Disclaimer

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

The final version of this standardized protocol: *Prospective longitudinal cohort study of newborns and infants born to mothers exposed to Zika virus during pregnancy* will be published as soon as the ethical review has been completed.

Standardized Protocol:

Prospective longitudinal cohort study of newborns and infants born to mothers exposed to Zika virus 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

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Acknowledgements

This protocol outline is based on the cohort study protocols generated by the Microcephaly Epidemic Research Group (MERG), the US Centers for Disease Control and Prevention, the Centre d'Investigations Cliniques (CIC) Antilles-Guyane Inserm 1424 (i.e., Caribbean Cohort studies) and the International Research Consortium of Dengue Risk Assessment, Management and Surveillance (IDAMS).

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.

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PROTOCOL SUMMARY

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

The geographic scope of the current ZIKV outbreak is vast, extending throughout the Americas and the Caribbean and into parts of Africa. The use of standardized research protocols will ensure that results from these studies can be compared across regions and countries and can potentially improve the quality of observational studies by identifying and minimizing biases.

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

The protocol described below is a prospective longitudinal cohort study, designed to collect data to estimate the risk of conditions related to a pathogen in a population. This standardized study protocol outlines methods to follow a cohort of infants born to ZIKV infected and ZIKV non-infected women in order to identify, describe and quantify the spectrum of congenital manifestations, including microcephaly, among the infants included in this cohort. The data collected from this study will be used to refine and update recommendations for surveillance and case definitions for microcephaly, to help understand spread, severity and spectrum of the disease and to adapt public health measures, especially for pregnant women and couples planning a pregnancy.

Other protocols currently under development include:

- Case-control study to assess potential risk factors related to microcephaly including Zika virus infection
- Case-control study to assess potential risk factors related to Guillain-Barré Syndrome including Zika virus infection
- Prospective longitudinal cohort study of women and newborns exposed to Zika virus during the course of pregnancy
- Prospective longitudinal cohort study of Zika-infected patients to measure the persistence of Zika virus in body fluids
- Cross-sectional seroprevalence study of Zika virus infection in the general population

- Clinical characterization protocol for Zika virus infection in the context of co-circulating arboviruses

Study groups may decide to implement several protocols during a ZIKV epidemic. In this case, participants may be enrolled in several studies (e.g. cohort of pregnant women and cohort of newborns or clinical characterization study). However, pregnant women should not be enrolled in multiple cohort studies of pregnant women and each study group needs to consider carefully the burden on each participant.

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

CONTENTS

LIST OF ABBREVIATIONS

ABR	Auditory brainstem response
CGA	Corrected gestational age
CHIKV	Chikungunya virus
CIC	Centre d'Investigations Cliniques
CMV	Cytomegalovirus
CONSISE	Consortium for the Standardization of Influenza Seroepidemiology
CSF	Cerebrospinal fluid
CT	Computed tomography
CNS	Central nervous system
DENV	Dengue virus
HIV	Human Immunodeficiency virus
HSV	Herpes Simplex virus
IDAMS	International Research Consortium of Dengue Risk Assessment, Management and Surveillance
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHR	International Health Regulations
IRB	Institutional Review Board
ISARIC	International Severe Acute Respiratory and Emerging Infection Consortium
LCMV	Lymphocytic choriomeningitis virus
MERG	Microcephaly Epidemic Research Group
MRI	Magnetic resonance imaging
OAE	Otoacoustic emissions
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
YFV	Yellow Fever virus
ZIKV	Zika virus

1.0 INTRODUCTION

In the months that have followed the WHO declaration of a Public Health Emergency of International Concern on 1st February 2016, increasing evidence of the association between exposure to ZIKV in pregnant women and microcephaly and other congenital defects in the fetus, has been published (Besnard, Eyrolle-Guignot et al. 2016, Brasil, Pereira et al. 2016, Broutet, Krauer et al. 2016, Cauchemez, Besnard et al. 2016, Driggers, Ho et al. 2016, Kleber de Oliveira, Cortez-Escalante et al. 2016, Mlakar, Korva et al. 2016, Musso and Gubler 2016, Schuler-Faccini, Ribeiro et al. 2016).

ZIKV RNA has 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). As has been seen with other infections (i.e., TORCHS infections - toxoplasmosis, other (e.g., varicella, etc.), rubella, cytomegalovirus, herpes, HIV, syphilis) which can be transmitted mother-to-child and which cause congenital defects such as microcephaly, ZIKV exposure during pregnancy may cause a range of possible effects, in addition to microcephaly (Jones, Lopez et al. 2003, Naing, Scott et al. 2016, Yazigi, De Pecoulas et al. 2016). Other complications in newborns, such as brainstem dysfunction and severe cerebral lesions, have been reported with ZIKV exposure in-utero (Besnard, Eyrolle-Guignot et al. 2016, Brasil, Pereira et al. 2016, Broutet, Krauer et al. 2016, Driggers, Ho et al. 2016, Musso and Gubler 2016).

However, the spectrum of associated adverse pregnancy outcomes and congenital defects has not yet been clearly described or quantified. In addition, other abnormalities which may develop during the first few months or years of life are unknown. Drawing on the example of other maternal viruses that are known to affect the fetus in-utero (e.g., cytomegalovirus (CMV), toxoplasmosis, rubella), it is possible that various degrees of cognitive, developmental and sensory abnormalities may arise in infants who appear healthy at birth (Jones, Lopez et al. 2003, Naing, Scott et al. 2016, Townsend, Forsgren et al. 2013, Yazigi, De Pecoulas et al. 2016).

The following prospective cohort study protocol is a follow-up of a minimum of two years of infants born to mothers exposed to ZIKV during pregnancy in order to address the following public health questions:

1. What are the characteristics, grade of neurological impairment, evolution, complications, and mortality of newborns born to mothers exposed to ZIKV during pregnancy?
2. What are the longer-term health consequences for infants born, both with and without microcephaly, to mothers exposed to ZIKV during pregnancy?

Comment: Although this newborn cohort protocol is a standalone protocol, it is strongly recommended that the implementation of this protocol be preceded by a cohort study of pregnant women exposed to ZIKV. If this protocol follows the cohort study of pregnant women, the infants born to the women included in that cohort study could represent the cohort for the protocol described below.

Comment: Technical, financial or capacity limitations in country may mean that including all infants born to pregnant women exposed to ZIKV may not be feasible. In this case, a cohort study of newborns with fetal or other abnormalities may be conducted. However, this study design may introduce selection bias, and will no

longer be measuring the risk of adverse fetal outcomes of ZIKV infection during pregnancy as described in this protocol. This will exclude the revised study from inclusion in any aggregate/pooled analysis.

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

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

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

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 epidemiologic study will be used to refine and update recommendations for surveillance and case definitions for ZIKV and related conditions, to help understand the spread, severity and spectrum of ZIKV infection and to adapt public health measures, particularly for pregnant women and couples planning a pregnancy.

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

- Identify, describe and quantify the spectrum of congenital manifestations, including microcephaly, in the live newborns born to ZIKV infected and ZIKV non-infected women
- Compare post-natal incidence of developmental abnormalities and outcomes between live newborns born to ZIKV infected and ZIKV non-infected women
- Evaluate the prognosis of babies born with microcephaly and other congenital abnormalities associated with ZIKV infection in the mother
- Estimate the relative risk of abnormalities or outcomes in the fetuses/newborns born to women infected with ZIKV compared to those born to women not infected with ZIKV

Comment: Cohort studies, such as the one described here, provide the opportunity to assess **secondary objectives.** These secondary objectives may be added to the protocol, as defined by the research group and as informed by the outbreak characteristics and by the local context.

2.0 STUDY PROCEDURES

Overview: This is a prospective longitudinal cohort study that follows the newborns born to mothers exposed to ZIKV, for a minimum of 2 years after birth. Ideally, the cohort study will follow the development of the children up to the age of 5 years, as resources permit.

The figure below presents the design of the cohort study, as it relates to the protocol of pregnant women:

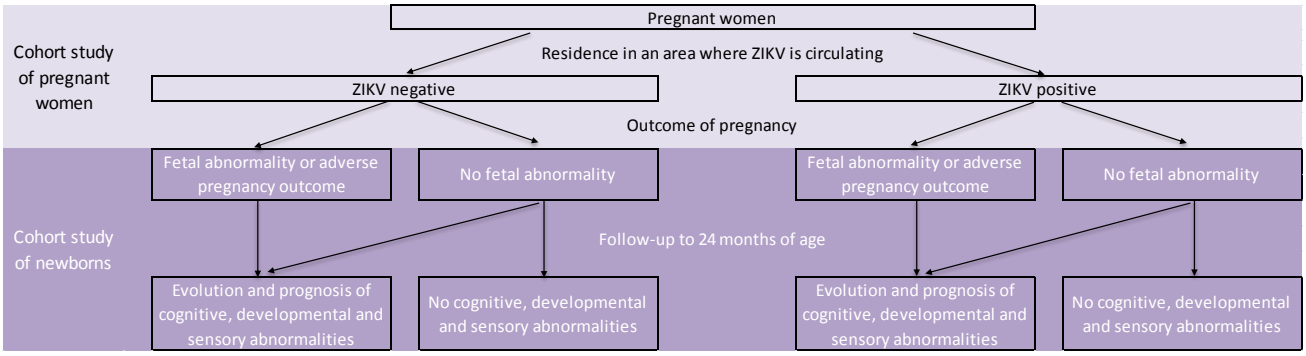

Figure 1: Study design of prospective longitudinal cohort study of newborns and infants born to mothers exposed to Zika virus during pregnancy

Ideally, newborns recruited will be those born to mothers previously enrolled in a cohort study of pregnant women. However, newborns born to mothers not enrolled in a cohort study of pregnant women may also be included - both apparently healthy newborns and those with fetal abnormalities.

2.1 STUDY SETTINGS

2.1.1 TIMING OF STUDY

This study will include infants born during, and up to 9 months after, a ZIKV outbreak in the study region. Ideally, this study should be implemented in areas with an ongoing ZIKV outbreak. This will increase the chances of including pregnant women with ZIKV infection and identifying fetal abnormalities. However, this should not exclude this study from being conducted in areas in which the disease is well established.

Comment: The timing of the study will need to be clearly defined by the study group and will depend on the epidemiology of the outbreak and the local setting.

2.1.2 STUDY AREA

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

2.2 SELECTION AND RECRUITMENT OF STUDY PARTICIPANTS

2.2.1 STUDY POPULATION

Selection of participants: In the event that a cohort study of pregnant women has also taken place in the region of study, it is strongly recommended and preferred (logistically and scientifically) to follow-up the newborn of mothers enrolled in that study.

The recruitment of *pregnant women* is most feasible:

- Antenatal clinics or during antenatal visits at health facilities, or with community health workers attended by women prior to childbirth. At this moment, women could be sensitized to the study and enrolled.

Alternatively, the recruitment of *newborns* may be most feasible in:

- Health facilities, clinics or hospitals within one week following childbirth, ideally before the mother is discharged from a health care facility, if she is hospitalized

Comment: The location of recruitment of newborns will depend on the local setting in which this study is carried out. It is important to clearly define your study area and, if using recruitment at health care facilities, to detail the catchment area of the health care facilities.

Comment: If the setting where the study is to be implemented has a substantial proportion of births at home, the study group will need to develop a strategy to recruit these home deliveries into the study.

2.2.2 PARTICIPANT FOLLOW-UP SCHEDULE:

Recruitment and baseline visit: Recruitment of mother/newborn pairs will take place prior to or as soon as possible after birth (within 1 week of birth). Baseline data will be collected during the enrollment visit. This should include information about the pregnancy which can be obtained from pregnancy notes, according to national guidelines. The mother will have a blood sample drawn and, ideally, swab of amniotic fluid at childbirth. A clinical examination at baseline will include neonatal measurements, physical examination, reflexes, tone, and evidence of seizures.

Follow-up: After enrollment, follow-up visits will occur at 1, 3, 6, 9, 12, 18 and 24 months. As much as possible, the follow-up schedule will overlap with the standard of care in the region of study, in order to minimize burden of study involvement for the participant. At each visit, (neuro)developmental evaluations will include assessments of epilepsy, hearing, vision, swallowing and spasticity/movement in the infant, following WHO guidelines.

Comment: For premature newborns, corrected gestational age (CGA) approach should be used for follow-up schedule.

Comment: Technical, financial or capacity limitations may mean that adhering to the follow-up schedule may not be feasible. In this case, efforts should be made by the study group to follow the schedule as closely as possible, with visits at months 3, 9 and 24 at a minimum.

Comment: The figure below presents the design of the cohort study, detailing the points of data collection from the newborns recruited into the study. This is an example, but represents the time points for data collection recommended in this protocol.

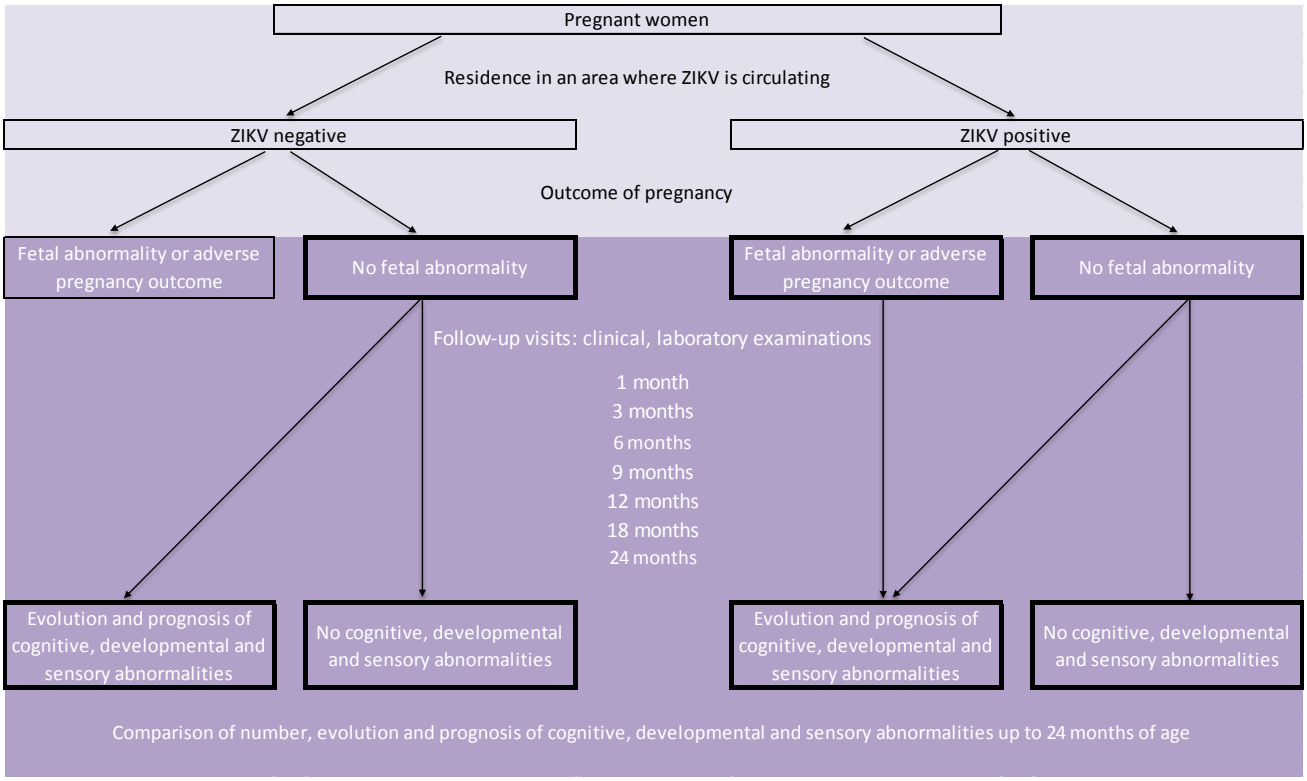

Figure 2: Study design of prospective longitudinal cohort study of newborns and infants born to mothers exposed to Zika virus during pregnancy, indicating data collection time points

2.2.3 ELIGIBILITY CRITERIA

- **Inclusion criteria:** All newborns from the study area with or without congenital abnormalities, who are less than 1 week old at the time of recruitment and born to mothers with confirmed ZIKV infection during pregnancy; all newborns from the study area, less than 1 week old and born to mothers without ZIKV infection during pregnancy.
- **Exclusion criteria:** Any newborn for whom the mother/guardian is unable to give informed consent, or with any contraindication to venipuncture.

2.2.4 INFORMED CONSENT

Written informed consent will be collected for all newborn participants from parent/legal guardian. Written informed assent witnessed by a parent or legal guardian will be collected for all pregnant women who are minors.

During the first interview with the mother/guardian following childbirth, the purpose of the study will be explained and consent/assent will be obtained from the mother/guardian upon enrollment of his or her newborn into the study by a trained member of the investigation team. Each study participant must be informed that participation in the study is voluntary and that s/he will be free, without justification, to his/her infant from the study at any time without consequences and without this affecting the clinical care of the newborn/infant. Data contributed to the study up until the point of withdrawal will remain with the study group, unless stated by the withdrawing participant.

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

Comment: The 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 in the infant may be notified to national authorities under the International Health Regulations (IHR) requirements.

If the mother/guardian agrees to enroll his/her newborn, the consent form must be completed legibly, with both surname and first name, dated and signed by the mother/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 assent of the newborn participant.

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

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

2.2.5 INCENTIVES TO PARTICIPATE AND COMPENSATION

The primary benefit of this study is the extended medical care and intensified (i.e., beyond routine) follow-up of infants with exposure to ZIKV, which will allow for timely detection and intervention for any arising abnormalities. 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 offer financial compensation (e.g., for expenses to attend medical visits) will depend on the context of the study and local policies and should be determined on a study-by-study basis. This will need to be detailed in the information provided to the participant and in the informed consent.

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

2.2.4 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

The primary benefit of this study is the extended medical care and intensified (i.e., beyond routine) follow-up of newborns born to women with potential exposure to ZIKV. This will allow for timely detection of any abnormality. Indirectly, data collected from this study 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.

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 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.

All mothers/guardians will be informed of the results of any individual testing of his or her infant. Results of any testing are the property of each participant and should be provided to each participant as promptly as possible.

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

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

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 has been obtained from the mother/guardian of an eligible newborn, a standardized questionnaire will be administered to each study participant. Information to be collected from each **newborn** at baseline includes:

- Clinical evaluation, including laboratory evaluations, cranial ultrasound, neurological signs, ophthalmology, auditory screening, reflexes
- 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]

At baseline, the following information will also be collected from the **mother**:

- Information on 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]

At each of the follow-up visits, the following information will be collected from the **infant**:

- Clinical evaluation including anthropometry, neurodevelopment reflexes, imaging, ophthalmology, auditory screening, reflexes. Please see WHO Screening, assessment and management of neonates and infants with complications associated with Zika virus exposure in utero (August 2016).

- Laboratory evaluations, if indicated by standard of care

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

- ISARIC (International Severe Acute Respiratory and Emerging Infection Consortium) Case Report Forms
- Centre d'Investigations Cliniques (CIC) Antilles-Guyane Inserm 1424: 'Observational studies on the consequences of being infected by Zika virus while pregnant during the epidemic period in the French Overseas Departments in 2016.'
- International Research Consortium of Dengue Risk Assessment, Management and Surveillance (IDAMS)

The questionnaire contains the core data variables that should be collected from the study participants to address the objectives of this study. Further questions may be added at the discretion of the research group. The questionnaire is designed to be implemented by trained study personnel, without advanced or specialized medical degrees.

Comment: Details of examinations and testing to be conducted at each of the follow-up visits are currently being discussed by WHO and technical partners. The questionnaire will need to be updated once these guidelines become available.

2.4.1 PHYSICAL AND NEUROLOGICAL INVESTIGATIONS

Different physical, neurological and biological tests will be performed at the follow-up visits 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 auditory 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, when possible
- MRI or CT scan, when possible
- Ocular exam, including fundoscopy

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

The principal samples to be collected at enrollment are listed in Table 1.

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

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

From **each newborn**: a range of biological samples will be collected according to the table below and the ISARIC/WHO/partners newborn algorithm found in Appendix D. This will also include a cranial ultrasound of newborns at birth.

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

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

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

	Specimen	Volume	Container	Timing	Remarks
Mother	Blood	7.5 mL	Dry tube (serum)	Within 2 days of birth	Minimal volume needed: 0.5 mL of whole blood.
Newborn	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 (if indicated)	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.
	Amniotic fluid	Swab of head or of saliva		At birth	Swab of newborn's head after delivery of swab of newborn's saliva after delivery

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:

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

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

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

2.5.2 LABORATORY PROCEDURES

Laboratory testing will be carried out in the country of the research institution collecting biological samples or in collaboration with an external laboratory partner as needed. At least two aliquots of sample will be made and at least one will be kept for future analysis. The principal tests described for ZIKV infection detection and differential diagnosis are listed in Table 2. Extended lab testing algorithms for newborns and mothers are included in Appendix C.

The serum will be tested for antibodies against suspected infectious pathogens, such as dengue virus, ZIKV, and Leptospira species bacteria. 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 thus needs to be considered on a study-by-study basis.

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

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

	Specimen	Type of test	Targeted pathogen
Mother	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
Newborn	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.*
	CSF (if indicated)	• Real-time RT-PCR • Serology (IgM only) • Biochemical & cytological analysis (proteins, glucose, cells) • Cytobacteriology	• **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

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

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

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 diagnosis 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.

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

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

3.0 STUDY ENDPOINTS AND STATISTICAL ANALYSES

3.1 SAMPLE SIZE CONSIDERATIONS

Sample size calculations for this study are driven by the assumptions related to absolute risk and the assumptions related to seroconversion rate – a proxy for ZIKV exposure – during pregnancy. Any sample size calculation should use two-tailed statistical tests, with 90% of statistical power and 5% significance level.

Table 4: *Example sample sizes calculations as a function of ZIKV infection among mothers*

Proportion of ZIKV infection among mothers	Assumptions	Sample size
70%	1. The incidence of abnormalities is greater in the exposed group (i.e. ZIKV+ women) compared to unexposed group (ZIKV- women) by an odds ratio of at least 3 2. The prevalence of congenital and developmental abnormalities in the unexposed group is at least 1%. 3. The rate of lost-to-follow-up is 10% or less	2803 pregnant women: • 841 ZIKV- • 1962 ZIKV+
50%		2284 pregnant women: • 1142 each ZIKV-, ZIKV+
30%		2581 pregnant women: • 1807 ZIKV- • 774 ZIKV+

3.2 STUDY OUTCOME MEASURES

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

3.2.1 CASE DEFINITIONS

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

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

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

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

Confirmed case:

- A person with laboratory confirmation of recent ZIKV infection:

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

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

3.3 PRIMARY OUTCOMES AND STATISTICAL ANALYSES

Statistical tests, as appropriate, will be used to test for statistical differences and describe 95% confidence intervals will be used to test for statistical differences in the incidence of each possible pregnancy outcome, and the incidence and range of congenital defects which are seen between infants born to mothers with ZIKV infection and to those without ZIKV infection.

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

Objective	Outcomes	Statistical analysis
Identify, describe and quantify the spectrum of congenital manifestations, including microcephaly, in the live newborns born to ZIKV infected and ZIKV non-infected women	Demographic characteristics of newborns with congenital manifestations, as well as of their mothers (number of exposed and unexposed; median age, sex, area of residence) Clinical spectrum: microcephaly, cranial facial disproportions, dysphagia, calcifications morbidity/mortality, birth weight, vital signs, neurological reflexes, spasticity and tone, hearing/vision impairment, epilepsy and seizures, cerebral palsy, contractures, hospitalization, and central nervous system (CNS) imaging characteristics, etc.	Provide descriptive epidemiology for each demographic characteristic and for each manifestation (mean/median, SD, percentages, etc.)
Compare post-natal incidence of developmental abnormalities and outcomes between newborns born to ZIKV infected and ZIKV non-infected women	Total number of developmental abnormalities or outcomes arising over the course of the study for both infants born to ZIKV infected and non-infected mothers.	Odds ratio or risk ratio of arising developmental abnormalities and other outcomes in the infants born to ZIKV+ vs. ZIKV- women.
Evaluate the prognosis of infants born with microcephaly and other congenital abnormalities associated with ZIKV infection in the mother	Description of developmental trajectory for infants born with microcephaly and other congenital abnormalities in the ZIKV exposed group.	Logistic regression or Cox regression
Estimate the relative risk of abnormalities or adverse outcomes in the fetuses/newborns born to women infected with ZIKV compared	Total number of microcephaly cases and other abnormalities associated with ZIKV over the entire course of the study, including those that appeared at or	Logistic regression obtaining odds ratio of total abnormalities and other outcomes in the infants of

to those born to women not infected with ZIKV	before birth.	ZIKV+ vs. ZIKV- women.

Comment: For the first outcome measure, this is a sample table to guide the standardized reporting of results

Infant abnormality	Entire cohort	ZIKV positive (mother – laboratory confirmed)	ZIKV negative
Microcephaly			
Facial disproportion			
Hearing and/or visual impairments			
Dysphagia			
Calcifications – CNS imaging			
Low birth weight			
Epilepsy and seizures			
Spasticity and tone			
Neurological reflexes			
Cerebral palsy			
Hospitalization			

Comment: Abnormalities may be updated as the results of further case-control studies are published. Further cohort studies may also wish to investigate quality of life, baby interaction, social impact on mothers etc.

4.0 REPORTING OF FINDINGS

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

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

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

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

5.0 COMPLEMENTARY STUDIES

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

Additional standardized protocols for ZIKV are available and include:

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

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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, Netherlands), 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).

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

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APPENDICES

Appendix A: Description of investigation and informed consent template

Appendix B: Standardized questionnaire/DRAFT undergoing review

Appendix C: Biological sampling algorithms

Appendix D: List of published primers for detection and quantification of Zika virus by real-time RT-PCR (Cao-Lormeau, Blake et al. 2016)

APPENDIX A: INFORMED CONSENT FORM FOR STUDY PARTICIPATION

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

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

INFORMATION FOR THE PARTICIPANT

Dear Mr/Mrs/Ms/Miss,

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

Cohort of newborns born to mothers with and without Zika virus infection to evaluate the risk of adverse outcomes

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. How often this occurs and how the Zika virus affects the development of babies is not 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:

- Identify, describe and quantify the spectrum of congenital manifestations, including microcephaly, in the newborns born to ZIKV infected compared to those born to non-infected women
- Compare post-natal incidence of developmental abnormalities and outcomes between newborns born to ZIKV infected compared to those born to non-infected women
- Evaluate the prognosis of babies born with microcephaly and other congenital abnormalities associated with ZIKV infection in the mother
- Estimate the relative risk of abnormalities or outcomes in the fetuses/newborn in infants born to women infected with ZIKV compared to those born to non-infected women

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

RESEARCH PROCESS

Your baby will be followed up at 7 time points: just after birth (within the first few days of life) and at 1, 3, 6, 9, 12, 18, and 24 months of age. Each time, your infant will be examined and information collected according to the medical standard of care for follow-up of infants born to mothers exposed to Zika virus during pregnancy. No intervention or invasive procedure will be done on you or your baby that is not designed for the purpose of this study.

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 infection with 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.

During follow-up visits, we will assess your child's acquired skills (e.g. movement, language) as well as their neurological and sensory development. Your child will also have hearing and visual testing at follow-up visits. Other examinations, such as imaging or laboratory analysis, will only be done at follow-up visits if there is a medical indication, or if it fits the standard of care in the study location.

You and your infant's participation in this research will last from the moment of your enrollment until your child is 2 years of age.

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 in your location for the follow-up of infants born to mothers who were exposed to Zika virus while pregnant. The primary benefit of this study is the extended medical care and intensified (i.e., beyond routine) follow-up of your infant- who may have had exposure to Zika virus. This will allow for timely 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

We will need to enter your data 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 under strict protection only to your doctor or to a small number of researchers 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 your baby participates, the study group will seek your permission to keep the data contributed up to the point at which you withdraw from the study, or to destroy all data.

INFORMATION ON YOUR SAMPLES DURING AND AFTER THIS STUDY

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

At any time, and without consequence to your participation in the present study or to your medical care, you may withdraw your consent for the use of your samples for these other research 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/GUARDIAN

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:

Cohort of newborns born to mothers with and without Zika virus infection to evaluate the risk of adverse outcomes

- ☐ I have read or orally received all the necessary information to understand the topic and enrollment process of the study.
- ☐ I was able to ask questions and received clear and adequate responses.
- ☐ I confirm my participation in this study, which includes responding to a questionnaire and allowing the taking of biological samples from me.
- ☐ I acknowledge that these samples may need to be shipped and/or 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 this research protocol.

I have been informed that my biological samples may be stored even after the end of the study period, in order to conduct further research on Zika virus infection or on other infections transmitted by mosquitos. Other research teams, private or public, national or international, may carry out this research. This 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:

- ISARIC (International Severe Acute Respiratory and Emerging Infection Consortium)
- Centre for Clinical Investigation Antilles-Guyane Inserm 1424: 'Observational studies on the consequences of being infected by Zika virus while pregnant during the epidemic period in the French Overseas Departments in 2016.'
- International Research Consortium of Dengue Risk Assessment, Management and Surveillance (IDAMS)

Purpose of the standardized questionnaire and instructions for its use

This questionnaire has been designed to collect the minimum amount of data to address the primary objectives of the standardized cohort study of pregnant women. Additional questions may be added to the questionnaire, as determined by the financial and technical capacity of the study group and by the outbreak characteristics. The questionnaire is designed to be implemented by trained study personnel, without advanced or specialized medical degrees.

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

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 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 full stops, they will already be present in the field if appropriate
  - Do no leave any space empty, enter a zero if necessary

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

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

For example: Yes ☐ No ☒

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

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

For the Primary Investigators 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: ____ / ____ / _____ (DD/MM/YYYY)

Interviewer name: _____

IDENTIFICATION: STUDY PARTICIPANT

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

VERIFICATION OF ELIGIBILITY

INCLUSION CRITERIA	Yes	No
Infant born to mother confirmed ZIKV+	☐	☐
Infant born to mother suspected ZIKV+	☐	☐
Infant born to mother ZIKV-	☐	☐
EXCLUSION CRITERIA	**Yes**	**No**
Mother/guardian unable or unwilling to give informed consent	☐	☐
Infant with a contraindication to venipuncture	☐	☐

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

Date of inclusion:	____ / ____ / _____ (DD/MM/YYYY)
Number of days after birth:	
Name of site/clinic/hospital:	
City or town of mother's residence: **If unknown, hospital:**	
State:	
Country:	

This case report form consists of two parts:

Part 1: To be completed at the time of enrollment of the newborn (within 1 week of birth) and consists of questions related to:

1a. Labor and delivery, and information about the mother

1b. Baseline information about the newborn (demographics, clinical, laboratory examinations)

Part 2: Specific forms to be completed during each follow-up visit between the periods of 1 week and 2 years after the child's birth.

Comment: The standardized protocol includes follow up forms for up to 2 years, but can adapted in order to follow up infants beyond 2 years of age, using age-appropriate milestones.

PART 1A: PREGNANCY DETAILS AND MOTHER'S BASELINE DATA

To be collected within 1 week of birth, ideally on day of birth

Comment: Part 1A only needs to be collected from those mothers who have not participated in a prospective longitudinal cohort study of women and newborns exposed to Zika virus during the course of pregnancy. It is recommended that the implementation of this protocol be preceded by the implementation of a cohort study of pregnant women exposed to ZIKV.

1.0 LABOUR AND DELIVERY

Date of delivery (DD/MM/YYYY)**:**	____ / ____ / _____
If multiple pregnancy, birth order of infant:	
Gestational age at time of birth:	
Onset of labor: (tick one box only)	☐ Spontaneous ☐ Induced ☐ No labor ☐ Unknown
Prelabor premature rupture of membranes (PPROM):	☐ Yes ☐ No ☐ Unknown
Place of delivery:	☐ Home ☐ Health facility ☐ Unknown
Mode of delivery:	☐ Vaginal spontaneous ☐ Vaginal assisted (e.g. forceps, vacuum) ☐ Caesarean section ☐ Assisted breech or breech extraction
If labor was induced, or caesarean section performed, please specify reason:	
Fetal presentation at delivery:	☐ Cephalic ☐ Breech ☐ Other (specify):
Color of amniotic fluid:	☐ Clear ☐ Bloody ☐ Meconium-stained ☐ Other (specify): ☐ Unknown
Placental weight:	_____ (Unit used=____)

Placental abnormalities: - If yes, specify: - Calcifications:	☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown
Intrapartum complications: - If yes, specify:	☐ Yes ☐ No ☐ Unknown
Postpartum complications: - If yes, specify:	☐ Yes ☐ No ☐ Unknown

2.0 MATERNAL DEMOGRAPHICS

Date of birth (DD/MM/YYYY)	____/ ____/ _____
Area of residence during pregnancy	
Maternal primary 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 ☐ Laborer/factory worker ☐ Without profession ☐ Retired ☐ Does not wish to respond ☐ Other (specify): _____
Ethnicity	(Add check boxes according to national guidelines)
Household income:	(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:	 _____ _____ _____ _____ ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown

Television: Refrigerator: Watch: Type of vehicle: At least five items of furniture: –Table –Chair –Sofa –Bed –Armoire –Cabinet Persons per sleeping room: Ownership of agricultural land and size: Ownership of farm animals by type and number: Domestic servant: Telephone (fixed and mobile): Cooking fuel: 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 ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Unknown

3.0 LIFESTYLE INFORMATION DURING PREGNANCY

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

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

Do you drink alcoholic beverages?	☐ Yes ☐ No ☐ Unknown
If yes, how frequently?	☐ Every day ☐ Less than every day, but at least weekly ☐ Less than weekly, but at least monthly ☐ On rare occasions
Do you currently smoke tobacco?	☐ Yes ☐ No ☐ Unknown
Have you smoked tobacco daily in the past?	☐ Yes ☐ No ☐ Unknown

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

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

4.0 EXPOSURE INFORMATION DURING PREGNANCY

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	
Do you wear/have you worn long trousers/long sleeves during your pregnancy?	☐Yes ☐ No If yes: ☐Sometimes ☐ Often ☐ Always
During your pregnancy, have you used a mosquito net while you sleep during the day or at night?	☐Yes ☐ No If yes: ☐Sometimes ☐ Often ☐ Always
Do you use/have you used essential oils to rid your home of mosquitos?	☐Yes ☐ No If yes: ☐Sometimes ☐ Often ☐ Always
Do you use/have you used mosquito repellent spray during your pregnancy?	☐Yes ☐ No If yes: ☐Sometimes ☐ Often ☐ Always
Do you use/have you used insecticides to remove mosquito larvae from your home?	☐Yes ☐ No If yes: ☐Sometimes ☐ Often ☐ Always
Do you use/have you used other methods to rid you home of mosquitos during your pregnancy? - If so, 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	☐Yes ☐ No	_____

5.0 MEDICAL HISTORY OF MOTHER

Have you ever been diagnosed with:	
High blood pressure:	☐ Yes ☐ No ☐ Unknown

Diabetes:			☐ Yes ☐ No ☐ Unknown	
Sickle cell disease:			☐ Yes ☐ No ☐ Unknown	
Blood transfusion: - If yes, date			☐ Yes ☐ No ☐ Unknown ____ / ____ / _____	
Other medical history: - If yes, specify:			☐ Yes ☐ No ☐ Unknown	
Surgical history: - If yes, specify:			☐ Yes ☐ No ☐ Unknown	
Obstetric history:	# Gravidity ___	# Parity ___	# Living ___	#Abortion____
Known familial genetic abnormalities - If yes, specify:			☐ Yes ☐ No ☐ Unknown	
Infants born with congenital abnormalities - If yes, specify:			☐ Yes ☐ No ☐ Unknown	
Pregnant woman's head circumference:			_____ cm ☐ Unknown	

6.0 LABORATORY TESTING OF MOTHER

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

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

PART 1B: NEWBORN BASELINE DATA

To be collected within 1 week of birth

1.0 CLINICAL EVALUATION OF THE NEWBORN

1.1 DEMOGRAPHICS OF THE NEWBORN

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): _____

1.2 PHYSICAL MEASUREMENTS OF NEWBORN

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) If measured before 24h after birth, indicate the number of hours after birth the measurement was taken:	_____ (cm) _____ (hrs)	☐ Unknown

1.3 VITAL SIGNS OF NEWBORN DAY 0 (≤ 24 HOURS POST-DELIVERY)

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	If abnormal, ☐ 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

	☐ Focal paralysis Specify part of body: _____
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	☐Yes ☐No ☐Unknown

of onset (DD/MM/YYYY):	__ __ / __ __ / 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

1.4 BIRTH ABNORMALITIES PRESENT AT THE TIME OF EXAM DAY 0 (≤ 24 HOURS POST-DELIVERY)

Fontanelle anterior - If yes, bulging, depressed or large:	☐Yes ☐No ☐Unknown
Fontanelle posterior - If yes, bulging, depressed or large:	☐Yes ☐No ☐Unknown
Facial dysmorphism - If yes, describe:	☐Yes ☐No ☐Unknown
Cleft lip/cleft palate	☐Yes ☐No ☐Unknown

Eye abnormalities - If yes, describe:	☐Yes ☐No ☐Unknown _____
Ear abnormalities	☐ Anotia/microtia ☐ Other (describe): _____ ☐No ☐Unknown
Cephalohaematoma	☐Yes ☐No ☐Unknown
Subgaleal hemorrhage	☐Yes ☐No ☐Unknown
Craniosynostosis - If present, specify/ describe	☐Yes ☐No ☐Unknown
Excessive head skin - If present, specify/ describe:	☐ Present ☐ Absent _____
Prominent occiput	☐ Present ☐ Absent
Down syndrome features - If present, specify/ describe:	☐Yes ☐No ☐Unknown
Neural tube defects, e.g. spina bifida, meningocele	☐Yes ☐No ☐Unknown
Haemangiomas	☐ Present ☐ Absent ☐ Facial ☐ Rest of body
Congenital heart defects - If yes, specify:	☐ Yes ☐ No ☐ Unknown

	_____
Gastroschisis	☐Yes ☐No ☐Unknown
Omphalocele	☐Yes ☐No ☐Unknown
Umbilical hernia	☐Yes ☐No ☐Unknown
Hand abnormalities	☐ Clinodactyly ☐ Missing digits ☐ Other (specify):_____ ☐ No ☐Unknown
Foot abnormalities	☐ Wide spaced toes ☐ Clubfoot ☐ Other (specify):_____ ☐No ☐Unknown
Upper and/or lower limb abnormalities - If yes, specify/describe which limb/s:	☐ Yes ☐ No ☐ Unknown
Other significant abnormalities - If yes, describe all:	☐ Yes ☐ No ☐ Unknown

AUDIO-VISUAL

(If abnormal, please describe and enclose images/report) tests for newborn

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	

2.0 IMAGING OF NEWBORN

(If abnormal, please describe and enclose images/report) of the newborn

Neuroimaging	**Results**	**If abnormal, please summarize key results from report:**		**Images attached**	**Report attached**
		Localization	**Findings**		
Cranial Ultrasound scan	☐ Normal ☐ Abnormal ☐ Not done			☐ Yes ☐ No	☐ Yes ☐ No
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

3.0 LABORATORY EVALUATIONS OF NEWBORN

3.1 BLOOD TESTS OF THE NEWBORN

Date of sampling (DD/MM/YYYY): __ / __ / 20 __

Test	**Value**	**Specify unit**		
C-reactive protein		mg/L		other:_____
Erythrocyte sedimentation rate		mm		other:_____
Procalcitonin		ng/mL		other:_____
Haemoglobin		g/L	g/dL	other:_____
Haematocrit		%		other:_____
White blood cell count		$x10^9$/L	$x10^3$/µL	other:_____
Neutrophils		$10^3/mm^3$	%	other:_____
Lymphocytes		$10^3/mm^3$	%	other:_____
Monocytes		$10^3/mm^3$	%	other:_____
Eosinophils		$10^3/mm^3$	%	other:_____
Basophils		$10^3/mm^3$	%	other:_____

MCV		μm^3	other:_____
RBC count		$x10^9$/L *or* $x10^3/\mu L$	other:_____
Platelets		$x10^9$/L *or* $x10^3/\mu L$	other:_____
APTT		seconds	
PT (seconds)		seconds	
Urea nitrogen		mmol/L mg/dL	other:_____
Albumin		g/L	other:_____
Sodium		mEq/L	other:_____
Potassium		mEq/L	other:_____
Calcium		mmol/L	other:_____
Phosphate		mg/dL	other:_____
Magnesium		mmol/L	other:_____
Total protein		g/dL	other:_____
Creatinine		µmol/L mg/dL	other:_____
Glucose		mmol/L mg/dL	other:_____
Amylase		U/L	other:_____
Bilirubin		µmol/L mg/dL	other:_____
AST/SGOT		U/L	other:_____
ALT/SGPT		U/L	other:_____
ALP		U/L	other:_____
GGT		U/L	other:_____
Creatine kinase		U/L	other:_____
Other biochemistry result (specify):		Unit:_____	
Other biochemistry result (specify):		Unit:_____	
Blood film results - If yes , describe results:	☐Yes ☐Not done ☐Unknown		

3.2 CSF SAMPLE

(If available as part of clinical care) of the newborn:

Date of lumbar puncture (DD/MM/YYYY):___ / ____ / 20 ___ ☐Not done

CSF appearance	☐Clear and colorless ☐Cloudy ☐Blood stained ☐Unknown
Gram stain	☐Negative ☐Positive ☐Not done

Test	Value	Unit	Other unit
CSF glucose		mmol/l	
Plasma glucose at time of lumbar puncture*		mmol/l	

CSF WBC count		per mm^3	
WBC Lymphocytes		%	
WBC Neutrophils		%	
CSF RBC count		per mm^3	
CSF protein		mg/dl	

*Must be taken within 4 hours of the lumbar puncture. Record capillary blood glucose measurement if laboratory plasma glucose not requested.

3.3 DIFFERENTIAL DIAGNOSTIC TESTS FOR THE NEWBORN

Test		**Date of testing** (DD/MM/YYYY)	**Result**
RT-PCR ZIKA: **- Blood** **- Urine** **- Placenta**	☐ Yes ☐ No ☐ Yes ☐ No ☐ Yes ☐ No	I__I__I/I__I__I/I__I__I__I__I I__I__I/I__I__I/I__I__I__I__I I__I__I/I__I__I/I__I__I__I__I	☐ Positive ☐ Negative ☐ Positive ☐ Negative ☐ Positive ☐ Negative
ZIKA Serology	☐ Yes ☐ No	I__I__I/I__I__I/I__I__I__I__I	IgM: ☐ Positive ☐ Negative ☐ Unknown
			IgG: ☐ Positive ☐ Negative ☐ Unknown
Dengue Serology	☐ Yes ☐ No	I__I__I/I__I__I/I__I__I__I__I	IgM: ☐ Positive ☐ Negative ☐ Unknown
			IgG: ☐ Positive ☐ Negative ☐ Unknown
Toxoplasmosis	☐ Yes ☐ No	I__I__I/I__I__I/I__I__I__I__I	☐ Positive ☐ Negative
Rubella	☐ Yes ☐ No	I__I__I/I__I__I/I__I__I__I__I	☐ Positive ☐ Negative
Cytomegalovirus	☐ Yes ☐ No	I__I__I/I__I__I/I__I__I__I__I	☐ Positive ☐ Negative
Syphilis	☐ Yes ☐ No	I__I__I/I__I__I/I__I__I__I__I	☐ Positive ☐ Negative
Herpes Simplex	☐ Yes ☐ No	I__I__I/I__I__I/I__I__I__I__I	☐ Positive ☐ Negative
BVDV	☐ Yes ☐ No	I__I__I/I__I__I/I__I__I__I__I	☐ Positive ☐ Negative
Other (specify)	☐ Yes ☐ No	I__I__I/I__I__I/I__I__I__I__I	☐ Positive ☐ Negative

PART 1 COMPLETED BY

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

PART 2A: INFANT FOLLOW UP VISIT: 1 MONTH

Infant ID#: __ ____ ___ _ ___-_____ __

Visit date (DD/MM/YYYYY)**:** ____ / ____ /_____

Weight: _____ g

Height: _____ cm

Head circumference: _____ cm

Screening		**Date (DD/MM/YYYY)**	**Result**
Phenylketonuria:	☐ Yes ☐ No ☐ Unknown	____ / ____ /_____	
Hypothyroidism:	☐ Yes ☐ No ☐ Unknown	____ / ____ /_____	
Congenital adrenal hyperplasia:	☐ Yes ☐ No ☐ Unknown	____ / ____ /_____	

Neurological development:	
Apnea	☐ Yes ☐ No ☐ Unknown
Seizures	☐ Yes ☐ No ☐ Unknown
Film	☐ Yes ☐ No ☐ Unknown
EEG (attach results)	☐ Yes ☐ No ☐ Unknown
Other abnormalities	☐ Yes ☐ No ☐ Unknown

Signs of sensory development:	
If abnormalities, specify:	

AUDIO-VISUAL

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	

PART 2B: IMAGING (1 MONTH)

IMAGING

(If abnormal, please describe and enclose images/report if possible)

Neuroimaging	**Results**	**If abnormal, please summarize key results from report:**		**Images attached**	**Report attached**
		Localization	**Findings**		
Cranial ultrasound scan	☐ Normal ☐ Abnormal ☐ Not done			☐ Yes ☐ No	☐ Yes ☐ No
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

PART 2 COMPLETED BY

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

PART 3A: INFANT FOLLOW UP VISIT: 3 MONTHS

Infant ID#: __ ____ ___ _ ___-_____ __

Visit date (DD/MM/YYYYY)**:** ____ / ____ /_____

Weight: _____ g

Height: _____ cm

Head circumference: _____ cm

Screening		**Date (DD/MM/YYYY)**	**Result**
Phenylketonuria: (If not collected at 1 month)	☐ Yes ☐ No ☐ Unknown	____ / ____ /_____	
Hypothyroidism:	☐ Yes ☐ No ☐ Unknown	____ / ____ /_____	
Congenital adrenal hyperplasia:	☐ Yes ☐ No ☐ Unknown	____ / ____ /_____	

Skills acquired:	
Grabs at objects when given	☐ Yes ☐ No
Sits without support and holds up head	☐ Yes ☐ No
On stomach; raises their head	☐ Yes ☐ No
Other abnormalities - If yes, specify:	☐ Yes ☐ No _____

Neurological development:	
Apnea	☐ Yes ☐ No
Seizures	☐ Yes ☐ No
Film	☐ Yes ☐ No
EEG (attach results)	☐ Yes ☐ No
Other abnormalities If yes, specify:	☐ Yes ☐ No _____

Signs of sensory development:	
If abnormalities, specify:	

AUDIO-VISUAL

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	

PART 3B: IMAGING (3 MONTHS)

IMAGING

(If abnormal, please describe and enclose images/report if possible)

Neuroimaging	**Results**	**If abnormal, please summarize key results from report:**		**Images attached**	**Report attached**
		Localization	**Findings**		
Cranial ultrasound scan	☐ Normal ☐ Abnormal ☐ Not done			☐ Yes ☐ No	☐ Yes ☐ No
CT Scan	☐ Normal ☐ Abnormal			☐ Yes ☐ No	☐ Yes ☐ No

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

PART 3 COMPLETED BY

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

PART 4A: INFANT FOLLOW UP VISIT: 6 MONTHS

Infant ID#: __ ___ ___ _ ___-_____ __

Visit date (DD/MM/YYYYY)**:** ____ / ____ /_____

Weight: _____ g

Height: _____ cm

Head circumference: _____ cm

Screening		**Date (DD/MM/YYYY)**	**Result**
Phenylketonuria:	☐ Yes ☐ No ☐ Unknown	____ / ____ /_____	
Hypothyroidism:	☐ Yes ☐ No ☐ Unknown	____ / ____ /_____	
Congenital adrenal hyperplasia:	☐ Yes ☐ No ☐ Unknown	____ / ____ /_____	

Skills acquired:	
Grabs at objects when given	☐ Yes ☐ No
Grabs at objects on own	☐ Yes ☐ No
Sits without support and holds up head	☐ Yes ☐ No
On stomach; raises their head	☐ Yes ☐ No
Shows a finger	☐ Yes ☐ No
Able to sit, with help	☐ Yes ☐ No
Able to sit without help	☐ Yes ☐ No
Crawls on belly	☐ Yes ☐ No
Craws on all-fours	☐ Yes ☐ No
Reacts to their name	☐ Yes ☐ No
Babbles	☐ Yes ☐ No
Other abnormalities - If yes, specify:	☐ Yes ☐ No _____

Neurological development:	
Trouble swallowing	☐ Yes ☐ No
Swallowing 'wrong way'	☐ Yes ☐ No
Spastic pyramidal syndrome of the upper limbs	☐ Yes ☐ No
Spastic pyramidal syndrome of the lower limbs	☐ Yes ☐ No
Damaged to cranial nerve pairs - If yes, describe:	☐ Yes ☐ No _____
Badly coordinated movements	☐ Yes ☐ No
Myoclonus tremors	☐ Yes ☐ No
Epilepsy - If yes, date of start of epilepsy: - If yes, type of seizures: - If yes, anti-epileptic treatment:	☐ Yes ☐ No _____ ☐ Generalized ☐ Partial ☐ Neonatal ☐ Syndrome de West ☐ Lennox ☐ Febrile ☐ Yes ☐ No
Film	☐ Yes ☐ No
EEG (attach results)	☐ Yes ☐ No
Other abnormalities If yes, specify:	☐ Yes ☐ No _____

Signs of sensory development:	
If abnormalities, specify:	

PART 4B: CLINICAL EXAMS AND IMAGING (6 MONTHS)

AUDIO-VISUAL

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	

IMAGING

(If abnormal, please describe and enclose images/report if possible)

Neuroimaging	Results	If abnormal, please summarize key results from report:		Images attached	Report attached
		Localization	Findings		
Cranial ultrasound scan	☐ Normal ☐ Abnormal ☐ Not done			☐ Yes ☐ No	☐ Yes ☐ No
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

PART 4 COMPLETED BY

Name and role:	

Signature:		**Date** (DD/MM/YYYY)	____ / ____ / ____

PART 5A: INFANT FOLLOW UP VISIT: 12 MONTHS

Infant ID#: __ ____ ___ _ ___-_____ __

Visit date (DD/MM/YYYYY)**:** ____ / ____ /_____

Weight: _____ g

Height: _____ cm

Head circumference: _____ cm

Skills acquired:	
Grabs at objects when given	☐ Yes ☐ No
Grabs at objects on own	☐ Yes ☐ No
Sits without support and holds up head	☐ Yes ☐ No
On stomach; raises their head	☐ Yes ☐ No
Shows a finger	☐ Yes ☐ No
Press together finger-thumb	☐ Yes ☐ No
Throws objects	☐ Yes ☐ No
Give objects	☐ Yes ☐ No
Put a cube in a box	☐ Yes ☐ No
Sits up with help	☐ Yes ☐ No
Sits up without help	☐ Yes ☐ No
Crawls on belly	☐ Yes ☐ No
Craws on all-fours	☐ Yes ☐ No
Walk	☐ Yes ☐ No
Puts up arms to be carried	☐ Yes ☐ No
Repeat a syllable	☐ Yes ☐ No
Repeat a word	☐ Yes ☐ No
Reacts to their name	☐ Yes ☐ No
Understands a simple instruction	☐ Yes ☐ No
Eat alone with a spoon	☐ Yes ☐ No
Other abnormalities - If yes, specify:	☐ Yes ☐ No

	_____

Neurological development:	
Hyperactivity	☐ Yes ☐ No
Aggressiveness	☐ Yes ☐ No
Spastic pyramidal syndrome of the upper limbs	☐ Yes ☐ No
Spastic pyramidal syndrome of the lower limbs	☐ Yes ☐ No
Ataxia	☐ Yes ☐ No
Suprabulbar syndrome	☐ Yes ☐ No
Dysmetria	☐ Yes ☐ No
Dystonia	☐ Yes ☐ No
Oculo-motor apraxia	☐ Yes ☐ No
Choreathetosis	☐ Yes ☐ No
Damaged to cranial nerve pairs - If yes, describe:	☐ Yes ☐ No _____
Epilepsy - If yes, date of start of epilepsy: - If yes, type of seizures: - If yes, anti-epileptic treatment:	☐ Yes ☐ No _____ ☐ Generalized ☐ Partial ☐ Neonatal ☐ Syndrome de West ☐ Lennox ☐ Febrile ☐ Yes ☐ No
Film	☐ Yes ☐ No
EEG (attach results)	☐ Yes ☐ No
Other abnormalities If yes, specify:	☐ Yes ☐ No _____

Signs of sensory development:	
Reacts to noise	☐ Yes ☐ No
Follows an object with eyes	☐ Yes ☐ No
If abnormalities, specify:	

PART 5B: CLINICAL EXAMS AND IMAGING (9 MONTHS)

AUDIO-VISUAL

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	

IMAGING

(If abnormal, please describe and enclose images/report if possible)

Neuroimaging	Results	If abnormal, please summarize key results from report:		Images attached	Report attached
		Localization	Findings		
Cranial ultrasound scan	☐ Normal ☐ Abnormal ☐ Not done			☐ Yes ☐ No	☐ Yes ☐ No
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

PART 5 COMPLETED BY

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

PART 6A: INFANT FOLLOW UP VISIT: 12 MONTHS

Infant ID#: __ ____ ___ _ ___-_____ __

Visit date (DD/MM/YYYY)**:** ____ / ____ /_____

Weight: _____ g

Height: _____ cm

Head circumference: _____ cm

Skills acquired:	
Grabs at objects when given	☐ Yes ☐ No
Grabs at objects on own	☐ Yes ☐ No
Sits without support and holds up head	☐ Yes ☐ No
On stomach; raises their head	☐ Yes ☐ No
Shows a finger	☐ Yes ☐ No
Press together finger-thumb	☐ Yes ☐ No
Throws objects	☐ Yes ☐ No
Give objects	☐ Yes ☐ No
Put a cube in a box	☐ Yes ☐ No
Sits up with help	☐ Yes ☐ No
Sits up without help	☐ Yes ☐ No
Crawls on belly	☐ Yes ☐ No
Craws on all-fours	☐ Yes ☐ No
Walk	☐ Yes ☐ No
Puts up arms to be carried	☐ Yes ☐ No
Repeat a syllable	☐ Yes ☐ No
Repeat a word	☐ Yes ☐ No
Reacts to their name	☐ Yes ☐ No
Understands a simple instruction	☐ Yes ☐ No
Eat alone with a spoon	☐ Yes ☐ No

Other abnormalities - If yes, specify:	☐ Yes ☐ No _____

Neurological development:	
Hyperactivity	☐ Yes ☐ No
Aggressiveness	☐ Yes ☐ No
Spastic pyramidal syndrome of the upper limbs	☐ Yes ☐ No
Spastic pyramidal syndrome of the lower limbs	☐ Yes ☐ No
Ataxia	☐ Yes ☐ No
Suprabulbar syndrome	☐ Yes ☐ No
Dysmetria	☐ Yes ☐ No
Dystonia	☐ Yes ☐ No
Oculo-motor apraxia	☐ Yes ☐ No
Choreathetosis	☐ Yes ☐ No
Myoclonus tremors	☐ Yes ☐ No
Damaged to cranial nerve pairs - If yes, describe:	☐ Yes ☐ No _____
Epilepsy - If yes, date of start of epilepsy: - If yes, type of seizures: - If yes, anti-epileptic treatment:	☐ Yes ☐ No _____ ☐ Generalized ☐ Partial ☐ Neonatal ☐ Syndrome de West ☐ Lennox ☐ Febrile ☐ Yes ☐ No
Film	☐ Yes ☐ No
EEG (attach results)	☐ Yes ☐ No
Other abnormalities If yes, specify:	☐ Yes ☐ No _____

Signs of sensory development:	
Reacts to noise	☐ Yes ☐ No
Follows an object with eyes	☐ Yes ☐ No
If abnormalities, specify:	

PART 6B: CLINICAL EXAMS AND IMAGING (12 MONTHS)

AUDIO-VISUAL

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	

IMAGING

(If abnormal, please describe and enclose images/report if possible)

Neuroimaging	Results	If abnormal, please summarize key results from report:		Images attached	Report attached
		Localization	Findings		
Cranial ultrasound scan	☐ Normal ☐ Abnormal ☐ Not done			☐ Yes ☐ No	☐ Yes ☐ No
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

PART 6 COMPLETED BY

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

PART 7A: INFANT FOLLOW UP VISIT: 18 MONTHS

Infant ID#: __ ____ ___ _ ___-_____ __

Visit date (DD/MM/YYYY)**:** ____ / ____ /_____

Weight: _____ g

Height: _____ cm

Head circumference: _____ cm

Skills acquired:	
Grabs at objects when given	☐ Yes ☐ No
Grabs at objects on own	☐ Yes ☐ No
Sits without support and holds up head	☐ Yes ☐ No
On stomach; raises their head	☐ Yes ☐ No
Shows a finger	☐ Yes ☐ No
Press together finger-thumb	☐ Yes ☐ No
Throws objects	☐ Yes ☐ No
Give objects	☐ Yes ☐ No
Able to match shapes and forms	☐ Yes ☐ No
Imitate a movement/gesture	☐ Yes ☐ No
Put a cube in a box	☐ Yes ☐ No
Sits up with help	☐ Yes ☐ No
Sits up without help	☐ Yes ☐ No
Crawls on belly	☐ Yes ☐ No
Craws on all-fours	☐ Yes ☐ No
Walk	☐ Yes ☐ No
Puts up arms to be carried	☐ Yes ☐ No
Repeat a syllable	☐ Yes ☐ No
Repeat a word	☐ Yes ☐ No
Reacts to their name	☐ Yes ☐ No

Understands a simple instruction	☐ Yes ☐ No
Identify and indicate objects if asked	☐ Yes ☐ No
Help when getting dressed	☐ Yes ☐ No
Eat alone with a spoon	☐ Yes ☐ No
Other abnormalities - If yes, specify:	☐ Yes ☐ No _____

Neurological development:	
Hyperactivity	☐ Yes ☐ No
Aggressiveness	☐ Yes ☐ No
Spastic pyramidal syndrome of the upper limbs	☐ Yes ☐ No
Spastic pyramidal syndrome of the lower limbs	☐ Yes ☐ No
Ataxia	☐ Yes ☐ No
Suprabulbar syndrome	☐ Yes ☐ No
Dysmetria	☐ Yes ☐ No
Dystonia	☐ Yes ☐ No
Oculo-motor apraxia	☐ Yes ☐ No
Choreathetosis	☐ Yes ☐ No
Myoclonus tremors	☐ Yes ☐ No
Damaged to cranial nerve pairs - If yes, describe:	☐ Yes ☐ No _____
Epilepsy - If yes, date of start of epilepsy: - If yes, type of seizures: - If yes, anti-epileptic treatment:	☐ Yes ☐ No _____ ☐ Generalized ☐ Partial ☐ Neonatal ☐ Syndrome de West ☐ Lennox ☐ Febrile ☐ Yes ☐ No
Film	☐ Yes ☐ No
EEG (attach results)	☐ Yes ☐ No
Other abnormalities If yes, specify:	☐ Yes ☐ No _____

Signs of sensory development:	
Reacts to noise	☐ Yes ☐ No

Follows an object with eyes	☐ Yes ☐ No
If abnormalities, specify:	

PART 7B: CLINICAL EXAMS AND IMAGING (18 MONTHS)

AUDIO-VISUAL

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	

IMAGING

(If abnormal, please describe and enclose images/report if possible)

Neuroimaging	Results	If abnormal, please summarize key results from report:		Images attached	Report attached
		Localization	Findings		
Cranial ultrasound scan	☐ Normal ☐ Abnormal ☐ Not done			☐ Yes ☐ No	☐ Yes ☐ No
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

PART 7 COMPLETED BY

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

PART 8A: INFANT FOLLOW UP VISIT: 24 MONTHS

Infant ID#: __ ____ ___ _ ___-_____ __

Visit date (DD/MM/YYYYY)**:** ____ / ____ /_____

Weight: _____ g

Height: _____ cm

Head circumference: _____ cm

Skills acquired:	
Grabs at objects when given	☐ Yes ☐ No
Grabs at objects on own	☐ Yes ☐ No
Sits without support and holds up head	☐ Yes ☐ No
On stomach; raises their head	☐ Yes ☐ No
Shows a finger	☐ Yes ☐ No
Press together finger-thumb	☐ Yes ☐ No
Throws objects	☐ Yes ☐ No
Give objects	☐ Yes ☐ No
Able to match shapes and forms	☐ Yes ☐ No
Imitate a movement/gesture	☐ Yes ☐ No
Holds a pencil and scribble	☐ Yes ☐ No
Puts a cube in a box	☐ Yes ☐ No
Sits up with help	☐ Yes ☐ No
Sits up without help	☐ Yes ☐ No
Crawls on belly	☐ Yes ☐ No
Craws on all-fours	☐ Yes ☐ No
Walks	☐ Yes ☐ No
Runs	☐ Yes ☐ No
Climbs and descends stairs	☐ Yes ☐ No

Holds out arms to be held	☐ Yes ☐ No
Repeats a syllable	☐ Yes ☐ No
Repeats a word	☐ Yes ☐ No
Matches coupled words	☐ Yes ☐ No
Reacts to their name	☐ Yes ☐ No
Understands a simple instruction	☐ Yes ☐ No
Identify and indicate objects if asked	☐ Yes ☐ No
Names an image	☐ Yes ☐ No
Helps when getting dressed	☐ Yes ☐ No
Asks to go to the toilet	☐ Yes ☐ No
Eats alone with a spoon	☐ Yes ☐ No
Other abnormalities - If yes, specify:	☐ Yes ☐ No _____

Neurological development:	
Hyperactivity	☐ Yes ☐ No
Aggressiveness	☐ Yes ☐ No
Spastic pyramidal syndrome of the upper limbs	☐ Yes ☐ No
Spastic pyramidal syndrome of the lower limbs	☐ Yes ☐ No
Ataxia	☐ Yes ☐ No
Suprabulbar syndrome	☐ Yes ☐ No
Dysmetria	☐ Yes ☐ No
Dystonia	☐ Yes ☐ No
Oculo-motor apraxia	☐ Yes ☐ No
Choreathetosis	☐ Yes ☐ No
Myoclonus tremors	☐ Yes ☐ No
Damaged to cranial nerve pairs - If yes, describe:	☐ Yes ☐ No _____
Epilepsy - If yes, date of start of epilepsy: - If yes, type of seizures: - If yes, anti-epileptic treatment:	☐ Yes ☐ No _____ ☐ Generalized ☐ Partial ☐ Neonatal ☐ Syndrome de West ☐ Lennox ☐ Febrile ☐ Yes ☐ No
Film	☐ Yes ☐ No
EEG (attach results)	☐ Yes ☐ No
Other abnormalities If yes, specify:	☐ Yes ☐ No _____

Signs of sensory development:	
Reacts to noise	☐ Yes ☐ No
Follows an object with eyes	☐ Yes ☐ No
If abnormalities, specify:	

PART 8B: CLINICAL EXAMS AND IMAGING (24 MONTHS)

AUDIO-VISUAL

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	

IMAGING

(If abnormal, please describe and enclose images/report if possible)

Neuroimaging	Results	If abnormal, please summarize key results from report:		Images attached	Report attached
		Localization	Findings		
Cranial ultrasound scan	☐ Normal ☐ Abnormal ☐ Not done			☐ Yes ☐ No	☐ Yes ☐ No
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

PART 8 COMPLETED BY

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

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

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

DRAFT
Version 5
6/MAY/2016

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

Sampling algorithm for research purposes

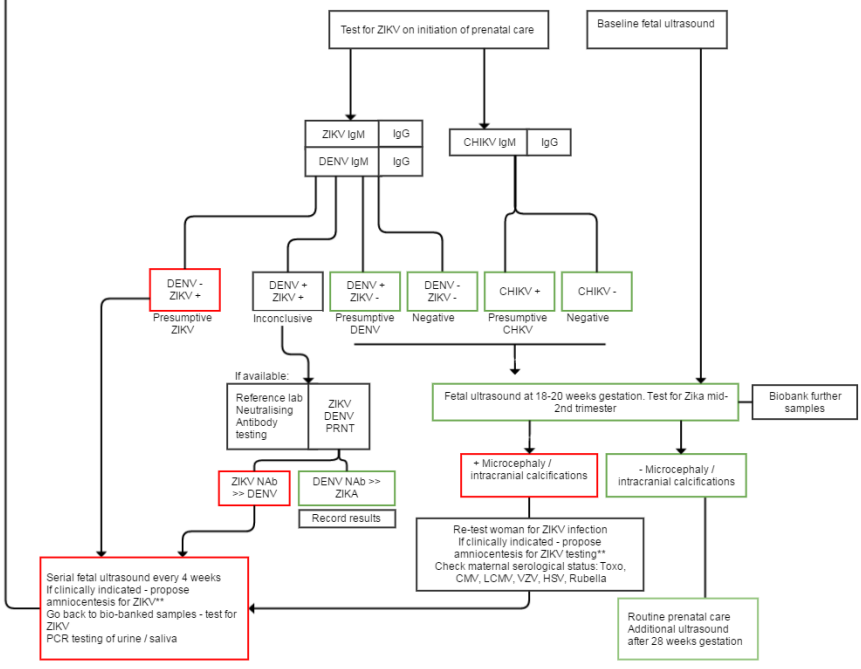
Tier Sampling approach

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

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

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

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



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

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

World Health Organization

DRAFT
Version 5
6/MAY/2016

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

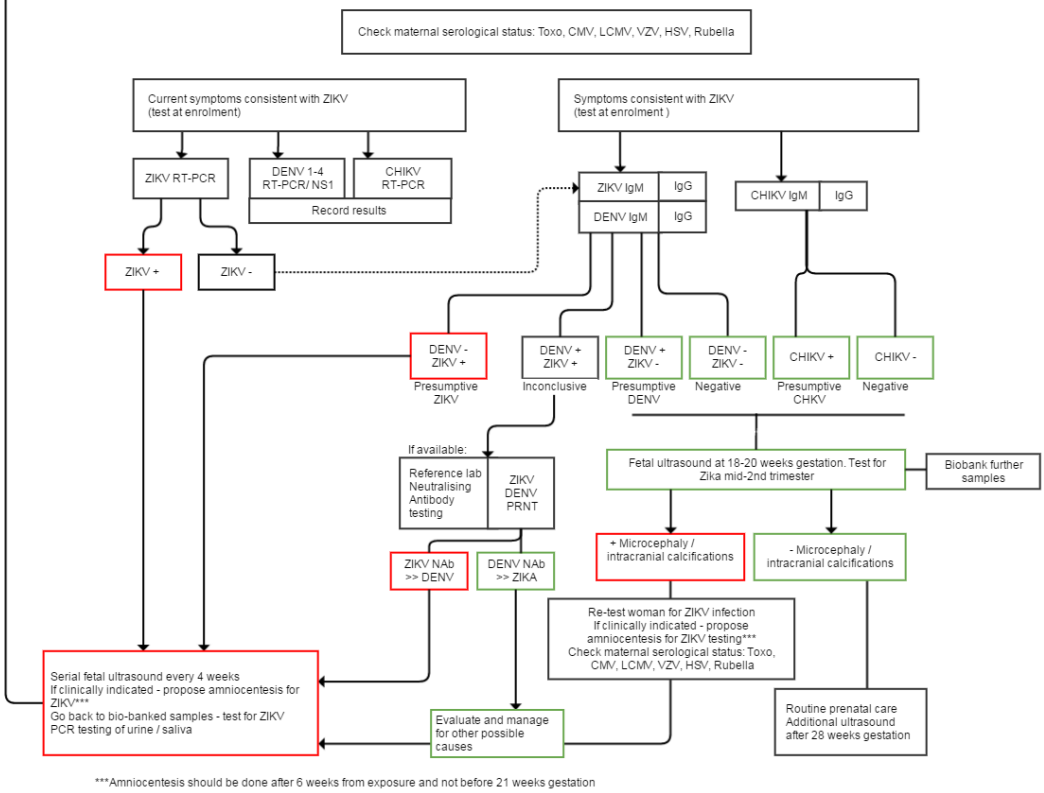
Sampling algorithm for research purposes

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)

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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

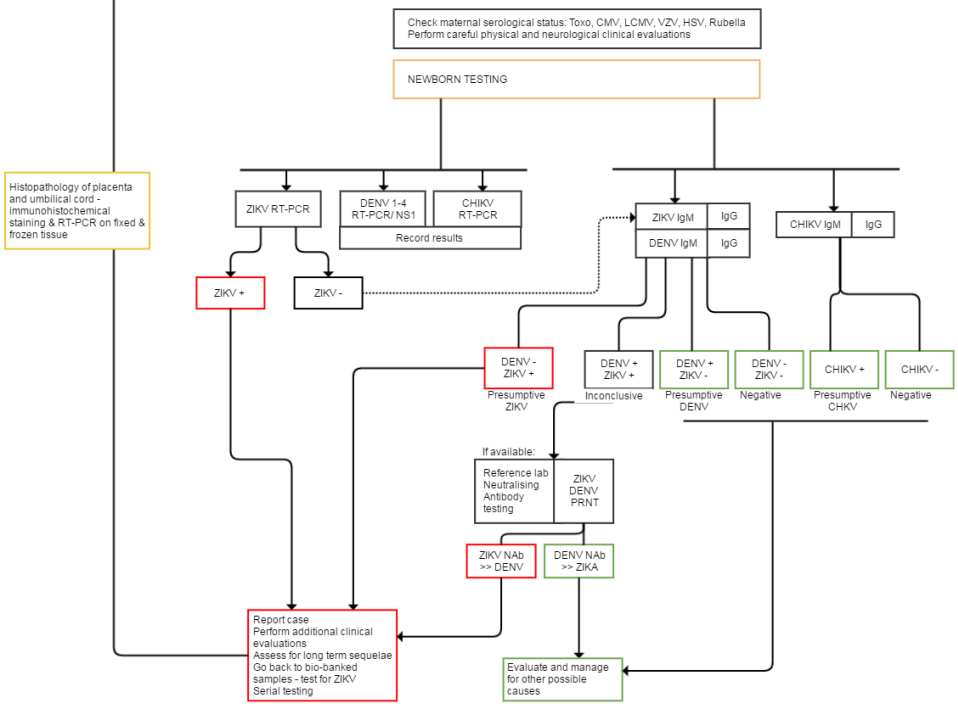
Tier Sampling approach

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)

World Health Organization

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

Sampling algorithm for research purposes

DRAFT

Version 5
6/MAY/2016

Tier Sampling approach

Tier 1: minimum sample set - single sample set taken at recruitment
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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
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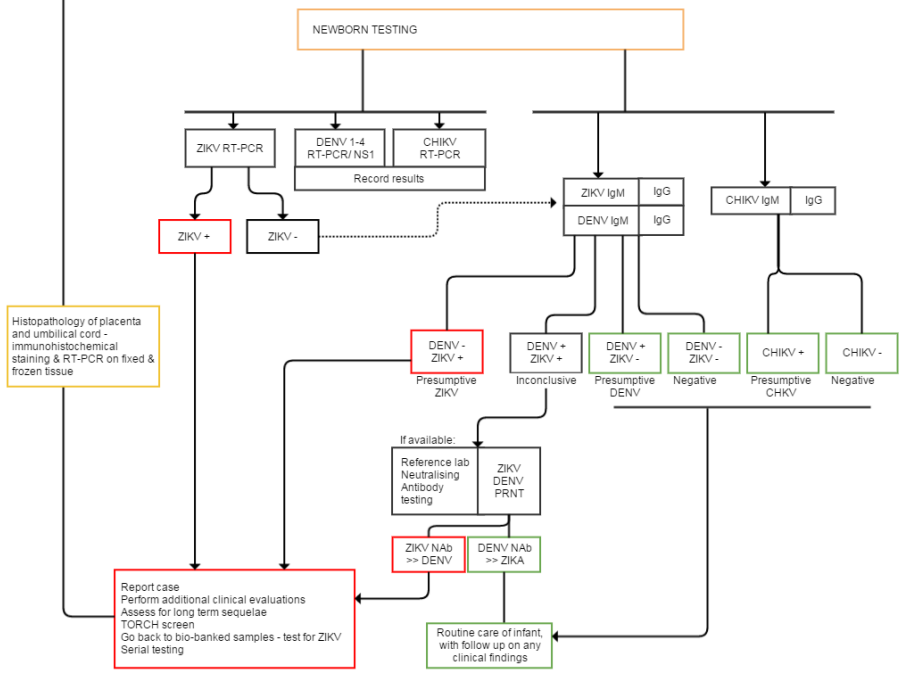

Serology
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APPENDIX D: LIST OF PUBLISHED PRIMERS FOR DETECTION AND QUANTIFICATION OF ZIKA VIRUS BY REAL-TIME RT-PCR (CAO-LORMEAU, BLAKE ET AL. 2016)

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