

**Emergency Zika Package II:
Persistence of zika virus in body fluids and case-control
investigation of etiologic agents associated with Guillain-
Barré Syndrome**

Request for OMB approval of a new ICR

Supporting Statement B
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1. Respondent Universe and Sampling Methods

PROJECT ONE (Shedding study):

Participants will include patients with ZIKV infection of all ages identified through the Sentinel Enhanced Dengue Surveillance System (SEDSS) that was established in 2012 at Saint Luke’s Episcopal Hospital in Ponce, Puerto Rico. Participants will also include household contacts of persons with RT-PCR-positive ZIKV infection. These contacts will be recruited into the study with the use of recruitment coupons that will be provided to the ZIKV RT-PCR-positive cases (Attachment M). The patient coverage area for this hospital consists of residents from 20 municipalities (county equivalent) who seek care at these facilities. These 20 municipalities have a combined population of 853,389 residents, and have a demographic profile similar to that of Puerto Rico at large which consists of 78 municipalities.

Participant inclusion criteria

1. Residents of Puerto Rico of all ages with RT-PCR-positive ZIKV infection in any body fluid or,
2. Household contacts of RT-PCR-positive ZIKV cases with a valid coupon.

Participant exclusion criteria

1. Having previously participated in the ZIKV persistence study,

We aim to recruit a total of 350 symptomatic participants with RT-PCR-positive ZIKV infection. Symptomatic participants with RT-PCR-positive ZIKV infection can refer up to 5 household contacts for a total of 1,750 contacts. We estimate that not all coupons will be used and that we will get on average 3 recruits per participant for a total of 1,050 contacts. An estimated 20% will be ineligible or decline to participate for a total of 840 household contacts. Based on surveys among Chikungunya contacts, we expect 5% of contacts to have ZIKV RNA (n=42) in body fluids. We present the following scenarios on the point prevalence of shedding among contacts and the confidence interval around the estimate based on different sample sizes. We used the formula $n=Z^2P(1-P)/d^2$, where Z is the Z statistic for a level of confidence (Z=1.96), P is the estimated prevalence (P ranged from 1%-10%, Table 4) and d is the precision (d ranged from 0.0005 – 0.003).

Estimated sample size for different prevalence and margin of error scenarios.

Sample size to estimate confidence interval

two sided, alpha=0.05, Z=1.96

Margin of	Prevalence	LCI*	UCI**	Sample
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Error (d)	(P)			Size
		0.50		
0.50%	1.00%	%	1.50%	1521
		0.00		
1.00%	1.00%	%	2.00%	380
		4.00		
1.00%	5.00%	%	6.00%	1825
		3.00		
2.00%	5.00%	%	7.00%	456
		8.00	12.00	
2.00%	10.00%	%	%	864
		7.00	13.00	
3.00%	10.00%	%	%	384

PROJECT TWO (Case-control investigation):

GBS patients at participating hospitals will be identified prospectively through surveillance that is being established by PRDH. Cases will be selected from residents of Puerto Rico with a diagnosis of GBS meeting levels 1-3 of diagnostic certainty for the Brighton Collaboration criteria case definition for GBS since January 1, 2016.

Control ascertainment

A minimum of two controls will be pair-matched to each case by age group (<5, 5–20, 21–39, 40–64, and >65 years). Controls will be randomly selected from households within a one kilometer radius of the matching case-patient’s residence. A wireless device (e.g., tablet, iPhone) will be brought to the case-patient toward identifying the place of residence using GoogleEarth. A one kilometer radius will be drawn around the case-patient’s residence, and a random number generator will be used to calculate a direction (i.e., between 0 and 360 degrees) from the case-patient household as well as a random distance (i.e., between 0 and 1,000 meters) away from the case-patient household. Selected locations that are clearly uninhabited (e.g., forests, industrial areas, ocean) will be excluded. The process will be repeated until an apparent household is selected. If no age-matched control is available on the day of investigation, the field team will spin a one-sided object (e.g., bottle, pen) and proceed to the closest household in which the one-sided object points. This process will be repeated until either all households immediately adjacent to the randomly selected household have been visited or an age-matched control can be ascertained. If an age-matched control is identified but is not willing to participate in the investigations or all households that are immediately adjacent to the randomly selected household are visited and an age-matched control is not identified, another household will be visited by random selection using the described method toward finding a control. Only one control will be enrolled per randomly selected household. The team will continue until at least two age-matched controls per case are enrolled. All cases and controls may be revisited one month after the initial visit to collect a second blood specimen to assist in interpretation of diagnostic test results.

2. Procedures for the Collection of Information

PROJECT ONE (Shedding study):

Identification of symptomatic participants

Participants will be identified through routine procedures for SEDSS, a nurse-initiated system with potential participants identified by triage nurses who take vital signs including body temperature in the

triage area. The triage nurse identifies all patients with an AFI defined by presence of fever at time of triage ($\geq 38.0^{\circ}\text{C}$ or 100.5°F) or complaint of having fever lasting seven days or less. Every patient who meets the inclusion criteria for symptomatic participants (i.e., fever or history of fever for less than or equal to 7 days) is invited to participate in SEDSS. It has been reported that only a subset of patients with confirmed ZIKV infection present with fever. In order to capture potential ZIKV cases during the duration of the ZIKV persistence study, the eligibility criteria for SEDSS will be expanded to include rash, conjunctivitis and arthralgia. Patients for SEDSS may first seek services at the emergency room, affiliated outpatient clinic, or they may be a direct admission to the inpatient service. All age group patients are included in SEDSS, however, enrollment is limited to infants after they are discharged from the hospital after birth. This means that infants are not enrolled right after birth during their birth hospitalization. If an infant goes home and is re-admitted 1 week after birth, this infant is eligible for enrollment. Infants weighing less than 10 pounds will not be enrolled in the study, due to the limit of blood collection per kg. Based on Puerto Rico laws, adults are defined as age 21 years or older.

As part of SEDSS, physicians order blood, nasopharyngeal swabs and urine to be collected for all participants. Specimens are sent for testing at the Dengue Branch Laboratory in San Juan where testing is done for dengue, Zika and other infectious diseases (*Leptospira*, *Burkholderia pseudomallei*, enterovirus, and chikungunya). In SEDSS, Zika testing is performed in blood (serology and RT-PCR) and urine (RT-PCR). Any RT-PCR-positive cases of ZIKV will be contacted over the phone by study staff and offered enrollment in the ZIKV persistence study.

In SEDSS, all orders for laboratory testing are given a special code upon entry into the hospital computer system so that specimens can be tracked via the hospital electronic Meditech system. In the ZIKV persistence study a new participant study ID will be assigned; however, the code from SEDSS will also be retained in order to link SEDSS laboratory and clinical results to data collected in the ZIKV persistence study.

Enrollment

As part of SEDSS procedures, patients are requested to attend a 7-day convalescent follow-up visit or receive a phone call from study staff to collect follow-up information. RT-PCR-positive ZIKV cases will be invited at that time to participate in the ZIKV persistence study. Upon contact with the patient, the study objectives and methods will be explained and eligibility assessed (Attachment G). The person will be asked whether they would like to participate in the study. Study staff will offer consent (Attachments H and I) among those interested in participating. At this same visit, participants will be enrolled in the study, which will be participants' week 1 visit.

A study ID will be assigned to each participant to link the forms and specimens. A base-line questionnaire (Attachment J) will be administered by interviewers in laptop computers or tablets that includes questions on socio-demographics, symptoms, and sexual activity among adults.

Specimen Collection

The convalescent blood sample for SEDSS (second specimen collected 7 days after enrolment in SEDSS) will be used to test for ZIKV serologic and molecular diagnostic testing. In addition, all participants will provide an oral swab, and urine specimen. Adult participants will be asked to self-collect a vaginal swab if female or provide a semen specimen if male. The study staff will label and mark all specimen containers before collection, with the study ID number. Procedures for specimen collection will be explained by study staff (Attachment L) and specimen collection will be completed in a secure, private space at the study site using appropriate infection control precautions.

Study kits will be prepared with all the materials necessary for specimen collection. Study kits will be contained in a large zip lock bag and will include: 1 tiger top blood collection tube, 2 sterile polyester swabs, 1 urine sterile collection container or collection bag for children, one semen collection container, two sterile transport media and a laboratory specimen collection biohazard bag. Study kits will be stored in the study office and distributed to designated area. At enrollment each participant (children and adults) will have 7 ml of blood collected in one tiger top tube following standard procedures. If the participant weighs less than 37 pounds, the amount of blood drawn will depend on their weight, but will not be more than 3 ml/kg during any 8-week period, and the volume will not exceed 7 ml at one time. All participants will have oral swabs collected separately with sterile polyester specimen collection swabs and will receive instructions on how to collect urine specimens. Among infants and non-toilet trained children urine specimens will be collected with a sterile urine collection bag. The study staff will instruct the participant to collect the vaginal swab and semen sample for adult participants only. Oral and vaginal swabs will be placed in viral transport media. Semen will be collected into a sterile container. Women will be offered pregnancy testing at baseline, and if positive they will be referred to antenatal care (ANC) for services.

Follow-up

After the initial visit, participants will have to attend follow-up visits as described in Attachment P. They will be provided the option to: 1) come to the study site to complete study procedures; or 2) have a study team (phlebotomist and an interviewer) visit their home at a date and time previously agreed upon. If a participant chooses to come to the study site to do the interview and provide specimens, they will still be provided the option to self-collect the semen or vaginal swab (adults only), and urine specimens at home and bring them to the clinic within 2 hours after specimen collection. At each visit, study staff will complete a follow-up questionnaire (Attachment J), review the participant’s health status since the previous specimen collection(s), report any available testing results, review the next specimen collection needs and provide counseling on how to prevent Zika and answer any questions the participant may have (Attachment K). For bodily fluids (blood, saliva, urine and semen/vaginal secretions), participants will repeat specimen collection weekly for at least 4 weeks. Beginning at week 6, PCR testing for the presence of Zika virus will continue bi-weekly until all fluids have 2 consecutive negative tests. In addition, every subject will have blood and other body fluids for serology and RT-PCR collected at 2, 4, 6, and 9 months. If all fluids test negative on 2 consecutive visits in the first 4 weeks, additional visits to collect specimens will be done at 2, 4, 6, and 9 months.

If at least one fluid remains positive after 4 weeks, bi-weekly visits will continue until all fluids have two consecutive negative tests (Table 1). For subjects who are still undergoing biweekly visits at months 2, 4, 6 and 9 months, only one set of specimens will be collected.

RT-PCR test results are estimated to be available within one week of specimen collection and will be shared with the study participant during follow-up visits or phone calls. Any specimen positive for ZIKV RNA by RT-PCR will be processed for culture/viral isolation at one of laboratory.

Because we do not yet know how long Zika virus persists in body fluids, we do not have a set time frame or a maximum number of visits for the study. At present, the study is designed to obtain body fluids at 2, 4, 6, and 9 months for all subjects, even if they no longer have detectable virus in their samples. The minimum number of visits will be 8, and there could be as many as 20 or more, if subjects still have detectable virus at 9 months.

Table 1. Collection of specimens by study week, Zika Persistence Study, 2016.

Specimen	Week
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	0	1	2	3	4	6	8	16	24	36
Blood	X	X	Y	Y	Y	*	Y	Y	Y	Y
Saliva	Y	Y	Y	Y	Y	*				
Urine	X	Y	Y	Y	Y					
Semen/vaginal swab	Y	Y	Y	Y	Y					

X = specimen collected as part of SEDSS

Y = always collected

* = will continue every 2 weeks until has all samples have 2 consecutive negative tests

Follow-up study site visits or home visits will be scheduled and agreed upon at each preceding communication. Contact information will be reviewed during each contact or visit and reminder calls will be made if applicable. Study participants will be supported to fulfill their participation in the study by counseling offered during telephone contact, clinic visit or home visit together with assistance with referrals to clinical care as needed. Carefully timed reminder calls will be made to participants who have missed their scheduled calls or appointments. Flexibility will be provided in terms of appointment bookings and any needs for additional counseling or information (Attachment K). Participants who fail to attend a follow-up visit will be contacted by phone or home visits. After 5 failed attempts to contact them, participants will be considered lost to follow-up. Women will be offered pregnancy testing by study staff, and if positive they will be referred to antenatal care, pending on gestational age and needs. Care for such patients will be recommended in line with national guidelines.

Identification of household contacts

Every RT-PCR-positive ZIKV case identified through SEDSS will receive five study coupons (Attachment M) to invite up to 5 household contacts to participate in the study. Recruitment coupons have been used successfully in other fields such as in surveillance among populations at high risk for HIV. ZIKV symptomatic participants will be instructed to provide a coupon to any member of their household irrespective of age or sex. If they have fewer than 5 household contacts they will receive an appropriate number of coupons based on the number of household contacts. They will be instructed that when recruiting: “participation should be completely voluntary and no one should be influenced or pressured into participating.” We will not provide a recruitment incentive for participants referring household contacts. If the referrals report having had symptoms compatible with ZIKV (fever, rash, arthralgia or conjunctivitis) they will still be offered participation in the study. If referred household contacts are eligible (Attachment G) and consent to participate, the number on the coupon will be entered into a database to keep track of ZIKV symptomatic participants and their household contacts.

Upon contact with the potential participant, an introduction to the study will be given and the requirements of participation in the study will be explained. If agreeable to participation, study staff will seek consent for screening (Attachment I) for the collection of blood, oral, urine and among adults, semen/vaginal secretions specimens. Household contacts with symptoms compatible with Zika who have a positive PCR result in any body fluid will be invited to participate in the cohort study. Results for asymptomatic household contacts will be provided by phone and study staff will answer questions participants may have (Attachment K). When providing the results over the phone to symptomatic household contacts they will be asked to return to the study site to invite them to participate in the cohort study, explain study procedures, and administer consent.

PROJECT TWO (Case-control investigation):

Retrospective medical record review

The billing department of participant hospitals will identify case-patients with a discharge ICD-9 or -10 code associated with GBS from 2010–2015. Medical records for such patients will be reviewed, and a standardized chart abstraction form will be utilized to determine if the case met the clinical criteria for GBS (Attachment F). GBS patients will be defined as a patient that presented or was transferred to one of the participating hospitals and met levels 1–4 of diagnostic certainty for the Brighton Collaboration criteria case definitions for GBS. For patients that meet the GBS case definition, if possible, the subtype of GBS will be defined.

GBS patient identification and description

GBS patients at participating hospitals will be identified prospectively through surveillance that is being established by PRDH. Case reporting will require submission of a serum specimen and a case report form that collects descriptive demographic, epidemiologic, and clinical data. GBS patients will be contacted by project field epidemiologists, who will explain the purpose of the investigation. For those that give written consent to participate in the investigation (Attachment D), remaining clinical specimens (e.g., cerebrospinal fluid (CSF), urine, stool or rectal swabs) will be collected, and retrospective medical chart review will be performed to collect detailed information on clinical characteristics (Attachment F).

Case definition

A case will be defined as a person that resided in Puerto Rico continuously for the two months prior to onset of GBS and presented or was transferred to a participating institution in 2016.

Case and control enrollment

Potential participants will be introduced to the investigation following a script that explains the reasons the investigation is being conducted, the activities involved in the evaluation, and the risks and benefits of participation (Attachment D). Written consent will be obtained from all participants for the following: 1) participation in the survey and collection of blood specimen on the day of the survey; 2) storage of specimens for future diagnostic testing; 3) retrieval of clinical specimens and review of medical records from any illness for which the individual sought medical care in the previous two months; and 4) willingness to be contacted in the future depending on test results or if additional studies are proposed. If a second specimen is needed one month after the initial investigation visit, a second consent form will be obtained (Attachment E). For participants meeting the definition of a minor in Puerto Rico (i.e., individuals <21 years of age, unmarried, without children, and living with their parents), written permission to participate will be obtained from a parent or guardian. Verbal assent will be obtained from participants 8–12 years of age.

Data collection and analysis

Case and control interviews will be conducted using the questionnaire developed by the investigation team (Attachment C). 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 matched controls. A calendar will be used to orient cases and controls to the time period of interest.

Sera, urine, and saliva will be collected from cases and controls at the time of interview using standard techniques. The sera will be tested for antibodies against suspected infectious pathogens, such as ZIKV, dengue virus, chikungunya virus, influenza virus, human immunodeficiency virus, and *Leptospira* species bacteria. Urine specimens will be tested by rRT-PCR to identify ZIKV, dengue virus, or chikungunya virus. Serum will also be tested for anti-GM1 antibodies that have been previously associated with specific sub-types of GBS.

If any residual specimens are available from cases, those will also be obtained and undergo testing for infectious pathogens. It is not expected that matched controls will have any previously collected clinical specimens; however, in cases where controls had specimens collected while seeking medical care for an acute illness experienced within two months of GBS symptom onset of the matching case, these specimens will also be collected and tested for evidence of infection with the aforementioned pathogens. Residual samples will be stored after infectious testing is complete at the U.S. CDC 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. As with cases, written consent will also be obtained to review controls' medical records, where applicable and available, using a standardized chart abstraction form (Attachment F). Diagnostic test results will be securely transmitted from CDC to PRDH, which will then transmit diagnostic test results to participants by telephone or mail, as they prefer.

3. Methods to Maximize Response Rates and Deal with No Response

PROJECT ONE (Shedding study):

In the past, SEDSS participants received an incentive of \$20 to return for a convalescent study visit; however, only 30% returned for the follow-up visit. Informal conversations with participants suggested that increasing incentive could help with attrition. Thus, participants 14 years or older will receive \$50 dollars for each study visit. For participants between 7 and 13 years of age the incentive will be split between the parent and the child, the child will receive \$30 dollars for participation and the parents will receive \$20. For participants under 7 years of age the parent will receive the full incentive. In a family that has multiple children participating above and below 14 years of age, the study staff will use their discretion in deciding whether the child gets the full incentive. Participants will receive compensation for attending the study visit even if there is incomplete specimen collection.

PROJECT TWO (Case-control investigation):

None

4. Tests of Procedures or Methods to be Undertaken

No pilot testing will be done for either project.

5. Individuals Consulted on Statistical Aspects and Individuals Collecting and/or Analyzing Data

No individuals were consulted on statistical aspects of these projects.