Disclaimer

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

The final version of this standardized protocol: *Case-control study to assess potential risk factors related to Guillain-Barré Syndrome including Zika virus infection* will be published as soon as the ethical review has been completed.

Standardized Protocol:

Case-control study to assess potential risk factors related to Guillain-Barré Syndrome including Zika virus infection

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

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

More information on CONSISE can be found on their website.

PROTOCOL SUMMARY

The World Health Organization and Pan American Health Organization, Institut Pasteur, the networks of Fiocruz, CONSISE and ISARIC and many other international research groups have generated standardized clinical and epidemiological research protocols and questionnaires to address key public health questions for Zika virus (ZIKV).

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

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

The protocol described below is a case-control study, designed to examine the differences in types of exposures between patients and controls. This standardized study protocol outlines methods to guide data collection in order to evaluate exposures and risk factors for Guillain-Barré syndrome (GBS) between patients diagnosed with GBS and controls without evidence of GBS and to quantify the strength of these associations with ZIKV infection. The data collected from this standardized protocol will help characterize demographic features or exposures associated with the development of GBS and guide the public health response.

Other protocols currently under development include:

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

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

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

1.0 INTRODUCTION

There is increasing evidence that Zika virus (ZIKV) infection may cause Guillain-Barré syndrome (GBS) - an autoimmune disease causing a highly diverse neurological syndrome, characterized by a rapidly progressive, symmetrical weakness of the limbs in combination with hyporeflexia or areflexia (Araujo, Ferreira et al. 2016, Cao-Lormeau, Blake et al. 2016, Malkki 2016). Typically, approximately 25% of the GBS patients develop a respiratory insufficiency requiring artificial ventilation and subsequent intensive medical care (van den Berg, Walgaard et al. 2014). Other pathogens, including viruses such as Dengue, Chikungunya virus, Cytomegalovirus (CMV) or Epstein Barr virus and bacteria such as *Campylobacter jejuni* or *Mycoplasma pneumoniae,* have been described as increasing the risk for GBS (Ravi, Taly et al. 1994, Jacobs, Rothbarth et al. 1998, Solomon, Dung et al. 2000, Hadden, Karch et al. 2001, Lebrun, Chadda et al. 2009, Islam, Jacobs et al. 2010, Leis and Stokic 2012).

Temporal coincidence between a ZIKV outbreak and an increase in the incidence of GBS cases has recently been reported in French Polynesia and in Brazil. Cao-Lormeau and colleagues (2016) provided the first evidence that ZIKV infection may cause GBS, independently from eventual co-infection with other *flavivirus* like Dengue virus. Given that the neurological conditions associated with GBS usually require admission into intensive care, public health preparedness is crucial. At the same time, further epidemiological studies are urgently needed to confirm whether GBS is related to ZIKV infection and to determine the risk factors for GBS.

The following standardized research protocol outlines methods to compare GBS patients with non-GBS patients. This study will address the following public health questions:

1. What are the risk factors for Guillain-Barré syndrome?
2. What are the risk factors for the larger spectrum of neurological damage associated with ZIKV infection?

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

1.1 OBJECTIVES

The data collected from this standardized study will be used to refine and update recommendations for surveillance and case definitions for Guillain-Barré syndrome (GBS). This will help characterize demographic features or exposures associated with the development of GBS and guide the public health response.

Comment: To date, GBS is the most common neurological complication that has been linked to ZIKV infection. As such, this protocol investigates the risk factors specific to GBS. Other neurological symptoms have been reported as being potentially linked to ZIKV infection, including bilateral facial palsy, acute myelitis, encephalitis or atypical GBS manifestations (Carteaux, Maquart et al. 2016, Mecharles, Herrmann et al. 2016, Roze, Najioullah et al. 2016). Should the epidemiological context

require the investigation of broader neurological complications, methodological information can be found as an addendum to this protocol (see Appendix A).

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

- Identify and quantify risk factors for GBS
- Quantify the strength of the association between GBS and ZIKV infection

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

- Estimate relative risk of GBS associated with infection with ZIKV and/or other pathogens
- Estimate the attributable risk of GBS associated with infection with ZIKV and/or other pathogens
- Describe the clinical, laboratory and imaging characteristics and outcome of patients with GBS that is associated with infection with ZIKV and/or other pathogens

2.0 STUDY PROCEDURES

STUDY SETTINGS

The study uses a case-control design that examines the differences in types of exposures between patients diagnosed with GBS and controls without evidence of GBS. Serum, urine and cerebrospinal fluid (CSF) will be collected at the time of GBS onset and will be tested for ZIKV and other pathogens known to cause GBS and known to be circulating concomitantly in the area of interest (Dengue, Chikungunya, West Nile, etc.).

Comment: Some of the pathogens known to cause GBS are ubiquitous, while others are unequally distributed geographically. The list of the pathogens to be tested will therefore need to be determined on a case-by-case basis.

The study may be prospective (i.e., only incidental cases are included) or retrospective (i.e., cases are enrolled retrospectively using a list of identified GBS cases in the area of interest), depending on the geographical and epidemiological context.

SELECTION AND RECRUITMENT OF STUDY PARTICIPANTS

2.2.1 STUDY POPULATION

Study participants will be selected from areas in which ZIKV has been reported to circulate (i.e., exposed populations). In case of a prospective study design, incident cases will be recruited at the time of diagnosis in hospitals located in or within an immediate proximity to the area(s) of interest. Exposed populations to ZIKV are defined here as populations residing in areas infested with the vector (i.e, *Aedes aegypti* and/or *Aedes albopictus* mosquitoes) and where confirmed ZIKV infection cases are documented.

Potential participants will receive information on the study at the time of GBS diagnosis and will be asked for their consent to participate in the study.

2.2.2 CASE AND CONTROL DEFINITION

- **Case:** a patient residing in the study area of interest with GBS meeting levels 1-3 of diagnostic certainty for the Brighton Collaboration criteria case definitions for GBS (Sejvar, Kohl et al. 2011).
- **Control:** for each case, two to four controls will be recruited and will be matched by age, sex and area of residence

Comment: The minimum duration of the participant's residence in the study area of interest needs to be discussed on a study-by-study basis as some countries or regions are more subject to intense

migration or population movements than other regions, and this needs be taken into consideration. Nevertheless, a minimum duration of six weeks of residence in the area of interest is recommended.

The decision of matching controls by sex should also be considered on a study-by-study basis, depending on the epidemiological context of the study. Matching by sex would prevent statistical analyses from determining whether sex is a risk factor for GBS in the context of ZIKV infection.

The study context will help determine the nature of the control group: hospital-based versus community-based controls, or combining both types of controls when feasible.

2.2.3 ELIGIBILITY CRITERIA

- **Inclusion criteria:** Any individual 18 years old or above [modify to be specific to the local age of consent], who gives informed consent either personally or by proxy; any child under the age of consent for whom a parent or guardian provides written informed consent; any minor under the local age of consent who can provide assent in the presence of a witnessing adult.
- **Exclusion criteria:** Patients unable to give informed consent personally or via an appropriate proxy; patients with severe immunodepression; pregnant women (see Comment below); patients who do not permanently reside in the study area (i.e., < 6 months residency in the study area), as well as those with any contraindication to venipuncture.

Comment: The exclusion of pregnant women from the study should be discussed on a study-by-study basis. Many IRBs are likely to consider pregnant women as a vulnerable population, thus preventing their inclusion in this study.

2.2.4 INFORMED CONSENT

During the first interview with the participant or his/her parent or guardian/proxy, the purpose of the study will be explained and consent will be obtained from the participant by a trained member of the investigation team. Each study participant must be informed that his/her participation in voluntary and that he/she will be free, without needing to justify himself/herself, to withdraw at any time without consequences.

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

The original version of the consent form for each patient will be retained by the investigation team and kept in a secure place for a period of 15 years after end of research. Also, at the time of signing the informed consent, one copy will be made and will be given to the study participant.

Written informed consent will be collected from all eligible patients or legal guardian/proxy.

Information for participant and informed consent form template can be found in Appendix B.

2.2.5 COMPENSATION AND INCENTIVES TO PARTICIPATE

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

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

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.

ETHICAL CONSIDERATIONS

Ethical approval will be sought in accordance with local, regional and national authorities. 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 to the Ethics Review Committee of the World Health Organization.

2.3.1 RISKS/BENEFITS FOR STUDY PARTICIPANTS

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

The collection of a small amount of cerebro-spinal fluid (CSF), blood and urine poses minimal risk to participants. CSF analysis may be indicated as part of the differential diagnostic investigation of GBS 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 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.

The primary benefit of this study is indirect in that data collected will help improve and guide efforts to improve public health measures towards ZIKV-exposed populations as well as the ones the more at risk for GBS and inform ZIKV vaccination strategies should a vaccine become available in the coming years. Participants will be informed of their individual results (e.g., whether they have evidence of past infection with ZIKV).

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.

DATA COLLECTION AND MANAGEMENT

After informed consent has been obtained from eligible participants, cases and controls will be interviewed using a standardized questionnaire. All cases and controls will be asked questions about activities, antecedent signs and symptoms of illness, and exposures in the two months prior to onset of neurologic illness for cases and the same time period for their age-matched controls. A calendar will be used to orient cases and controls to the time period of interest. Cases will also be asked specific questions on the development of GBS, including clinical evaluation. This will be repeated at a number of specific times across the course of the disease.

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

- Clinical report form for the investigation of GBS in relation to arboviral infections (provided by James Sejvar, US CDC)
- Clinical report form and clinical guidelines for the characterization of a GBS (provided by Benoît Rozé, Centre Hospitalier Martinique)
- Clinical report form for the investigation of GBS in relation to arboviral infections (provided by José Guerrero-Cantera, Intituto Mexicano Del Seguro Social)
- Clinical report form of a case-control study protocol conducted in French Polynesia (Institut Louis Mallardé & Institut Pasteur)
- 'Zika virus detection in urine from patients with GBS on Martinique, January 2016,' Rozé et al. 2016, Eurosurveillance.

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

Comment: A separate standardized protocol, called the Clinical Characterization Protocol, has been developed to collect clinical information required to describe important features of the natural history of Zika virus infection such as incubation period, period of infectivity, case definitions, clinical course, and risk factors for severe disease and congenital infection.

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

Diagnostic test results will be securely transmitted to the centre in charge of data centralization and analysis, which will then be responsible for making the tests results available to the investigation 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. Original data collection forms will be kept in locked storage in accordance with national regulations for an extended period of time after the end of the study. An identification log will be implemented and will be kept in a secure, locked facility within the study country.

SPECIMEN COLLECTION AND LABORATORY INVESTIGATIONS

2.5.1 SPECIMEN COLLECTION, STORAGE AND TRANSPORTATION

Table 1 lists the principal samples to be collected from cases and controls are listed in Table 1.

Serum and urine from cases and controls will be collected using standard techniques at the time of interview, and CSF analysis will be performed only on cases.

Matched controls are unlikely to have any samples that were previously collected; however, if these samples, collected during medical care for any illness experienced within two months of GBS symptom onset of the matching case, these residual specimens should be used and diagnostically tested for evidence of infection with the aforementioned pathogens.

Sample collection should be first performed at the time of onset of GBS to target the acute phase of the syndrome. If possible, serum should be collected before administration of any form of treatment (IV immunoglobulin, plasmapheresis).

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

Upon consent, any residual samples will be stored after infectious testing is complete at [name of testing facility] with an identification number for possible additional testing for GBS-associated biological markers or other infectious pathogens as clinically indicated. If a participant does not provide consent to store the specimens, all specimens for that participant will be destroyed once testing for infectious disease pathogens has been completed.

Comment: The full list of testing for the biological samples will be determined by the individual countries/researchers conducting the research.

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

	Specimen	Volume*	Container	Timing	Remarks
Case	Blood	7.5 mL	Dry tube (serum)	At GBS symptoms onset; if not possible, as early as possible**	Volume to be adjusted for weight and age in children. Minimal volume needed: 0.5 ml of whole blood.
	Urine	1 mL	Sterile collection tube	At GBS symptoms onset; if not possible, as early as possible	Minimal volume needed: 0.5 ml of urine.
	CSF	1.5 mL	Sterile collection tube	At GBS symptoms onset; if not possible, as early as possible	Volume to be adjusted for weight and age in children. Minimal volume needed: 0.5 ml of CSF.
Control	Blood	7.5 mL	Dry tube (serum)	At enrollment	Volume to be adjusted for weight and age in children. Minimal volume needed: 0.5 ml of whole blood.
	Urine	1 mL	Sterile collection tube	At enrollment	Minimal volume needed: 0.5 ml of urine.

**Suggested volume to collect, may be increased if clinically acceptable. **Ideally before administration of any kind of IV immunological treatment.*

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

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

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

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

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

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

2.5.2 LABORATORY PROCEDURES

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

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

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

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

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

	Specimen	Type of test	Targeted pathogen	Remarks
Case	Serum	• Real-time RT-PCR/PCR • Serology (IgM / IgG) • Biochemical and hematological analysis	• RT-PCR & serology: ZIKV and other locally circulating arboviruses • Serology only: Anti-ganglioside antibodies, enterovirus, HSV, VZV, CMV, HIV, HTLV-1, HVB, HVC, HVA, HVE, EBV, Influenza virus, measles, rubella, *Treponema pallidum*, Campylobacter*	In case of a positive result for ZIKV with serology, use same sample for plaque-reduction neutralization test (PRNT). For the list of biochemical and hematological analysis, see the questionnaire (Appendix C).

	Urine	Real-time RT-PCR	ZIKV and other locally circulating arboviruses	
	CSF	• Real-time RT-PCR/ PCR • Serology (IgM / IgG) • Biochemical & cytological analysis	• RT-PCR & serology: ZIKV and other locally circulating arboviruses • PCR/RT-PCR only: Enterovirus, HSV, VZV, CMV	In case of a positive result for ZIKV with serology, use same sample for plaque-reduction neutralization test.
Control	Serum	• Real-time RT-PCR/PCR • Serology (IgM / IgG) • Biochemical and hematological analysis	• RT-PCR & serology: ZIKV and other locally circulating arboviruses • Serology only: Enterovirus, HSV, VZV, CMV, HIV, HTLV-1, HVB, HVC, HVA, HVE, EBV, Influenza virus, measles, rubella, *Treponema pallidum, Campylobacter pylori, Mycoplasma pneumoniae, Chlamydia pneumoniae, Leptospira sp.*	In case of a positive result for ZIKV with serology, use same sample for plaque-reduction neutralization test. For the list of biochemical and hematological analysis, see the questionnaire (Appendix C).
	Urine	Real-time RT-PCR	ZIKV and other locally circulating arboviruses	

**For the diagnosis of syphilis, VDRL (Venereal Disease Research Laboratory) or TPHA (Treponema Pallidum Hemagglutinations Assay) may be used instead of serology.*

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, hepatic and renal enzymes, reactive protein C, etc. (for a complete list, see the questionnaire in Appendix C).

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 a 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 antibodies cross-reaction with other genetically related viruses is minimal during primary

infection, sera of individuals with a previous history of infection from other flaviviruses (especially dengue, yellow fever and West Nile) may cause cross-reactivity. Although neutralization by plaque reduction (PRNT) offers greater specificity in the detection of neutralizing antibodies (IgG), cross-reactions have also been documented. In fact, some patients with a previous history of infection by other flaviviruses have shown up to a fourfold increase in neutralizing antibody titers when infected with ZIKV. Thus, primary screening should be performed by enzyme-linked immunosorbent assays, immunoassays or immunofluorescence assays and confirmation will need to include virus neutralization assay.

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

2.5.3 PHYSICAL AND NEUROLOGICAL INVESTIGATIONS

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

- Full physical examination including the evaluation of neurological reflexes
- Evaluation of the Hughes score, the MRC breathlessness score and the GBS score using the Brighton criteria
- Electrophysiological analysis, when possible
- MRI of the spine and/or other imaging techniques when possible

3.0 STUDY ENDPOINTS AND STATISTICAL ANALYSES

3.1 SAMPLE SIZE CONSIDERATIONS

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

Comment: Sample size calculations should be performed as two-tailed statistical tests, with 90% of statistical power and 5% significance level. Table 4 shows different scenarios of sample size in case and control groups, depending on the proportion of exposure to ZIKV infection among cases and on the number of controls that will be matched to each case. The calculation was performed using Stata 13 software, with an odds ratio superior or equal to 2.

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

Proportion of ZIKV infection among cases	# of controls per case	# of cases	# of controls
70%	2	152	304
	3	135	405
	4	126	504
50%	2	140	280
	3	125	375
	4	117	468
30%	2	213	426
	3	190	570
	4	178	712

3.2 STUDY OUTCOME MEASURES

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

- Provide descriptive epidemiology: demographic characteristics of cases and controls (number of cases and controls; median age, sex, area of residence)
- Description of the clinical spectrum of GBS among enrolled cases:
    - Outcomes: discharge home with no sequelae, discharge home with sequelae, discharge to rehabilitation facility, discharge to long-term care facility, death
    - Duration of hospitalization, duration of each GBS phases, interval between infectious symptoms and onset of neurological symptoms, interval between GBS onset and nadir
    - ICU stay frequency and duration, mechanical ventilation frequency and duration, dysautonomia frequency
    - Hughes GBS disability score
    - Frequency of IV Immunoglobulin treatment, frequency of plasmapheresis
    - CSF findings among cases (red blood cell count, protein and glucose dosage)
    - Electrophysiological findings including GBS subtypes (AMAN, AIDP, AMSAN, Fisher syndrome)
    - Presence/frequency of anti-ganglioside antibodies
- Assessment of risk factors for GBS by calculating the odds of different exposures between GBS cases and controls, followed by logistic regression. This will include assessment of the frequency of ZIKV infection among cases and controls. ZIKV infection will be assessed by a positive test for anti-ZIKV antibody and/or detection of ZIKV nucleic acid using RT-PCR as defined by the standards of the laboratory.
- Calculating overall incidence of GBS and if possible calculating attributable risk of GBS among laboratory confirmed ZIKV cases.
- Assessing the frequency of infection with other pathogens than ZIKV among cases and controls

3.3 STATISTICAL ANALYSES

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

Data analysis will focus on potential environmental, behavioural, medical, or other risk factors for developing GBS, as well as laboratory evidence for infection with the aforementioned pathogens.

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

Objective	Outcomes	Statistical analysis
Describe the clinical, laboratory and imaging characteristics and outcome of patients with GBS that is associated with infection with ZIKV and/or other pathogens	Demographic characteristics of cases and controls (number of cases and controls; median age, sex, area of residence) Clinical spectrum of GBS among enrolled cases: disease outcome, duration of hospitalization, duration of each GBS phases, interval between infectious symptoms and onset of neurological symptoms, interval between GBS onset and nadir, ICU stay frequency and duration, mechanical ventilation frequency and duration, dysautonomia frequency, Hughes GBS disability score, frequency of IV Immunoglobulin treatment, frequency of plasmapheresis, CSF findings among cases (red blood cell count, protein and glucose dosage), electrophysiological findings including GBS subtypes (AMAN, AIDP, AMSAN, Fisher syndrome), presence/frequency of anti-ganglioside antibodies, etc.	Provide descriptive epidemiology for each outcome (mean/median, SD, etc.)
Identify and quantify risk factors for Guillain-Barré syndrome	Participant demographics, medical characteristics and exposures to different potential risk factors will be collected using a questionnaire (CRF) and laboratory tests	The risk factors for GBS will be determined by calculating the odds of different exposures between GBS cases and controls, followed by logistic regression
Estimate the relative risk of GBS associated with infection with ZIKV and/or other pathogens and quantify the strength of the association between GBS and ZIKV infection	ZIKV infection will be assessed by a positive test for anti-ZIKV antibody and/or detection of ZIKV nucleic acid using RT-PCR as defined by the standards of the laboratory. Infection with other pathogens will also be assessed using serology and/or PCR.	The increase of risk of GBS if infected with ZIKV that is associated with ZIKV will be estimated by the odds-ratio (OR) of the association between patient infection with ZIKV and GBS.
Estimate incidence of GBS associated with infection with ZIKV and/or other pathogens	Total number of GBS cases over the course of the study.	The incidence of GBS will be estimated by dividing the total number of GBS cases recruited during the study by the general population from which the cases come from and the duration of the study.
Estimate the attributable risk of GBS associated with infection with ZIKV	ZIKV infection will be assessed by a positive test for anti-ZIKV antibody and/or detection of ZIKV nucleic acid using RT-PCR as defined by the	The fraction of GBS that is attributable to ZIKV infection will be calculated as follows: $\frac{OR-1}{OR} * Pe$, where *Pe* is the

and/or other pathogens	standards of the laboratory. Infection with other pathogens will also be assessed using serology and/or PCR.	proportion of cases with ZIKV infection.

4.0 REPORTING OF FINDINGS

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

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

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

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

5.0 COMPLEMENTARY STUDIES

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

Additional standardized protocols for ZIKV are available and include:

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

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

6.0 ACKNOWLEDGEMENTS

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

James Sejvar (Center for Disease Control and Prevention, Brazil), Thais dos Santos (Pan American Health Organization), Benoit Rozé (Centre Hospitalier Universitaire Martinique), Jose Guerrero Cantera (Instituto Mexicano del Seguro Social, México).

Comment: This list will be amended, adding individuals and affiliations as appropriate.

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APPENDICES

Appendix A: Addendum to the case-control study protocol for GBS to the attention of researchers wishing to broaden the studied clinical spectrum to other neurological complications due to ZIKV infection

Appendix B: Description of investigation and informed consent template

Appendix C: Standardized questionnaire/DRAFT undergoing review

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: ADDENDUM TO THE CASE-CONTROL STUDY PROTOCOL FOR GBS TO THE ATTENTION OF RESEARCHERS WISHING TO BROADEN THE STUDIED CLINICAL SPECTRUM TO OTHER NEUROLOGICAL COMPLICATIONS DUE TO ZIKV INFECTION

To date, GBS is the most common neurological complication that has been linked to ZIKV infection. However, other neurological complications have been reported as potentially linked to ZIKV infection, including bilateral facial palsy, acute myelitis, encephalitis or atypical GBS manifestations (Carteaux, Maquart et al. 2016, Mecharles, Herrmann et al. 2016, Roze, Najioullah et al. 2016). In addition, animal models studies have recently provided evidence of ZIKV neurotropism (Garcez, Loiola et al. 2016, Lazear, Govero et al. 2016). The clinical spectrum of the neurological conditions caused by ZIKV is still poorly described and comprehensive epidemiological studies are urgently needed to provide additional knowledge on this phenomenon.

If research groups are interested in broadening the scope of the ZIKV-related neurological complications in this study, a few methodological changes would be required to the main protocol:

- The objectives would need to include the description and quantification of each neurological complication addressed by the study and the measurement of the association with ZIKV;
- The case definition would need to be adapted in order to include other types of neurological complications than GBS. The main difficulty would be in the decision to include all patients with neurological symptoms, which may be too large, or only those with 'GBS-like syndrome' which may be too restrictive;
- The endpoints would need to be adapted according to the objectives;
- The questionnaire would need to include specifically the neurological damages already described as being potentially related to ZIKV infection (listed below) but also leave open a dedicated space in the section 'Clinical examination' in order to give the possibility to the clinicians to describe the complete spectrum of neurological complications:
    - Myelitis
    - Encephalitis
    - Meningitis
    - Acute Disseminated Encephalomyelitis (ADEM)
    - Facial palsy / weakness
    - Hearing impair / loss
    - Optical nevritis

In the absence of a broader description of the ZIKV-associated neurological complications, the decision to expand the clinical scope of the study should be taken on a study-by-study basis, depending on the local epidemiological context. This addendum may be updated as further neurological findings are reported and published.

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

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

Comment: The language of this document is more technical than other 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:

Case-control study to assess potential risk factors related to Guillain-Barré Syndrome including Zika virus infection

International Sponsor: [_____]
Local Investigator: [_____]
International Collaborators: [_____]

INFORMATION

This document is meant to provide you with the written information necessary to make a decision regarding your participation in the study. We ask that you read this document carefully. Do not hesitate to ask the health care professional taking care of you any questions if you would like more information. You may take your time to reflect and consider your participation in this research, and discuss with your doctor and your close family and friends. At the end of this document, if 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 voluntary: you are free to accept or refuse to participate in this medical research. If you decide to participate, please keep in mind that you can withdraw your consent at any moment, without any consequences, ill-feeling or prejudice. A withdrawal will change nothing about the care you receive. We simply ask you to inform the health care professional who is in charge of your care. You will not be asked to justify or explain your decision.

GENERAL BACKGROUND AND RESEARCH OBJECTIVES

A Zika virus epidemic has been occurring in the [region of study] since [general time of ZIKV introduction into study region]. Zika virus is usually transmitted to people by mosquitoes. Most people who are infected with Zika virus do not get sick. About one in five people will have symptoms.

The most common symptoms of Zika virus infection are rash, fever, joint pain, and red eyes. Other symptoms may include headaches or muscle pains.

You are being asked to participate in a study which is attempting to determine whether Zika virus might be causing a neurological syndrome called Guillain-Barré syndrome – limb and facial paralysis which can lead to hospitalization in intensive care unit. Some patients with Guillain-Barré syndrome in French Polynesia and Brazil have been found to be infected with Zika virus. It is believed that these patients were infected when bitten by a mosquito with Zika virus.

The objectives of this research are to:

- Identify and quantify the risk factors for Guillain-Barré syndrome
- Quantify the strength of the association between Guillain-Barré syndrome and ZIKV infection
- Estimate the risk of developing Guillain-Barré syndrome in individuals infected with ZIKV and/or other pathogens compared to those who have not been infected with the cited pathogen(s).
- Describe the clinical, laboratory and imaging characteristics and outcome of patients with Guillain-Barré syndrome that is associated with infection with ZIKV and/or other pathogens

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

RESEARCH PROCESS

If you agree to participate in this study then you will be asked to answer questions about your health and daily life, such as the type of protection measures you use against mosquitoes. We will ask for access to your past and present medical records. We also would like to draw blood through a needle in your arm and collect a urine sample from you. We would like approximately 7.5ml of blood, 1 to 3 mL of urine (less than two teaspoons). There is a risk that you experience some discomfort when we take your blood. A small bruise may also appear. Some people might feel lightheaded when they have their blood drawn.

In case you have developed a Guillain-Barré syndrome, we would like to collect cerebrospinal fluid using a lumbar puncture. The volume collected would be less than one teaspoon. Cerebrospinal fluid collection can cause some pain and other complications, such as infection of the central nervous system or neurological injury; however this risk is minimal if the sampling is performed by a trained physician and under sterile conditions, such will be the case in this study.

The samples collected from you will be tested for Zika virus and other pathogens known to cause Guillain-Barré syndrome, such as influenza, cytomegalovirus, etc.

RISKS AND BENEFITS OF YOUR PARTICIPATION

This research does not present any foreseeable risk for you; no procedure will be done on you that is not designed for the purpose of this study. Furthermore, all procedures that are done will follow the current standard of care in your location for medical care of Guillain-Barré syndrome. The primary benefit of this study is the extended medical care and intensified (i.e., beyond routine) follow-up. This will allow for timely detection and intervention for any arising abnormalities.

RESEARCH RESULTS

The main results of this research will be shared with national and international authorities, such as the World Health Organisation. The results of this research may be presented in scientific conferences and publications. However, your personal data will not be identifiable by any means, as all data will be made 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

There will need to be a computer-based treatment of your data in order for us to analyse it. This analysis will eventually allow us to answer the research objectives. To this end, your medical data, and the data relating to your lifestyle and ethnic origins will be transmitted to your doctor or to persons working for the research group, in [country of study] or overseas in other countries.

If, during the course of the study, you no longer wish to participate, the research team will use any data that has been collected up until that point. You may refuse the use of your data, in which case, all data collected will be destroyed.

INFORMATION ON YOUR SAMPLES DURING AND AFTER THIS STUDY

If your samples are not completely used upon completion of the study, they may be stored and used for other research studies that are looking at Zika, and possibly for other viral infections that are transmitted by mosquitoes. As in this study, 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.

If, during the course of this study, you no longer wish to participate, any samples collected from you before the withdrawal of your consent could be stored and used for the purpose of the study. . You may refuse the use of your samples, in which case, all samples collected will be destroyed.

INFORMED CONSENT OF PARITICIPANTS

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

Case-control study to assess potential risk factors related to Guillain-Barré Syndrome including Zika virus infection

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

- ☐ I have read or orally received all the necessary information to understand the topic and enrolment process of the study.
- ☐ I was able to ask questions and received clear and adequate responses.
- ☐ I confirm my participation in this study, which includes responding to a questionnaire and allowing the taking of biological samples from me.
- ☐ 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 RELATED TO PERSONAL DATA

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

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

CONSENT RELATIVE TO THE USE OF MY BIOLOGICAL SAMPLES

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

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

SIGNATURES

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

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

LAST NAME, First name: Contact number:	Date: Signature:

APPENDIX C: STANDARDIZED QUESTIONNAIRE/DRAFT UNDERGOING REVIEW

Development of the draft questionnaire

This questionnaire has been designed by the Institut Pasteur, ISARIC, CONSISE, WHO, and partners and adapted from:

- Clinical report form for the investigation of GBS in relation to arboviral infections (provided by James Sejvar, US CDC)
- Clinical report form and clinical guidelines for the characterization of a GBS (provided by Benoît Rozé, Centre Hospitalier Martinique)
- Clinical report form for the investigation of GBS in relation to arboviral infections (provided by José Guerrero-Cantera, Intituto Mexicano Del Seguro Social)
- Clinical report form of a case-control study protocol conducted in French Polynesia (Institut Louis Mallardé & Institut Pasteur)
- 'Zika virus detection in urine from patients with GBS on Martinique, January 2016,' Rozé et al. 2016, Eurosurveillance.

Purpose of the standardized questionnaire and instructions for its use

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

Instructions for completing questionnaire

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

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

General guidance

- The questionnaire is designed to collect data obtained through patient examination, through parent/guardian/representative interview (for minors), and review of hospital charts.
- Patient ID codes should be filled in on all pages of the questionnaire.
- Complete every line of every section, except for where the instructions say to skip a section based on certain responses.
- It is important to indicate when the answer to a particular question is not known. Please mark the 'Unknown' box if this is the case.
- Some sections have open areas where you can write additional information. To permit standardized data entry, please avoid writing additional information outside of these areas.
- Place an (X) when you choose the corresponding answer. To make corrections, strike through (----) the data you wish to delete and write the correct data above it. Please initial and date all corrections.
- Please keep all of the sheets for each study participant together e.g. with a staple or in a folder that is unique to the patient.

- Please contact us if we can help with any CRF completion questions, if you have comments, and to let us know that you are using the forms.
- We recommend writing clearly in black or blue ink, using BLOCK-CAPITAL LETTERS.
- Do not use abbreviations; write out each letter.
- Complete the heading on each page.
- Use standard medical language.
- Write only one character per box (|__|)
- Numerical values :
  - Align numerical values to the right
  - Do not add commas, they will already be present in the field if appropriate
  - Do no leave any space empty, enter a zero if necessary

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

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

For example: Yes ☐ No ☒

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

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

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

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

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

Interviewer: _____

IDENTIFICATION: STUDY PARTICIPANT

Study code	Center code	Patient code	Initials (surname/first name)
I___I	I___I	I___I___I___I___I	I___I___I

CASE/CONTROL STATUS

CASE	Yes	No
Patient residing in the study area of interest with GBS meeting levels 1-3 of diagnostic certainty for the Brighton Collaboration criteria case definitions for GBS	☐	☐
CONTROL		
Patient with no sign of GBS	☐	☐

VERIFICATION OF ELIGIBILITY

INCLUSION CRITERIA	Yes	No
Adult 18 years old and over, who has given consent either personally or by proxy if the patient is too ill to give consent personally.	☐	☐
Patient under the age of 18, for whom a written permission to participate has been obtained from a parent or guardian.	☐	☐
EXCLUSION CRITERIA		
Patient unable to give informed consent in person or via an appropriate proxy	☐	☐
Patient with severe immunodepression	☐	☐
Patient who does not permanently reside in the study area (i.e., Add: minimum number of months residency in the study area)	☐	☐
Pregnant woman (Comment: To be decided on a study-by-study basis, depending on the status of pregnant women as vulnerable population in the country of the study.)	☐	☐
	☐	☐

Contraindication to venipuncture		

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

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

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

Interviewer: _____

1) DEMOGRAPHICS OF PARTICIPANT

Sex:	☐ Male ☐ Female
Date of birth:	____ / ____ / _____
Age at development of first neurological symptoms:	_____ years
Area of residence: (Or, enter GPS coordinates):	_____ _ _ . _ _ _ _ _ S, _ _ _ . _ _ _ _ _ E
Maternal language	(Add check boxes here)
Social-professional category Comment: Add occupation/professional categories that are appropriate for the country implementing the study	☐ Student ☐ Farmer ☐ Artisan, merchant, business owner ☐ Highly qualified professional (management) ☐ Employee ☐ Labourer/factory worker ☐ Without profession ☐ Retired ☐ Does not wish to respond ☐ Other (specify): _____
Ethnicity	(Add check boxes according to national guidelines)
Blood type:	☐ A ☐ B ☐ O ☐ Unknown
Rh type:	☐ +ve ☐ -ve ☐ Unknown

2) MEDICAL HISTORY

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

Participant has previously had GBS : - If yes, specify date:	☐ Yes ☐ No ☐ Unknown ____ / ____ / _____
Family history of GBS: - If yes, specify who and date:	☐ Yes ☐ No ☐ Unknown ____ / ____ / _____
History of other neurological illness: - If yes, specify condition and date**:**	☐ Yes ☐ No ☐ Unknown ____ / ____ / _____
History of cardiovascular disease: - If yes, specify condition and duration:	☐ Yes ☐ No ☐ Unknown
High blood pressure: - If yes, specify duration:	☐ Yes ☐ No ☐ Unknown
High cholesterol: - If yes, specify type and duration**:**	☐ Yes ☐ No ☐ Unknown
History of stroke: - If yes, specify date:	☐ Yes ☐ No ☐ Unknown ____ / ____ / _____
Diabetes: - If yes, specify type and duration**:**	☐ Yes ☐ No ☐ Unknown
Obesity:	☐ Yes ☐ No ☐ Unknown
Asthma:	☐ Yes ☐ No ☐ Unknown
COPD:	☐ Yes ☐ No ☐ Unknown
Kidney disease:	☐ Yes ☐ No ☐ Unknown
Liver disease:	☐ Yes ☐ No ☐ Unknown

Rheumatologic disease:	☐ Yes ☐ No ☐ Unknown
HIV infection:	☐ Yes ☐ No ☐ Unknown
Cancer: - If yes, specify type: - If yes, specify nature of treatment: - If yes, specify duration of treatment:	☐ Yes ☐ No ☐ Unknown ____ / ____ /_____ - ____ / ____ /_____
Other medical history: - If yes, specify condition and duration:	☐ Yes ☐ No ☐ Unknown
Surgical history: - If yes, specify:	☐ Yes ☐ No ☐ Unknown
Blood transfusion: - If yes, specify date:	☐ Yes ☐ No ☐ Unknown ____ / ____ /_____
Vaccination for Yellow Fever: - If yes, specify date**:** - If yes, indicate:	☐ Yes ☐ No ☐ Unknown ____ / ____ /_____ ☐ Information verified on vaccine card ☐ Information provided verbally
Vaccination within the last six weeks: - If yes, indicate: - If yes, specify type and date**:**	☐ Yes ☐ No ☐ Unknown ☐ Information verified on vaccine card ☐ Information provided verbally ____ / ____ /_____ ____ / ____ /_____
Specific or concomitant treatments(s) during the last six weeks: - If yes specify:	☐ Yes ☐ No ☐ Unknown

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

Over the past six weeks, have you been sick?	☐ Yes ☐ No ☐ Unknown
- If yes, specify date of onset of symptoms:	____/ ____/______
- If yes, indicate symptoms: (tick all that apply)	☐ Fever ☐ Chills ☐ Nausea or vomiting ☐ Diarrhoea ☐ Muscle pains ☐ Joint pains ☐ Skin rash ☐ Abnormally red eyes ☐ Headache ☐ Pain behind eyes ☐Stiff neck ☐ Confusion ☐ Abdominal pain ☐ Coughing ☐ Runny nose ☐ Sore throat ☐ Calf pain

	☐ Pruritus ☐ Other: Specify _____
- If yes, did you seek healthcare for these symptoms?	☐ Yes ☐ No ☐ Unknown
- If yes, date of consultation: - Name of medical doctor: - Specimen collected for testing: - Specimen aliquot remaining:	____ / ____ /_____ ☐ Yes ☐ No ☐ Unknown ☐Yes ☐ No ☐ Unknown

Comment: For biological, virological and bacteriological testing, please fill in Sections 6 and 7.

3) EXPOSURES

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

Protection against mosquitoes prior to symptom onset	
Do you wear long trousers/long sleeves?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
Do you use a mosquito net while you sleep during the day or at night?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
Do you use essential oils to rid your home of mosquitos?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
Do you use window or door screens to keep mosquitos out of your home?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
Do you use mosquito repellent spray?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
Do you use insecticides to remove mosquito larvae from your home?	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always
Do you use other methods to rid you home of mosquitos? - If yes, indicate here which methods you've used:	☐Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always

Has anyone you know had a Zika virus infection during the time of your pregnancy?		**Did this individual go to a health care clinic?**	**(DD/MM/YYYY)**
Husband/partner	☐Yes ☐ No	☐Yes ☐ No	_____
Children	☐Yes ☐ No	☐Yes ☐ No	_____
Neighbours	☐Yes ☐ No	☐Yes ☐ No	_____
Close friends/relative	☐Yes ☐ No	☐Yes ☐ No	_____
Other (specify): _____		☐Yes ☐ No	_____
In the last six weeks, has anyone in your household been sick at all?		**Did this individual go to a health care clinic?**	**(DD/MM/YYYY)**

Spouse/partner **Children** **Other (specify): _____**	☐Yes ☐ No ☐Yes ☐ No	☐Yes ☐ No ☐Yes ☐ No ☐Yes ☐ No _____	_____ _____ _____
If yes, indicate symptoms : (tick all that apply)	☐ Fever ☐ Chills ☐ Nausea or vomiting ☐ Diarrhoea ☐ Muscle pains ☐ Joint pains ☐ Skin rash ☐ Abnormally red eyes ☐ Headache ☐ Pain behind eyes ☐Stiff neck ☐ Confusion ☐ Abdominal pain ☐ Coughing ☐ Runny nose ☐ Sore throat ☐ Calf pain ☐ Pruritus ☐ Other: Specify _____		

In the last six weeks, what pets, farm or other animals have lived in your house or on your property? (tick all that apply)	☐ Dog(s) ☐ Cat(s) ☐ Pet rodent(s) ☐ Pet bird(s) ☐ Pet lizard/turtle(s) ☐ Goat(s) ☐ Sheep ☐ Cows ☐ Poultry ☐ Pigs ☐ Other: Specify _____
In the last year, did you eat any of the following foods raw or undercooked? (tick all that apply)	☐ Beef ☐ Lamb ☐ Chicken ☐ Fish ☐ Shellfish
In the last six weeks, have you taken your drinking water from the tap? - If yes, was the water boiled or treated?	☐ Yes ☐ No If yes: ☐ Sometimes ☐ Often ☐ Always ☐ Yes ☐ No ☐ Unknown
In the last six weeks, have you taken your drinking water from a well or river/stream/pond? - If yes, was the water boiled or treated?	☐ Yes ☐ No ☐ Unknown If yes: ☐ Sometimes ☐ Often ☐ Always ☐ Yes ☐ No ☐ Unknown
In the last six weeks, have you swam or wadded in a freshwater river, stream or pond?	☐ Yes ☐ No ☐ Unknown If yes: ☐ Daily ☐ Weekly ☐ Monthly ☐ Rarely

How much time do you spend outdoors each day?	☐ <1 hour ☐ 1-4 hours ☐ 5-8 hours ☐ >8 hours
- If yes, # of glasses / day	____ / day
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

4) CLINICAL EXAMINATION

Body weight:	_____ (kg)
Body temperature:	_____ (°C)
Respiratory rate:	_____ (kg)
Heart rate:	_____ (bpm)
Arterial blood pressure: Systolic/ Diastolic	_____ (mmHg)
Pulse:	_____ (bpm)
Pulse oximetry:	_____ (%)
Clinical characteristics indicative of infectious illness: - If yes, indicate symptoms: (tick all that apply)	☐ Yes ☐ No ☐ Unknown ☐ Fever ☐ Chills ☐ Nausea or vomiting ☐ Diarrhoea ☐ Muscle pains ☐ Joint pains ☐ Skin rash ☐ Headache ☐ Pain behind eyes ☐ Stiff neck ☐ Confusion ☐ Abdominal pain ☐ Coughing ☐ Runny nose ☐ Sore throat ☐ Calf pain ☐ Pruritus ☐ Bleeding ☐ Conjunctival hyperaemia ☐ Petechiae ☐ Limb swelling ☐ Other: specify _____
Specimen collection for testing: - If yes, indicate: (tick all that apply)	☐ Yes ☐ No ☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: specify _____

Comment: For biological and microbiological analyses, please fill in Sections 6 and 7.

5) GUILLAIN-BARRE SYNDROME DIAGNOSIS (CASES ONLY)

Has the patient been seen by a neurologist? - If yes, name of the neurologist: - If yes, date of the consultation (DD/MM/YYYY):	☐ Yes ☐ No ____ / ____ /_____
Date of <u>onset of neurological symptoms</u>: (DD/MM/YYYY)	____ / ____ /_____
Symptoms at onset: - (tick all that apply)	☐ Leg weakness ☐ Arm weakness ☐ Face weakness ☐ Facial palsy ☐ Leg numbness/parasthesias ☐ Arm numbness/parasthesias ☐ Face numbness/parasthesias ☐ Diploplia/Opthalmoplegia ☐ Gait imbalance (not weakness) ☐ Hand clumsiness (not weakness) ☐ Dysphagia ☐ Dysarthria ☐ Incapacity to walk ☐ Areflexia / decreased reflexes ☐ SOB/respiratory distress ☐ Other: specify _____
Date of <u>onset of hospital admission</u>: (DD/MM/YYYY)	____ / ____ /_____
Symptoms at hospital admission: - (tick all that apply)	☐ Leg weakness ☐ Arm weakness ☐ Face weakness ☐ Facial palsy ☐ Leg numbness/parasthesias ☐ Arm numbness/parasthesias ☐ Face numbness/parasthesias ☐ Diploplia/Opthalmoplegia ☐ Gait imbalance (not weakness) ☐ Hand clumsiness (not weakness) ☐ Dysphagia ☐ Dysarthria ☐ Incapacity to walk ☐ Areflexia / decreased reflexes

	☐ SOB/respiratory distress ☐ Other: specify _____
Hughes Disability Score at admission: Comment: Hughes Disability Score 0 = Health state; 1= Minor symptoms and capable of running; 2= Able to walk 10 meters or more without assistance but unable to run; 3= Able to walk 10 meters across an open space with help; 4= Bedridden or chairbound; 5= Requiring assisted ventilation for at least part of the day; 6= Dead (van Koningsveld, Steyerberg et al. 2007)	_____ (0 to 6) ☐ Unknown
MRC Breathlessness Scale at admission: Comment: MRC Breathlessness Scale 0 = No breathlessness; 1= Not troubled by breathlessness except on strenuous exercise; 2= Short of breath when hurrying on the level or walking up a slight hill; 3= Walks slower than most people on the level, stops after a mile or so, or stops after 15 minutes walking at own pace; 4= Stops for breath after walking about 100 yards or after a few minutes on level ground; 5= Too breathless to leave the house, or breathless when undressing (Stenton 2008)	_____ (0 to 5) ☐ Unknown
Date of <u>peak illness</u>: (DD/MM/YYYY)	____ / ____ /_____
Symptoms at peak illness: - (tick all that apply)	☐ Leg weakness ☐ Arm weakness ☐ Face weakness ☐ Facial palsy ☐ Leg numbness/parasthesias ☐ Arm numbness/parasthesias ☐ Face numbness/parasthesias

	☐ Diploplia/Opthalmoplegia ☐ Gait imbalance (not weakness) ☐ Hand clumsiness (not weakness) ☐ Dysphagia ☐ Dysarthria ☐ Incapacity to walk ☐ Areflexia / decreased reflexes ☐ SOB/respiratory distress ☐ Other: specify _____
Hughes Disability Score at peak illness: Comment: Hughes Disability Score 0 = Health state; 1= Minor symptoms and capable of running; 2= Able to walk 10 meters or more without assistance but unable to run; 3= Able to walk 10 meters across an open space with help; 4= Bedridden or chairbound; 5= Requiring assisted ventilation for at least part of the day; 6= Dead (van Koningsveld, Steyerberg et al. 2007)	_____ (0 to 6) ☐ Unknown
MRC Breathlessness Scale at peak illness: Comment: MRC Breathlessness Scale 0 = No breathlessness; 1= Not troubled by breathlessness except on strenuous exercise; 2= Short of breath when hurrying on the level or walking up a slight hill; 3= Walks slower than most people on the level, stops after a mile or so, or stops after 15 minutes walking at own pace; 4= Stops for breath after walking about 100 yards or after a few minutes on level ground; 5= Too breathless to leave the house, or breathless when undressing (Stenton 2008)	_____ (0 to 5) ☐ Unknown

Time from onset to most serious neurological symptom:	☐ Minutes ☐ Hours ☐ Days ☐Weeks
At the time of the most serious neurological symptom, the patient was: - (tick all that apply)	☐ Unable to walk without assistance (e.g., cane, walker) ☐ Unable to walk at all ☐ Admitted to the hospital ☐ Admitted to the ICU/CCU ☐ Intubated

MUSCLE & NERVE ELECTROPHYSIOLOGY

Nerve conduction study: - If yes, indicate date (DD/MM/YYYY): - If yes, are findings compatible with definition of GBS? - If yes, indicate type of GBS:	☐ Yes ☐ No ☐ Unknown ____/ ____/_____ ☐ Yes ☐ No ☐ AMAN ☐ AIDP ☐ AMSAN ☐ Fisher syndrome ☐ Uninterpretable
Has the nerve conduction study been repeated? - If yes, indicate date (DD/MM/YYYY): - If yes, are findings compatible with definition of GBS? - If yes, indicate type of GBS:	☐ Yes ☐ No ☐ Unknown ____/ ____/_____ ☐ Yes ☐ No ☐ AMAN ☐ AIDP ☐ AMSAN ☐ Fisher syndrome ☐ Uninterpretable
Other muscle & nerve electrophysiology test(s): - If yes, indicate test: - If yes, indicate date (DD/MM/YYYY): - If yes, indicate result:	☐ Yes ☐ No ☐ Unknown ____/ ____/_____

CENTRAL NERVOUS SYSTEM:

MRI of spinal cord: - If yes, indicate date (DD/MM/YYYY): - If yes, indicate result:	☐ Yes ☐ No ☐ Unknown ____/ ____/_____

Lumbar puncture performed: - If yes, indicate date of CSF collection: - If yes, indicate result: Proteins Erythrocytes Leukocytes Glucose	☐ Yes ☐ No ☐ Unknown ____ / ____ /_____ _____ (g/L) _____ (cells per mm^3) _____ (cells per mm^3) _____ (unit _____)

Comment: For biological and microbiological analyses, please fill in Sections 6 and 7.

SPECIFIC TREATMENT FOR GBS:

Has specific treatment for GBS been administered?	☐ Yes ☐ No ☐ Unknown
- Intravenous globulins:	☐ Yes ☐ No
- If yes, indicate date of treatment:	____ / ____ /_____
- If yes, indicate dosage:	_____ (unit _____)
- Plasmapheresis:	☐ Yes ☐ No
- If yes, indicate date of treatment:	____ / ____ /_____
- If yes, indicate volume:	_____ (unit _____)
- Other treatment, specify_____:	
- If yes, indicate date of treatment:	____ / ____ /_____
- If yes, indicate dosage:	_____ (unit _____)

INTENSIVE CARE:

Has the patient been admitted to intensive care? - If yes, indicate reason(s): Trouble swallowing: Need for respiratory assistance: Other, specify:	☐ Yes ☐ No ☐ Unknown ☐ Yes ☐ No ☐ Yes ☐ No
Duration of stay in intensive care: - Date of admission (DD/MM/YYYY): - Date of discharge (DD/MM/YYYY): (Please leave empty if patient is still in ICU)	 ____ / ____ /_____ ____ / ____ /_____
Duration of mechanical ventilation: - Date of intubation (DD/MM/YYYY): - Date of exubation (DD/MM/YYYY): (Please leave empty if patient is still under mechanical ventilation)	 ____ / ____ /_____ ____ / ____ /_____

Hughes Disability Score at time of last evaluation:	_____ (0 to 6) ☐ Unknown

Comment: Hughes Disability Score 0 = Health state; 1= Minor symptoms and capable of running; 2= Able to walk 10 meters or more without assistance but unable to run; 3= Able to walk 10 meters across an open space with help; 4= Bedridden or chairbound; 5= Requiring assisted ventilation for at least part of the day; 6= Dead (van Koningsveld, Steyerberg et al. 2007)	
GBS Brighton level at time of last evaluation:	_____ (1 to 5) ☐ Unknown

Comment: US CDC GBS Brighton level identification:

Level 1	**Level 2**	**Level 3**	**Level 4**	**Level 5** (Not a case)
Absence of an alternative diagnosis for weakness				
Acute onset of bilateral and relatively symmetric flaccid weakness of the limbs				
Decreased or absent deep tendon reflexes in affected limbs				
Monophasic illness pattern with weakness nadir between 12 hours and 28 days, followed by clinical plateau				
Albuminocytologic dissociation (elevation of CSF protein level above laboratory normal value and CSF total white cell count < 50 cells/mm3)				
CSF with a total white cell count < 50 cells/mm^3 (with or without CSF protein elevation above laboratory normal value) or if CSF not collected or results not available, and electrodiagnostic studies consistent with GBS				
Electrophysiologic findings consistent with GBS				

Duration of hospitalization: - Date of admission (DD/MM/YYYY): - Date of discharge (DD/MM/YYYY): (Please leave empty if patient is still in hospital)	____ / ____ /_____ ____ / ____ /_____

6) MICROBIOLOGICAL ANALYSIS

When were specimens collected:	☐ At the time of infectious illness onset ☐ At the time of neurological symptoms onset ☐ Other, specify: _____

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

Pathogen	Type of specimen	Date of collection	Type of test	Result
Zika virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Dengue virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Chikungunya virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Cytomegalovirus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Epstein-Barr virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Herpes simplex virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____ / ____ /_____	☐ PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ PCR result: ____ copies/ml

Hepatitis A virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/____/____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Hepatitis B virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/____/____	☐ PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ PCR result: ____ copies/ml
Hepatitis C virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/____/____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Hepatitis E virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/____/____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
HIV	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/____/____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
HTLV-1	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/____/____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Influenza virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/____/____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Varicella/Zona virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/____/____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Enterovirus	☐ Blood/Serum		☐ RT-PCR	☐ Positive

	☐ Urine ☐ CSF ☐ Other: _____	____/ ____/_____	☐ IgM ☐ IgG ☐ Other: _____	☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Measles virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/ ____/_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Rubella virus	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/ ____/_____	☐ RT-PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ RT-PCR result: ____ copies/ml
Mycoplasma pneumoniae	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/ ____/_____	☐ PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ PCR result: ____ copies/ml
Chlamydia pneumoniae	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/ ____/_____	☐ PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ PCR result: ____ copies/ml
Campylobacter jejuni	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/ ____/_____	☐ PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ PCR result: ____ copies/ml
Treponema pallidum	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/ ____/_____	☐ PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ PCR result: ____ copies/ml
Leptospira sp.	☐ Blood/Serum ☐ Urine ☐ CSF ☐ Other: _____	____/ ____/_____	☐ PCR ☐ IgM ☐ IgG ☐ Other: _____	☐ Positive ☐ Negative ☐ NK ☐ ND ☐ PCR result: ____ copies/ml
Other (specify): _____	☐ Blood/Serum ☐ Urine		☐ PCR/ RT-PCR ☐ IgM ☐ IgG	☐ Positive ☐ Negative

	☐ CSF ☐ Other: _____	____ / ____ /_____	☐ Other: _____	☐ NK ☐ ND ☐ PCR result: ____ copies/ml

NK: Unknown; ND: Not determined.

7) BIOLOGICAL AND IMMUNOLOGICAL ANALYSIS

When were specimens collected:	☐ At the time of infectious illness onset ☐ At the time of neurological symptoms onset ☐ Other, specify: _____

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

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

Vitamin B1	____/____/_____		
Anti-ganglioside antibodies in serum			
- Any	____/____/_____	☐ Yes ☐ No	
- GM1	____/____/_____	☐ Yes ☐ No	
- GA1	____/____/_____	☐ Yes ☐ No	
- GM2	____/____/_____	☐ Yes ☐ No	
- GD1a	____/____/_____	☐ Yes ☐ No	
- GD1b	____/____/_____	☐ Yes ☐ No	
- GQ1b	____/____/_____	☐ Yes ☐ No	
- GT1a	____/____/_____	☐ Yes ☐ No	
- GalNac-GD1a	____/____/_____	☐ Yes ☐ No	
Other (specify):	____/____/_____		
Other (specify):	____/____/_____		

8) ADMINISTERED TREATMENT(S)

List of all the drugs administered to the participant during the last six weeks					
Drug type	**Generic drug name**	**Dosage and frequency**	**Start of treatment**	**Duration (days)**	**Route of administration**
Antibiotics: ☐ Yes ☐ No			____/____/_____		☐ IV ☐ Oral ☐ Rectal ☐ Other: _____
			____/____/_____		☐ IV ☐ Oral ☐ Rectal ☐ Other: _____

Antivirals: ☐ Yes ☐ No			____ / ____ /_____		☐ IV ☐ Oral ☐ Rectal ☐ Other: _____
			____ / ____ /_____		☐ IV ☐ Oral ☐ Rectal ☐ Other: _____
Corticosteroids: ☐ Yes ☐ No			____ / ____ /_____		☐ IV ☐ Oral ☐ Rectal ☐ Other: _____
Anticonvulsants: ☐ Yes ☐ No			____ / ____ /_____		☐ IV ☐ Oral ☐ Rectal ☐ Other: _____
Diuretics: ☐ Yes ☐ No			____ / ____ /_____		☐ IV ☐ Oral ☐ Rectal ☐ Other: _____
Immunoglobulins: ☐ Yes ☐ No			____ / ____ /_____		☐ IV ☐ Oral ☐ Rectal ☐ Other: _____
Other (specify): _____			____ / ____ /_____		☐ IV ☐ Oral ☐ Rectal ☐ Other: _____

INTERVIEW COMPLETED BY

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

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	