☐ Not done			☐ Yes ☐ No	☐ Yes ☐ No
MRI	☐ Normal ☐ Abnormal ☐ Not done			☐ Yes ☐ No	☐ Yes ☐ No
Other (specify type of test):	☐ Normal ☐ Abnormal ☐ Not done			☐ Yes ☐ No	☐ Yes ☐ No

VISUAL AND HEARING EVALUATION

Test	**Result**	**If abnormal, describe abnormality:**
Fundoscopy	☐ Normal ☐ Abnormal ☐ Not done	
Red reflex	☐ Present ☐ Absent ☐ Not done	
Cataract	☐ Normal ☐ Abnormal ☐ Not done	
Chorioretinitis	☐ Present ☐ Absent ☐ Not done	
Hearing test, please specify test used:	☐ Normal ☐ Abnormal ☐ Not done	

5) BIOLOGICAL AND MICROBIOLOGICAL ANALYSIS

BIOLOGICAL ANALYSIS

When were specimens collected:	☐ At birth ☐ After birth (specify date or day of life): _____ ☐ Other, specify: _____

(Please complete a new form each time in case of repeated tests)

Test	Date (DD/MM/YYYY)	Value	Unit
Hemoglobin	____/____/_____		
Hematocrit	____/____/_____		
Total leukocytes	____/____/_____		
- Neutrophils (absolute count)	____/____/_____		
- Lymphocytes	____/____/_____		
- Monocytes	____/____/_____		
- Eosinophils	____/____/_____		
- Basophils	____/____/_____		
Platelets	____/____/_____		
PT (NIR)	____/____/_____		
Total bilirubin	____/____/_____		
Conjugated bilirubin	____/____/_____		
C reactive protein	____/____/_____		
Glucose	____/____/_____		
AST	____/____/_____		**IU**
ALT	____/____/_____		**IU**
Creatinine kinase	____/____/_____		
Thiamine	____/____/_____		
Thyroid-stimulating hormone	____/____/_____		
Sodium	____/____/_____		
Potassium	____/____/_____		
Phosphate	____/____/_____		
Magnesium	____/____/_____		

Vitamin B1	____/____/_____		
Newborn metabolic screen	____/____/_____	Normal: ☐ Yes ☐ No	
Lead	____/____/_____		
Mercury	____/____/_____		
Arsenic	____/____/_____		
Other (specify):	____/____/_____		

MICROBIOLOGICAL ANALYSIS

When were specimens collected:	☐ During pregnancy ☐ At the time of delivery ☐ Other, specify: _____

(Please complete a new form each time in case of repeated tests)

Pathogen	Type of specimen	Date of collection: (DD/MM/YYYY)	Type of test	Result
Zika virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/____/_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Dengue virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/____/_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Chikungunya virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/____/_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Cytomegalovirus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/____/_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
LCMV virus	☐ Blood/Serum		☐ RT-PCR	☐ Positive

	☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ IgM ☐ IgG ☐ Other: _____	☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Herpes simplex virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ PCR result: ____ copies/ml
HIV	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
HTLV	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Rubella virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Varicella/Zona virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Toxoplasma sp.	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Treponema pallidum	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
BVVD	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other:	____ / ____ /_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result:

	_____			____ copies/ml
Other, specify: _____	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml

6) OUTCOME AT DISCHARGE

Date of discharge (DD/MM/YYYY)**:**	____ / ____ /_____
Newborn's 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
If deceased please specify date of death:	____ / ____ /_____
Was an autopsy performed: **Report attached:**	☐Yes ☐No ☐ Unknown Date of autopsy (DD/MM/YYYY): ____ / ____ /_____ ☐Yes ☐No
Was placenta analyzed?	☐Yes ☐No ☐ Unknown
Placental weight:	_____ ☐grams ☐Other units (specify):_____ ☐ Unknown
Placental calcifications:	☐Yes ☐No ☐ Unknown
Other placental abnormalities: (describe)	☐Yes ☐No ☐ Unknown

PART I COMPLETED BY

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

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

Interviewer: _____

PART II - MOTHER

1) DEMOGRAPHICS OF MOTHER

Date of birth (DD/MM/YYYY)**:**	____ / ____ / _____
Area of residence: (Or, enter GPS coordinates):	_____ _ _ . _ _ _ _ _ S, _ _ _ . _ _ _ _ _ E
Maternal language	(Add check boxes here)
Social-professional category Comment: Add occupation/professional categories that are appropriate for the country implementing the study	☐ Student ☐ Farmer ☐ Artisan, merchant, business owner ☐ Highly qualified professional (management) ☐ Employee ☐ Labourer/factory worker ☐ Without profession ☐ Retired ☐ Does not wish to respond ☐ Other (specify): _____
Ethnicity	(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:	 _____ _____ _____ _____ ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown _____

–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: 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 ☐ 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

2) PHYSICAL EXAMINATION

Body weight before pregnancy:	_____ (kg)
Current body weight:	_____ (kg)
Height:	_____ (cm)
Body temperature:	_____ (°C)
Respiratory rate:	_____ (kg)
Heart rate:	_____ (bpm)
Arterial blood pressure: Systolic/ Diastolic	_____ (mmHg)
Pulse:	_____ (bpm)
Pulse oximetry:	_____ (%)
Clinical characteristics indicative of infectious illness: - If yes, indicate symptoms: (tick all that apply)	☐ Yes ☐ No ☐ Unknown ☐ Fever ☐ Chills ☐ Nausea or vomiting ☐ Diarrhoea ☐ Muscle pains ☐ Joint pains ☐ Skin rash ☐ Headache ☐ Pain behind eyes ☐ Stiff neck ☐ Confusion ☐ Abdominal pain ☐ Coughing ☐ Runny nose ☐ Sore throat ☐ Calf pain ☐ Pruritus ☐ Bleeding

	☐ Conjunctival hyperaemia ☐ Petechiae ☐ Limb swelling ☐ Other: specify _____
Other clinical symptoms: - If yes, specify:	☐ Yes ☐ No ☐
Blood type:	☐ A ☐ B ☐ O ☐ Unknown
Rh type:	☐ +ve ☐ -ve ☐ Unknown

3) INFORMATION ON THE COURSE OF THE PREGNANCY

Date of the start of the pregnancy (DD/MM/YYYY): (Estimation by ultrasound)	____ / ____ /_____
Estimated date of delivery (DD/MM/YYYY):	____ / ____ /_____
Medically assisted reproduction:	☐ Yes ☐ No
Any complications during pregnancy? - If yes, indicate: (Tick all that apply)	☐ Yes ☐ No ☐ Threatened miscarriage ☐ Threatened premature delivery ☐ Pregnancy-induced hypertension ☐ Pregnancy-induced diabetes ☐ Hemorrhaging in 2nd or 3rd trimester ☐ Pre-eclampsia / Eclampsia ☐ HELLP Syndrome ☐ Intrauterine Growth Restriction ☐ Other, specify: _____
Clinical characteristics indicative of infectious illness during pregnancy: - 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 _____
Date of onset of symptoms (DD/MM/YYYY):	____ / ____ /_____
Specimen collection for testing during pregnancy: - If yes, indicate date of collection (DD/MM/YYYY): - If yes, indicate nature of specimen:	☐ Yes ☐ No ☐ Unknown ____ / ____ /_____ ☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other, specify: _____
Fetal ultrasound during pregnancy:	☐ Yes ☐ No ☐ Unknown

If yes, please fill in the table below:

Date of ultrasound: (DD/MM/YYYY)	__ / __ /___	__ / __ /___	__ / __ /___	__ / __ /___	__ / __ /___
Term: (in WA)					
Intraparietal diameter: (mm) (%)	____ ____	____ ____	____ ____	____ ____	____ ____
Head circumference: (mm) (%)	____ ____	____ ____	____ ____	____ ____	____ ____
Cerebellum - transversal view: (mm) (%)	____ ____	____ ____	____ ____	____ ____	____ ____
Corpus callosum - sagittal view: (mm) (%)	____ ____	____ ____	____ ____	____ ____	____ ____
Lateral ventricle: (mm) (%)	____ ____	____ ____	____ ____	____ ____	____ ____
Height of cerebellar vermis: (mm) (%)	____ ____	____ ____	____ ____	____ ____	____ ____
Placenta thickness: (mm)					
Amniotic fluid index: (mm)					
Measure of Cisterna Magna: (mm)					
Calcifications (site): If yes, specify location:	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Fluid accumulation: If yes, specify location:	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Intestinal hyperechogenicity:	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Periventricular hyperechogenicity:	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Malformation: If yes, specify:	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No

Are the parents blood-related?	☐ Yes ☐ No ☐ Unknown

4) PREGNANCY OUTCOME:

Date of end of pregnancy: (DD/MM/YYYY)	____ / ____ / _____
If delivery, complete the fields below:	
Onset of labor: (tick one box only)	☐ Spontaneous ☐ Induced ☐ No labor ☐ Unknown
Prelabor premature rupture of membranes (PPROM):	☐ Yes ☐ No ☐ Unknown
Mode of delivery:	☐ Vaginal spontaneous ☐ Vaginal assisted (e.g. forceps, vacuum) ☐ Emergency caesarean section ☐ Scheduled caesarean section ☐ Assisted breech or breech extraction
If labor was induced, or caesarean section performed, please specify reason:	
Analgesia:	☐None ☐General ☐Epidural ☐Spinal
Fetal presentation at delivery:	☐ Cephalic ☐ Breech ☐ Other (specify):
Color of amniotic fluid:	☐ Clear ☐ Bloody ☐ Meconium-stained ☐ Other (specify): ☐ Unknown

5) FAMILY AND OBSTETRIC HISTORY

5.1 FAMILY HISTORY

Known familial genetic disorders: - If yes, specify:	☐ Yes ☐ No ☐ Unknown
Syndromic abnormalities identified by a physician? - If yes, specify:	☐ Yes ☐ No ☐ Unknown

5.2 OBSTETRIC HISTORY

Excluding the current pregnancy, indicate number of: - Previous pregnancies - Previous abortions - Livebirths - Died during first week of life - Died after first week of life - Alive	

6) BIOLOGICAL AND MICROBIOLOGICAL ANALYSIS

6.1 BIOLOGICAL ANALYSIS

When were specimens collected:	☐ At birth ☐ At the time of delivery ☐ Other, specify: _____

(Please complete a new form each time in case of repeated tests)

Test	**Date** (DD/MM/YYYY):	**Value**	**Unit**
Hemoglobin	____ / ____ / _____		
Hematocrit	____ / ____ / _____		
Total leukocytes	____ / ____ / _____		

- Neutrophiles (absolute count)	____ / ____ / _____		
- Lymphocytes	____ / ____ / _____		
- Monocytes	____ / ____ / _____		
- Eosinophils	____ / ____ / _____		
- Basophils	____ / ____ / _____		
Platelets	____ / ____ / _____		
TP (seconds)	____ / ____ / _____		
Total bilirubin	____ / ____ / _____		
Conjugated bilirubin	____ / ____ / _____		
C reactive protein	____ / ____ / _____		
Glucose	____ / ____ / _____		
AST	____ / ____ / _____		**IU**
ALT	____ / ____ / _____		**IU**
Creatinine kinase	____ / ____ / _____		
Thiamine	____ / ____ / _____		
Thyroid-stimulating hormone	____ / ____ / _____		
Sodium	____ / ____ / _____		
Potassium	____ / ____ / _____		
Phosphate	____ / ____ / _____		

Magnesium	____ / ____ / _____		
Vitamin B1	____ / ____ / _____		
Lead	____ / ____ / _____		
Mercury	____ / ____ / _____		
Arsenic	____ / ____ / _____		
Other (specify):	____ / ____ / _____		
Other (specify):	____ / ____ / _____		

6.2 MICROBIOLOGICAL TESTING

When were specimens collected:	☐ At birth ☐ At the time of delivery ☐ Other, specify: _____

(Please complete a new form each time in case of repeated tests)

Test		**Date of testing** (DD/MM/YYYY)	**Result**
RT-PCR ZIKA: **- Blood** **- Urine** **- Placenta**	☐ Yes ☐ No ☐ Yes ☐ No ☐ Yes ☐ No	____ / ____ / _____ ____ / ____ / _____ ____ / ____ / _____	☐ Positive ☐ Negative ☐ Positive ☐ Negative ☐ Positive ☐ Negative
ZIKA serology	☐ Yes ☐ No	____ / ____ / _____	IgM: ☐ Positive ☐ Negative ☐ Unknown
			IgG: ☐ Positive ☐ Negative ☐ Unknown
Dengue serology	☐ Yes ☐ No	____ / ____ / _____	IgM: ☐ Positive ☐ Negative ☐ Unknown
			IgG: ☐ Positive ☐ Negative ☐ Unknown
Chikungunya virus			IgM: ☐ Positive ☐ Negative

			☐ Unknown
Toxoplasmosis	☐ Yes ☐ No	____ / ____ / _____	☐ Positive ☐ Negative
Rubella	☐ Yes ☐ No	____ / ____ / _____	☐ Positive ☐ Negative
Cytomegalovirus	☐ Yes ☐ No	____ / ____ / _____	☐ Positive ☐ Negative
LCMV	☐ Yes ☐ No	____ / ____ / _____	☐ Positive ☐ Negative
Syphilis	☐ Yes ☐ No	____ / ____ / _____	☐ Positive ☐ Negative
Herpes simplex	☐ Yes ☐ No	____ / ____ / _____	☐ Positive ☐ Negative
HIV	☐ Yes ☐ No	____ / ____ / _____	☐ Positive ☐ Negative
HTLV	☐ Yes ☐ No	____ / ____ / _____	☐ Positive ☐ Negative
Varicella/Zona virus	☐ Yes ☐ No	____ / ____ / _____	☐ Positive ☐ Negative
Treponema pallidum	☐ Yes ☐ No	____ / ____ / _____	☐ Positive ☐ Negative
BVDV	☐ Yes ☐ No	____ / ____ / _____	☐ Positive ☐ Negative
Other (specify)	☐ Yes ☐ No	I__I__I/I__I__I/I__I__I ____ / ____ / _____	☐ Positive ☐ Negative

PART II COMPLETED BY

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

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

Interviewer: _____

PART III – EXPOSURES DURING PREGNANCY

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
Type of residence during pregnancy:	☐ Apartment ☐House ☐ Other, specify: _____
Location of residence during pregnancy:	☐ City/Urban ☐ Rural/Country-side ☐ Other, specify: _____
Air conditioning in residence during pregnancy: (tick all that apply)	☐ Local air conditioning (at least 1 room) ☐ Fans ☐ None

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

Do you use insecticides to remove mosquito larvae from your home?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
Do you use other methods to rid you home of mosquitos? - If yes, indicate here which methods you've used:	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always

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

Vaccination during pregnancy:	☐ Yes ☐ No ☐ Unknown

- If yes, indicate:	☐ Information verified on vaccine card ☐ Information provided verbally
- If yes, specify type and date: (DD/MM/YYYY)	____/ ____/________ ____/ ____/________
Specific or concomitant treatments(s) during the last six weeks: - If yes specify:	☐ Yes ☐ No ☐ Unknown

Medication		**Indication**	**Start date** (DD/MM/YYYY)	**End date** (DD/MM/YYYY)
International Non-Proprietary Name	**Daily dosage**			
	_______ (unit ______) ☐ Upon request		____/ ____/______	____/ ____/_____
	_______ (unit ______) ☐ Upon request		____/ ____/______	____/ ____/_____
	_______ (unit ______) ☐ Upon request		____/ ____/______	____/ ____/_____
	_______ (unit ______) ☐ Upon request		____/ ____/______	____/ ____/_____
	_______ (unit ______) ☐ Upon request		____/ ____/______	____/ ____/_____
	_______ (unit ______) ☐ Upon request		____/ ____/______	____/ ____/_____
	_______ (unit ______) ☐ Upon request		____/ ____/______	____/ ____/_____

The following questions aim to collect sensitive lifestyle information about the mother during her pregnancy

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/Cocaine ☐ Cannabis ☐ Opiods ☐ 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

PART III COMPLETED BY

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

INTERVIEW DATA VALIDATED BY

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

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

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

Sampling algorithm for research purposes

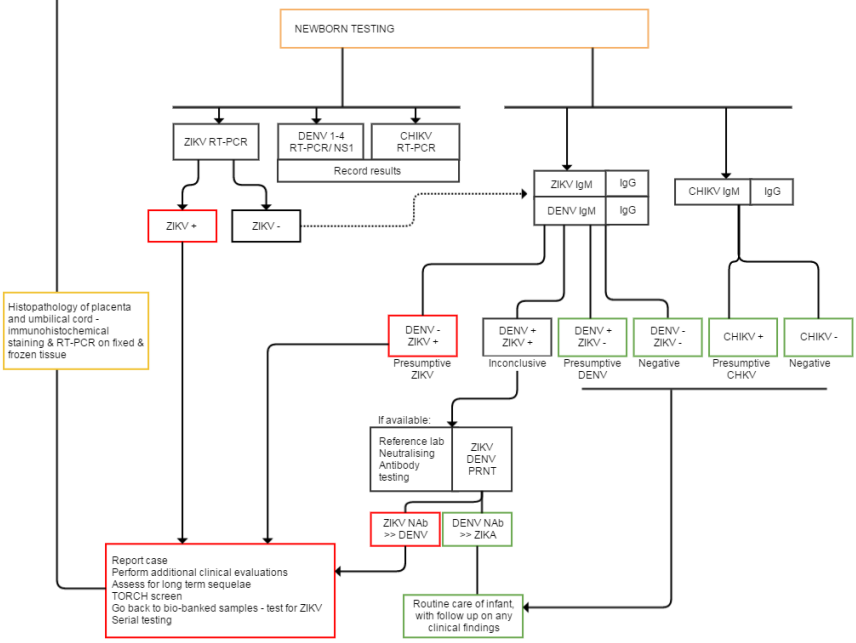
Tier Sampling approach

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)

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

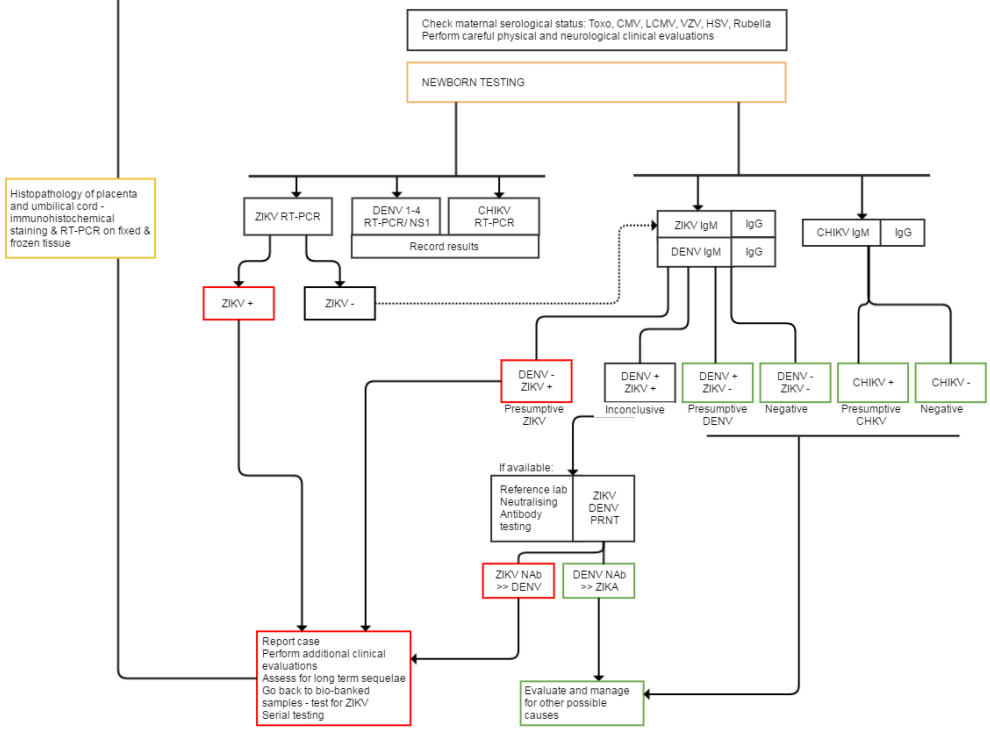
Tier Sampling approach

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)

DRAFT

Version 5
6/MAY/2016

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

Sampling algorithm for research purposes

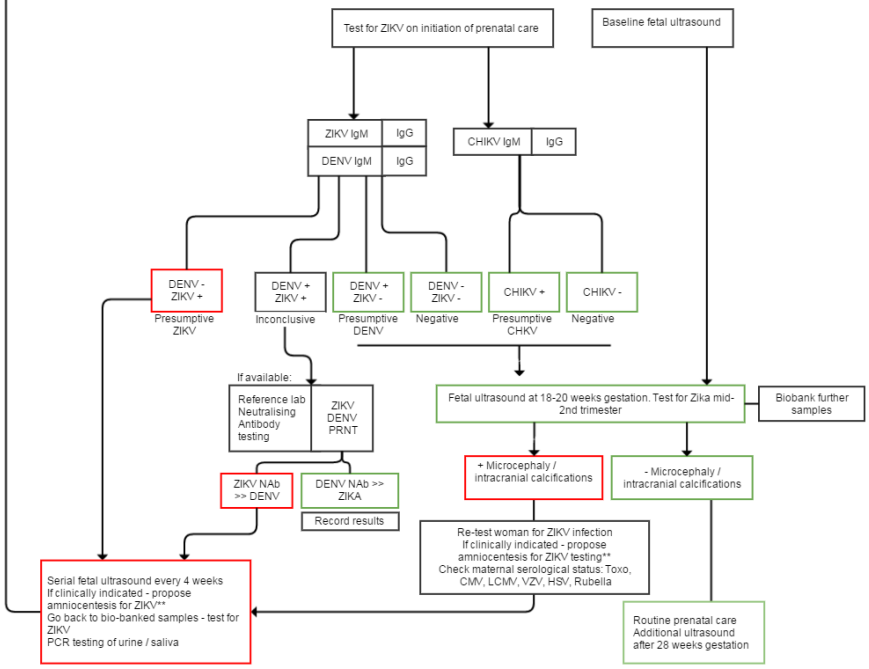
Tier Sampling approach

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

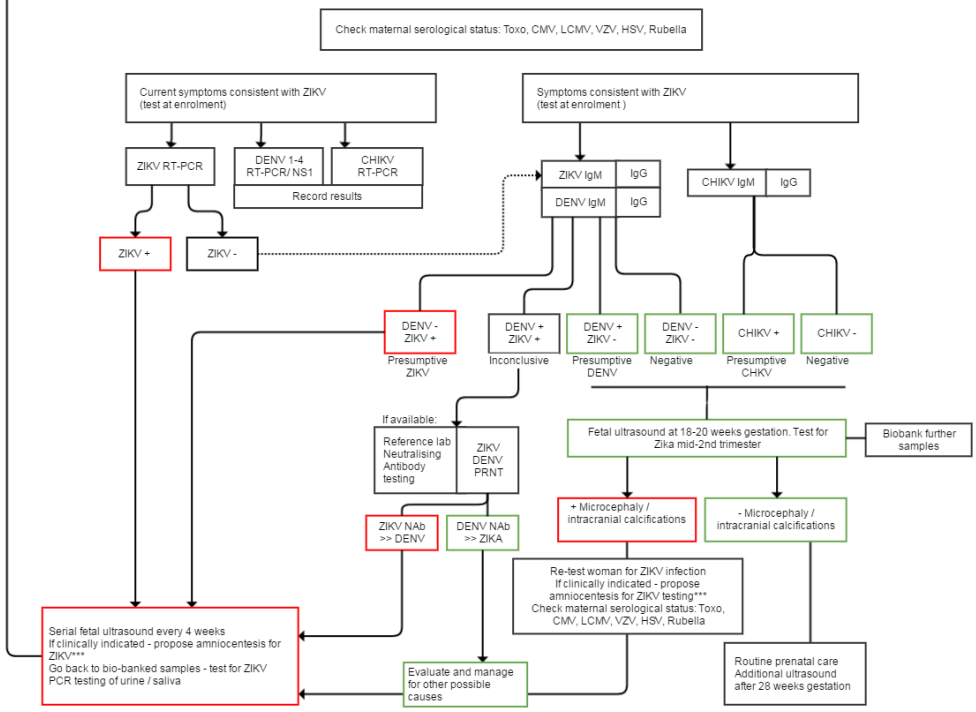
Sampling algorithm for research purposes

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)

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	