ZIRP Puerto Rico Study

Zika Virus RNA Persistence in Pregnant Women and Congenitally Exposed Infants in Puerto Rico

Supporting Statement: Part A

OMB # 0920-XXXX

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ABBREVIATIONS

CDC	Centers for Disease Control and Prevention
EOC	Emergency Operations Center
IgM	Immunoglobulin M
IRB	Institutional Review Board
mL	Milliliter
MRI	Magnetic Resonance Imagining
OB	Obstetric
PRDH	Puerto Rico Department of Health
PI	Principal Investigator
POC	Point of Contact
PRDH	Puerto Rico Department of Health
RNA	Ribonucleic acid
rRT-PCR	Real-time reverse transcription–polymerase chain reaction
USZPR	US Zika Pregnancy Registry
ZAPSS	Zika Active Pregnancy Surveillance System
ZIKV	Zika Virus

Goal of the study: This study aims to determine the prevalence and duration of ZIKV RNA persistence in pregnant women infected with ZIKV and their congenitally exposed infants.

Intended use: The data from the ZIRP Puerto Rico study will provide the data needed to establish ZIKV testing guidance for pregnant women and congenitally exposed infants. It will also provide information to assess the association between persistent ZIKV RNA in pregnant women or infants and adverse outcomes; this information is needed to determine the clinical implications for infants born to mothers with persistent ZIKV RNA.

Methods to collect: The proposed information collection is for a prospective cohort study following pregnant women diagnosed with ZIKV in academic hospitals and obstetric clinics (including symptomatic and asymptomatic women) and congenitally exposed infants in pediatric offices associated to the academic hospitals.

Questionnaires for pregnant women will be administered at study enrollment and follow-up visits at 2 week intervals from the date of ZIKV diagnosis until three months post-delivery. The interval of questionnaire administration will switch to monthly when the woman has either reached her one month post-delivery visit or has had two consecutive blood or urine samples test negative for ZIKV by RT-PCR. All women, irrespective of their ZIKV status will have samples collected up until one month post-delivery; women who continue to test positive will have samples collected every two weeks whereas women who have tested negative in two consecutive blood or urine samples will have samples collected monthly. . Questionnaires will collect data on demographics, medical/obstetric and sexual history.

Questionnaires for collecting information on infants will be administered to the infant's mother or father at the time the infant is enrolled and monthly intervals until the infant is 6 months of age. Questionnaires for obtaining information on infants will include data on demographics, medical history and adverse outcomes.

How data will be analyzed: This is a hypotheses testing study; as such we will carry out descriptive, univariate and multivariate analysis.

Descriptive analysis will outline the demographic characteristics of the cohort of pregnant women and infants and the prevalence and duration of ZIKV RNA persistence. Multivariable analysis will be used to assess whether ZIKV RNA persistence is associated with adverse outcomes and infant infection. Risk factors will be fit into a logistic regression model to test for possible association with persistent detection of ZIKV RNA among pregnant women. Given that both symptomatic and asymptomatic women are being enrolled, we will further stratify our analysis.

A. Justification

A.1. Circumstances Making the Collection of Information Necessary

This Information Collection Request is submitted as a "New" Information Collection Request, (**an Emergency Collection Request, OMB # 0920-1189, was approved on July 10, 2017 and expires on October 31, 2017**). The length of data collection request for Office of Management and Budget (OMB) approval is two years. The National Center on Birth Defects and Developmental Disabilities (NCBDDD) at the Centers for Disease Control and Prevention (CDC) is making this request as authorized by Section 301 of the Public Health Service Act (42 U.S.C. 241) **(Attachment A).**

The Puerto Rico Department of Health (PRDH) reported the first case of autochthonous transmission of Zika virus (ZIKV) in December 2015.[1] As of December 16, 2016, Puerto Rico reported 35,648 ZIKV cases, more than any other location in the U.S., and the number is expected to continue to rise. [1-3] Among the cases, 2,864 have been among pregnant women,[3] and the first case of microcephaly in a fetus with confirmed ZIKV infection was announced by the PRDH on May 13, 2016.[4] Currently, testing for ZIKV infection can be done by either using rRT-PCR to detect the presence of ZIKV RNA or by serologic testing to detect IgM and neutralizing antibodies. rRT-PCR testing is the preferred and suggested method for diagnosing ZIKV infection because it provides a definitive diagnosis and is not subject to the limitations (e.g., cross-reactivity) associated with serology testing. However because level of viremia is generally low [5] and RNA concentrations decline over time, ZIKV rRT-PCR has generally only been considered for a short testing window (2 weeks).[6]

Currently, the Centers for Disease Control and Prevention (CDC) and the PRDH recommend that all pregnant women living in areas with active ZIKV transmission such as Puerto Rico, be tested for ZIKV. Symptomatic pregnant women should have serum and urine tested for the presence of ZIKV RNA by rRT-PCR within two weeks of symptom onset.[8, 9] Symptomatic pregnant women tested more than two weeks after symptom onset and symptomatic women with negative rRT-PCR test results should have serologic testing. Asymptomatic pregnant women are recommended to have serologic testing at the initiation of prenatal care and again during their second and third trimesters as a part of routine care; serum and urine rRT-PCR testing are recommended after a positive or equivocal serological test result to identify persistent RNA and to provide a definitive diagnosis.[9-14] For infants, CDC currently recommends ZIKV testing within 2 days of life for infants born to women with laboratory evidence of possible ZIKV and for infants who have abnormal clinical or neuroimaging findings suggestive of congenital ZIKV syndrome, regardless of maternal ZIKV test results. [15-17]

Limited data from human studies also suggest that pregnant women have persistent detection of ZIKV RNA in serum. [25-27] In one case report, a pregnant woman tested positive for ZIKV RNA 107 days after symptom onset.[25] In another case report, a pregnant woman became symptomatic at 11 weeks gestation and was rRT-PCR-positive at 16 weeks gestation.[26] Fetal ultrasounds and magnetic resonance imagining (MRI) revealed a fetus with brain abnormalities; ZIKV was cultured

from the fetal brain postmortem. A recent case series found persistent detection of ZIKV RNA in five pregnant women. Symptomatic women had detectable virus at 17, 23, 44, and 46 days post symptom onset and one asymptomatic woman was still rRT-PCR positive 53 days after returning from travel. . In one symptomatic pregnant women with prolonged detection of ZIKV RNA, the pregnancy ended as a fetal loss and ZIKV RNA was found in the fetus.[27]Findings from these case reports and series led to the hypothesis that persistent detection of RNA in pregnant women may be a marker of fetal infection and thus, potentially a marker of adverse fetal outcomes including microcephaly and brain abnormalities [25-27]. However, more data are needed including whether the detection of IgM impacts the risk of adverse infant outcomes.

Even less is known about persistent detection of ZIKV RNA and IgM in infants. One case study reported persistent ZIKV RNA detection in a male child born in Brazil at 40 weeks gestation with brain abnormalities.[28] Fifty-four days after birth, the infant's serum, saliva, and urine all tested positive for ZIKV RNA; ZIKV RNA continued to be detected in the infant's serum on day 67 and had cleared by day 216. The infant exhibited no obvious illness or evidence of being immunocompromised when examined on day 54. However, he demonstrated neuropsychomotor developmental delay, with global hypertonia and spastic hemiplegia, by 6 months of age. The duration of IgM detection in infants is also important to determine the window of diagnostic utility of this test for infants not tested at birth.

Since receiving emergency OMB approval, the study protocol has been approved by the appropriate Puerto Rican scientific and ethics committees. Training of study staff took place on July 20th, 2017, Enrollment is expected to start on August 28th, 2017. We are also in the process of obtaining IRB approval to include pregnant women who have tested IgM positive; although this will not change our burden, it does broaden our population of interest.

During the Emergency Clearance period, protocol changes were made to reflect site-specific needs. These include changes to consent forms and questionnaires, previously approved in the emergency OMB application. These changes were made to improve comprehension by study participants, reduce participant burden, and capture additional information later found to be necessary for conducting the study.

A.2. Purpose and Use of Information Collection

CDC's goal in developing this study is to determine the prevalence and duration of ZIKV RNA persistence in pregnant women infected with ZIKV and their congenitally exposed infants.This information will be essential for establishing guidance for testing and clinical management of pregnant women and congenitally exposed infants with exposure to ZIKV.

The primary research objectives we aim to address with the ZIRP Puerto Rico study are:

1. To determine the proportion of pregnant women with continued ZIKV RNA detection in blood and/or urine and IgM in blood at each month following the first positive ZIKV test and up to three months post-delivery.

2. To determine the duration of ZIKV RNA and IgM detection in blood and/or urine among pregnant women infected with ZIKV and their congenitally exposed infants.

3. To evaluate the diagnostic utility of PCR testing for ZIKV RNA on capillary blood among symptomatic and asymptomatic pregnant women.

The secondary research objectives we aim to address with the ZIRP Puerto Rico study are:

1. To determine if persistent ZIKV RNA in pregnant women is associated with detection of ZIKV RNA in infants;.

2. To identify factors (such as: gestational age at infection, maternal age, maternal comorbidities, TORCH infections, previous flavivirus infections, symptom status, etc.) that are associated with persistent detection of ZIKV RNA and IgM in pregnant women and congenitally exposed infants.

This study is a part of an ongoing public health emergency response related to the Zika virus outbreak in Puerto Rico. Results of the ZIRP Puerto Rico study will provide critical data in establishing guidance for testing in pregnant women and congenitally exposed infants with exposure to ZIKV. It will also provide information on the association between persistent ZIKV RNA in pregnant women and adverse outcomes or infection in infants that can enable health care providers to determine the clinical implications on infants born from mother with persistent detection of ZIKV RNA.

During the Emergency Clearance period, protocol changes were made to adjust to the local setting. The current request is to continue data collection beyond the expiration of the Emergency Clearance period (October 31st, 2017). Enrollment is scheduled to begin August 28th, 2017 The expected total enrollment will be 150 pregnant women and their 150 congenitally infected infants.

A.3. Uses of Improved Information Technology and Burden Reduction

The ZIRP Puerto Rico study questionnaires have been designed to collect the minimum amount of information necessary to meet the study's objectives. Questions about other factors with the potential to have mediating or moderating effects on primary outcomes have been considered and included.. All data will be entered and stored on the password-protected and secure web-based data collection system which will be housed on a CDC server.

A.4. Efforts to Identify Duplication and Use of Similar Information

As noted in the 2016 CDC publication on prolonged detection of ZIKV RNA in pregnant women (9), there is an urgent need to conduct additional real-time rRT-PCR testing of pregnant women with laboratory evidence of ZIKV RNA infection to estimate the prevalence and duration of ZIKV RNA persistence. Through its prospective design, the ZIRP Puerto Rico study will systematically identify the symptomatic and asymptomatic ZIKV positive pregnant women and their infants at the time of diagnosis and monitor their viral load during a period of 15 months. The prospective design also allows estimation of prevalence and duration of ZIKV RNA detection among pregnant women infected with ZIKV and infants exposed to ZIKV and whether persistent ZIKV RNA in pregnant women is associated with detection of ZIKV RNA in infants. Previous studies that have examined ZIKV RNA persistence have been limited by their small sample sizes, lack of a prospective design and a lack of control for risk factors.

Information collection as part of this study are not conducted anywhere else within CDC or within the Department of Health and Human Services.

In general, the ZIRP Puerto Rico study is the only prospective cohort study of ZIKV positive pregnant women and exposed infants currently being undertaken by CDC. Although surveillance efforts focused on ZIKV in pregnancy are currently conducted in the U.S. (including territories), the ZIRP Puerto Rico study will be the first to examine prevalence and duration of ZIKV RNA persistence among pregnant women infected with ZIKV RNA

Ongoing projects in the CDC Zika response and how the ZIRP Puerto Rico study fill gaps not addressed by these existing efforts

Project	Contribution added by ZIRP
Persistence of Zika virus in semen and urine of adult men in the United States with confirmed Zika virus infection (OMB Control No. 0920-1139)	Not applicable- ZiRP does not examine the adult male population
Zika virus persistence in body fluids of patients with Zika virus infection in Puerto Rico (ZIPER Study) (OMB Control No. 0920-1140)	Study population includes all ages and sexes; ZIRP examines prolonged detection of ZIKV RNA in positive pregnant women and infants only Ensuring sufficient sample size in these special populations
The Effect of Community-Wide Vector Control Initiatives on Zika Virus Transmission in Puerto Rico, 2016 (0920-1137)	Not applicable – ZIRP does not address community-wide vector control

Evaluation of In2Care Traps during the Zika Outbreak in Puerto Rico (0920-1071)	Not applicable – ZIRP does not address community-wide vector control
Knowledge, Attitudes, and Practices related to a Domestic Readiness Initiative on Zika Virus Disease (0920-1136)	Not applicable – ZIRP does not address a specific domestic readiness objective
Integrated *Aedes aegypti* Vector Control Intervention in Caguas City, Puerto Rico to Prevent and Control Zika Virus Infections (PRA N/A)	Not applicable – ZIRP does not address community-wide vector control
Migrant Farm Workers Understanding and Use of Measures to Prevent Zika Transmission (0920-1126)	Not applicable – ZIRP does not address migrant farm workers
US Zika Pregnancy Registry (USZPR) (0920-1143)	This project focuses on U.S. pregnant women with laboratory evidence of Zika virus infection with the exception of Puerto Rico, USZPR data do not systematically include serial sampling to allow for assessment of ZIKV RNA persistence; ZIRP data can be linked with data in the US Zika Pregnancy Registry to assess an association with adverse outcomes.
Assessment of Interventions Intended to Protect Pregnant Women in Puerto Rico from Zika virus infections (0920-1118)	ZIRP can provide critical data in establishing guidance for testing and interventions in pregnant women and congenitally infected infants with exposure to ZIKV.
Assessment of Contraceptive Use and Needs, Puerto Rico, 2016 (0920-1114)	Not applicable – ZIRP does not assess contraceptive use and needs
Enhanced Surveillance of Pregnancy and Infant Outcomes following with Zika Virus infection in Pregnancy, Colombia (PRA N/A)	Enhanced surveillance data do not allow identification of absolute risk, because the base population is challenging to quantify. Further, because infections are identified retrospectively, exact timing of infection during pregnancy cannot be ascertained. ZIRP will prospectively monitor woman from the date of diagnosis until two consecutive samples test negative for ZIKV and will identify the risk factors associated with prolonged detection of ZIKV RNA.
Characterization of Guillain-Barré Syndrome Cases in the Setting of Zika Virus Transmission— Colombia, 2016 (PRA N/A)	Although ZIRP does not focus on Guillain-Barré Syndrome (GBS) in Colombia as an outcome specifically, ZIRP does examine potential outcomes from prolonged detection of ZIKV RNA and one of these potential

	outcome could be GBS which can guide governments internationally to revise the current testing algorithms.
Formative Evaluation of Zika Prevention Kits for Pregnant Women in Puerto Rico (0920-1071)	ZIRP does not propose evaluation of Zika Prevention Kits
Case-control microcephaly study in Brazil (0920-1011)	ZIRP will prospectively follow a defined population of pregnant women and their infants that are ZIKV positive which will estimate the prevalence and length of ZIKV RNA detection among pregnant women, this will provide information on risk factors that are associated with prolonged detection of ZIKV RNA in pregnant women. These risk factors can then be further examined in other settings such as Brazil.
Collection of serum and plasma from patients with antibodies reactive with Zika virus and other arboviruses (PRA N/A)	Although ZIRP collects serum and plasma, it does so only from ZIKV positive women and infants. ZIRP can contribute with data on cross reactivity between diagnosis of ZIKV and other arboviruses in a more niche population (pregnant women and infants)
Mosquito Surveillance Survey (0920-1101)	Not applicable – ZIRP does not propose to assess mosquito populations
American Samoa Zika Surveillance (0920-1011)	Surveillance data do not allow identification of absolute risk, because the base population is challenging to quantify. Further, because infections are identified retrospectively, exact timing of infection during pregnancy cannot be ascertained. ZIRP will prospectively monitor woman from the date of ZIKV diagnosis until two consecutive samples test negative for ZIKV and will identify the risk factors associated with prolonged detection of ZIKV RNA in both asymptomatic and symptomatic pregnant women.
Case-control GBS study in PR – Surveillance (0920-1106)	ZIRP cans shed light into whether or not GBS can be an outcome in prolonged detection of ZIKV RNA in infants.
Case-control GBS study in PR - Records Abstraction (PRA N/A)	Medical record abstraction can be biased to clinicians and staff filling out the forms and at times difficult to track given on where the subject is treated/followed up. ZIRP uses questionnaires to obtain clinical data from

	infected mothers and infants to determine possible adverse outcomes, one of them being Guillain-Barré Syndrome
Formative evaluation among partners of pregnant women about Zika in PR (0920-0572)	Not applicable – ZIRP does not include formative evaluation of any programs
Zika Postpartum Emergency Response Survey, Puerto Rico (0920-1127)	ZiRP will collect postpartum information on Zika symptoms to go along with the specimen collected as well as a follow-up questionnaire to assess changes in risk and behavior since the last study visit. These questions build on each other, rather than being a point-in-time survey.
Formative Assessment Regarding Contraception Use in the U.S. Virgin Islands (USVI) in the Context of Zika (0920-1148)	Not applicable – ZIRP does not include formative evaluation of any programs
Monitoring & Evaluation for the Zika Contraception Access Network (0920-1164)	Not applicable – ZiRP does not monitor and evaluate contraceptive use and needs

A.5. Impact on Small Businesses or Other Small Entities

Data will be collected from an academic hospital in Puerto Rico. Although the study focuses primarily on the OB units for enrollment there will be pediatrician's available for infant follow-up. The study data collection form are the absolute minimum required for the intended use of the data. The questionnaires will be presented in a clear and easy to complete format based on previous questionnaires and recommendations from questionnaire methodology research.

A.6. Consequences of Collecting the Information Less Frequently

Each woman will participate in the study from the date of ZIKV RNA positive diagnosis through the end of the study (15 months). Infants will participate in the study from the date of delivery through 6 months of age. It is important to monitor the progression of the virus since the date of diagnosis in pregnant women and infant; Following consent, enrolled pregnant women and infants will be serially tested for ZIKV RNA every 2 weeks and monthly, respectively.

For pregnant women who continue to test ZIKV positive, blood and urine samples will be collected every 2 weeks during pregnancy and up to 3 months post-delivery. If the pregnant woman tests negative by rRT-PCR on two subsequent collection dates, they will be followed monthly through 3 months post-delivery and blood and urine samples will be collected and tested for ZIKV until 1 month post-delivery

Live-born infants will be tested every month after birth up to 6 months of age until blood and urine samples are all rRT-PCR-negative on two subsequent collection dates to confirm negative results. Infants who test rRT-PCR- negative on two subsequent collection dates will continue to have monthly study visits in which only study forms will be completed until six months of age.

Collecting information less frequently may not permit us to quantify the reduction in viral load both in pregnant women and infants, nor will it allow us to calculate the prevalence and of ZIKV RNA persistence.

A.7. Special Circumstances Relating to the Guidelines of 5 CFR 1320.5

This information collection fully complies with all guidelines of 5 CFR 1320.5.

A.8. Comments in Response to the Federal Register Notice and Efforts to Consult Outside the Agency

A 60-Day Federal Registry Notice was published in the Federal Registry on April 19, 2017. Vol. 82, NO. 74, pp. 18462. No comments were received.

CDC has consulted with other public health agencies, e.g., NIH, to ensure there are no duplication of similar efforts in the study location.

A.9. Explanation of Any Payment or Gift to Respondents

At each clinic and laboratory visit, the participant or guardian of the infant participant will be provided with a 25 USD per diem to defer the outlay of expenses incurred by adult participants and infant guardians. In accordance with U.S guidelines, this value has been determined based on other research studies conducted in this population. The intent of the per diem is to minimize personal financial outlay of participants for their time, travel, and meals, and cover the cost of childcare should that be needed for women who continue in the study past delivery.

While the ZIRP Puerto Rico study clinic visits were designed to largely align with standard of care for prenatal or infant well-being visits, full compliance with prenatal and infant standard of care visit schedules does not always occur due to resource and access to care barriers. Moreover, this study focuses on a special population during a limited time period around the time of the birth of an infant when women indicate that participating in even simple activities is constrained by lifestyle changes, financial constraints, childcare duties, and fatigue (10,11).

Adult pregnant women participants and guardians of infant participants will receive the 25 USD for each clinic and laboratory visit in the form of a gift card.

A. 10. Protection of Privacy and Confidentiality of Information Provided by Respondents

The CIO's Information Systems Security Officer determined that the Privacy Act does apply. The compilation of individual research results and responses into a secure study database for the ZIRP Puerto Rico study will be used only for research purposes. Investigators have completed certifications in Information Security and Privacy Awareness and will put systems in place to meet Privacy Act requirements. Data collected for this study also will be protected by an Assurance of Confidentiality under Section 308(d) of the Public Health Service Act, and the System of Records Notice (SORN) # 09-20-1136. The sections below describe the protections in place to preserve privacy and confidentiality.

A. 10.1 Overview of the Data Collection System

Pregnant women of any gestational age at the time of ZIKV diagnosis and their infants will be invited to participate. The Pregnant Women Screening Form (Att **C**.1) will be administered to potential participants by trained ZIRP research staff, and will be used to confirm participant eligibility prior to consent.

The pregnant woman will consent for her participation. Following consent, information will be collected at baseline regarding demographics and risk factors for ZIKV through the Pregnant Woman Enrollment Questionnaire (Att **C**.2) and a Pregnant Woman Symptom Questionnaire (Att **C**.3). Pregnant women will then be serially tested for ZIKV RNA in blood (7.5mL) and/or urine samples by rRT-PCR and ZIKV Immunoglobulin M (IgM) every two weeks . The blood samples will consist of one venous blood collection (via venipuncture) every two weeks and a one-time collection of capillary blood (via finger prick) at enrollment. If these visits do not coincide with a typical prenatal care visit, they will be asked to come in for a laboratory-only visit to obtain samples and to complete the Pregnant Woman Follow-up questionnaire (Att **C**.4).

Samples will be collected for all women until 1 month post delivery. The frequency of sample collection will change depending on the woman's ZIKV status; if a woman tests rRT-PCR negative in blood and/or urine twice consecutively then she will only have samples collected monthly up until 1 month post deliveryIf a woman continues to be rRT-PCR positive she will have samples collected every two weeks..

Infants born to women enrolled in the pregnancy cohort will be enrolled when the pregnant woman and the infant's father sign the infant informed consent form. This can occur at any time following the enrollment of the pregnant woman but must occur prior to delivery. Blood and/or urine samples will be collected from these infants at birth and tested for the presence of ZIKV RNA by rRT-PCR. Infants will be tested every month after birth up to 6 months of age until blood and urine samples are all rRT-PCR-negative on two subsequent collection dates to confirm negative results. Infants who test rRT-PCR- negative on two subsequent collection dates will continue to have monthly study visits in which only study forms will be completed until six months of age.

A. 10.2 Description of Information to be Collected

Data collection includes maternal and infant demographic information, pregnancy history, medical history, obstetric history, sexual history, Zika virus infection, and pregnancy outcome information (including infant anomalies and birth defects). The follow-up questionnaire will contain laboratory information as well as any other medical conditions or adverse outcomes.

A. 10.3 How Information Will Be Shared and for What Purpose

There are four main entities involved in the conduct of the ZIRP Puerto Rico study. Puerto Rico's Department of Health (PRDH), Universtiy of Puerto Rico (UPR), CDC Dengue Branch in Puerto Rico, CDC Emergency Operations Center in Atlanta.

Patients' identifiable information (PII) will not be disclosed in any reports, statistical summaries, or shared or disclosed to public entities, external agencies, or other people or organizations outside the entities involved in the conduct of the ZIRP Puerto Rico study. Statistical summaries of de-identified data will be shared in peer-reviewed journals and conference presentations.

A. 10.4 Impact on the Respondent's Privacy

ZIRP study site staff will protect the confidentiality of participant data and research records by assigning a unique study Participant Identification Number (PIN) to study forms, specimens, and database.Since the laboratory will not be reporting any of the results to healthcare providers, the PIN will be used to track the participants specimen in the laboratory (the name of the participant will not be included in the study sample label).. Moreover, to ensure the anonymity of the research data, the PIN will be used for monitoring the study participant and their laboratory results. An electronic crosswalk file that is separate from the research data will be kept to provide the link between the unique subject PIN and associated PII (e.g. names, dates of birth, contact information, etc.). Resulting reports or publications regarding this research are to be reported in aggregate and ensure individuals cannot be identified. Patients' personally identifiable information (PII) will not be shared or disclosed to public entities, external agencies, or other people or organizations outside the entities conducting the ZIRP study. Additionally, CDC has obtained an Assurance of Confidentiality under Section 308(d) of the Public Health Service Act, which provides the highest level of protection for maintaining the confidentiality of individually identifiable data. All CDC, UPR and PRDH personnel who have access to personally identifiable information (PII) are required to go through training on confidentiality protections and to sign a nondisclosure agreement (Att D.1, D.2, D.3).

A. 10.5 Explaining Voluntary Nature of Participation

In accordance with the approved IRB protocol for this study (Att E.1), ZIRP study staff will explain to pregnant women that participation in the study is voluntary, and they can end their participation or the participation of their infant at any time without negative consequences.

A. 10.6 Opportunities to Consent to Sharing and Submission of Information

The informed consent documents explain that the study findings will be compiled and only presented on a group level, with no individuals identified. . In addition, within the informed consent document, there is a separate item where the participant must check "Yes" or "No" regarding their approval to allow researchers to store study samples and health information for future research.

A. 10.7 How Information Will Be Secured

Paper documentation, such as informed consent, will be stored in a designated and secured office area and similarly designated and locked filing cabinet within each study site. Research data will be kept secure and confidential on a CDC server, which will require user authentication and password protection. Laboratory data will be kept in a secure and confidential CDC Dengue Branch server, which requires authentication and password protection. Administrator controls in place include regular backups, security training, completion of a security certification and accreditation (C&A), security plans, and policies. Access to ZIRP Puerto Rico study data is limited to CDC, UPR and PRDH staff supporting the project. Requirements for adherence to privacy provisions and policies, as well as instructions for destruction of study data and files when the contract ends, are specified in the contract language.

A. 11. Institutional Review Board (IRB) and Justification for Sensitive Questions

The CDC Institutional Review Board (IRB) approved the study as research (Att E.1). The protocol was reviewed in accordance with the expedited review process outlined in 45 CFR 46.110(b)(1), Categories 2, 5, and 7. The IRB determined the study to be not greater than minimal risk to subjects. The IRB approved the inclusion of children under 45 CFR 46.404 and the inclusion of pregnant women under 45 CFR 46.204.

CDC's IRB will also conduct continuing reviews of routine annual data points as well as required review of any adverse events or protocol violations as needed. Some data to be collected for the ZIRP Puerto Rico study may be sensitive in nature to some respondents. To reduce the sensitivity of these questions, respondents will be completing questionnaires in private and will be reminded that they are not required to answer any questions to which they would prefer not to respond. Topics that

may be perceived by subjects as sensitive are: 1) patient demographic information including social-economic status; 2) patient medical history including number of pregnancies lost; 3) ZIKV symptom and diagnosis information; 4) sexual behavior and practice; 5) pregnancy outcome information; and 6) infant health information.

CDC developed the data collection requirements after extensive consultation with medical professionals and epidemiologists. There is consensus that the sensitive information collected is necessary to provide accurate information in order to determine the prevalence and duration of ZIKV RNA detection among pregnant women infected with ZIKV and its association to adverse outcomes or infection in infants.

A. 12. Estimates of Annualized Burden Hours and Costs

Burden Hours

Study participants are ZIKV positive pregnant women and their congenitally exposed infants.

ZIRP Puerto Rico study staff will use the Pregnant Woman Screening form (Att. C1) to confirm eligibility of pregnant women and their infants. Because the screening forms are administered prior to initiation of the consent process, the burden estimate for administration of the screening forms is based on a larger pool of ZIKV positive pregnant women and infants than will be eligible for the study and asked to complete, the enrollment, symptom and follow up forms. This assumes 10% of women screened will not meet eligibility criteria. The remainder of the burden estimate is based on completion of questionnaires by consented respondents: 150 pregnant women and their 150 infants.

As exhibited in Table A.12-1, each study questionnaire instrument is considered one response. The annualized burden hours for each questionnaire was calculated by multiplying the number of respondents by the number of responses (estimated number of times each questionnaire would be completed) per respondent by the average burden per response. The total estimated annualized burden for all information collection for ZIRP is 785 hours.

A. 12 – 1 Estimated Annualized Burden Hours

Respondents	Form Name	No. of Respondents	No. of Responses (Questionnaires) per Respondent	Average Burden per Response (in hours)	Total Burden Hours
ZIKV positive Pregnant women	Pregnant women screening form	150	1	2/60	5
	Pregnant women enrollment	150	1	8/60	20

	questionnaire				
	Pregnant women symptom questionnaire	150	1	8/60	20
	Pregnant women follow-up questionnaire	150	30	8/60	600
Parents of ZIKV positive Infants	Infant enrollment and delivery questionnaire	150	1	8/60	20
	Infant follow-up questionnaire	150	6	8/60	120
				Total	785

A. 12 – 2 Annualized Cost to Respondents

Due to the diversity of the pregnant woman population, we do not know what the wage rate category will be for the pregnant women enrolled in this study. Therefore, we used the minimum wage rate for Puerto Rico ($7.25 per hour) (available at https://www.dol.gov/whd/minwage/america.htm#PuertoRico. This wage rate will also be used for the hourly wage related to visits and study forms for the parents of all infants in the study

Type of Respondents	Form Name	Number of Respondents	Number of Responses per Respondent	Average Burden Hours per Response	Total Burden Hours	Hourly Wage Rate	Total Costs
ZIKV positive Pregnant women	Pregnant women screening form	150	1	2/60	5	$7.25 USD	$36.25
	Pregnant women enrollment questionnaire	150	1	8/60	20	$7.25 USD	$145
	Pregnant woman symptom questionnaire	150	1	8/60	20	$7.25 USD	$145
	Pregnant women follow-up questionnaire	150	30	8/60	600	$7.25 USD	$4350
	Infant enrollment and	150	1	8/60	20	$7.25 USD	$145

ZIKV positive infants	delivery questionnaire						
	Infant follow-up questionnaire	150	6	8/60	120	$7.25 USD	$870
					Total		**$5,691**

A. 13. Estimates of Other Total Annual Cost Burden to Respondents or Record Keepers

There are no additional costs to respondents other than their time.

A. 14. Annualized Cost to the Federal Government

The total estimated cost to the federal government is $1,608,106.00. This amount is based on the contractor's costs for carrying out the data collection activities and reporting and CDC personnel travel costs to provide project oversight and participate in analysis and dissemination of the results. The table summarizes expenses to the federal government. This project will be executed as part of Contract No. 200-2016-91591.

Expense Type	Expense Explanation	Annual Costs (dollars)
Contract	Contract No. 200-2016-91591 (Total Solutions, Inc.)	$1,001,674.00
	Subtotal, Contract Costs	**$1,001,674.00**
CDC Personnel	Principal Investigator, 20% of FTE	$30,322
	Team Lead, 50% of FTE	$50,304
	Study Lead, 100% of FTE	$100,608
	Epidemiologists (x3), 50% of FTE	$150,912
	Subtotal, CDC Personnel	$332,146
	TOTAL COST TO THE GOVERNMENT	**$1,333,820.00**

A. 15. Explanation for Program Changes or Adjustments

This is a new collection of information.

A. 16. Plans for Tabulation and Publication and Project Time Schedule

A 2-year OMB clearance is requested to cover all data collection activities, including enrollment, follow-up and data analysis. Analysis plans included conducting descriptive, univariate and multivariate analyses. Results of the study will be disseminated to the scientific community through

the published literature and presentation at meetings. In concert with dissemination to the scientific community, a roll-out plan will be created in collaboration with UPR, PRDH and CDC's communications teams to release lay versions of the study results to the public, as warranted. Routes of communication include press releases, media interviews, PRDH, UPR or CDC websites, and social media.

Activity	**Timeframe after approval**
Study	1 month after OMB approval
Enrollment completion of ZIKV positive pregnant women. Continue follow-up and testing of enrolled ZIKV positive pregnant women and congenitally-infected infants.	6th month after OMB approval
Active follow-up and testing of enrolled ZIKV positive pregnant women and their congenitally-infected infants.	9th month after OMB approval
End of study period, final data collected and laboratory results collected on all enrolled ZIKV positive pregnant women and their congenitally- infected infants	15th month after OMB approval
Clinical and Laboratory data base lock	13th month after OMB approval
Data analysis completed	14th month after OMB approval
Final report	15th month after OMB approval

A.17. Reason(s) Display of OMB Expiration Date is Inappropriate.

No exceptions from display of expiration date are requested.

A. 18. Exceptions to Certification for Paperwork Reduction Act Submissions

No exemptions to certification are sought.

A. 19. References

1. Dirlikov, E., et al., *Update: Ongoing Zika Virus Transmission - Puerto Rico, November 1, 2015-April 14, 2016.* MMWR Morb Mortal Wkly Rep, 2016. **65**(17): p. 451-5.
2. Tepper, N.K., et al., *Estimating Contraceptive Needs and Increasing Access to Contraception in Response to the Zika Virus Disease Outbreak - Puerto Rico, 2016.* MMWR Morb Mortal Wkly Rep, 2016. **65**(12): p. 311-4.
3. Estado Libre Asociado De Puerto Rico Departamento de Salud. *Informe Semanal de Enfermedades Arbovirales. 2016; http://www.salud.gov.pr/Estadisticas-Registros-y-Publicaciones/Informes%20Arbovirales/Reporte%20ArboV%20semana%2048-2016.pdf. Accessed 12/19/2016.*

4. Estado Libre Asociado De Puerto Rico Departamento de Salud, *Comunicado De Prensa: Presentan Informe Arboviral con Actuaizacion de Datos Relacionados al Virus del Zika. 2016. Accessed May 14, 2016.*
5. Lanciotti, R.S., et al., *Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007.* Emerg Infect Dis, 2008. **14**(8): p. 1232-9.
6. Rabe, I.B., et al., *Interim Guidance for Interpretation of Zika Virus Antibody Test Results.* MMWR Morb Mortal Wkly Rep, 2016. **65**(21): p. 543-6.
7. Adams L, Bello-Pagan M, Lozier M, et al. Update: Ongoing Zika Virus Transmission — Puerto Rico, November 1, 2015–July 7, 2016. MMWR Morb Mortal Wkly Rep 2016;65:774–779. DOI: http://dx.doi.org/10.15585/mmwr.mm6530e1
8. Centers for Disease Control and Prevention. *Memorandum: revised diagnostic testing for Zika, chikungunya, and dengue viruses in US public health laboratories*. 2016 10 May 2016]; Available from: http://www.cdc.gov/zika/pdfs/denvchikvzikv-testing-algorithm.pdf.
10. Oduyebo, T., et al., *Update: Interim Guidance for Health Care Providers Caring for Pregnant Women with Possible Zika Virus Exposure - United States, July 2016.* MMWR Morb Mortal Wkly Rep, 2016. **65**(29): p. 739-744.
11. Petersen, E.E., et al., *Update: Interim Guidance for Health Care Providers Caring for Women of Reproductive Age with Possible Zika Virus Exposure - United States, 2016.* MMWR Morb Mortal Wkly Rep, 2016. **65**(12): p. 315-22.
12. Oduyebo, T., et al., *Update: Interim Guidelines for Health Care Providers Caring for Pregnant Women and Women of Reproductive Age with Possible Zika Virus Exposure - United States, 2016.* MMWR Morb Mortal Wkly Rep, 2016. **65**(5): p. 122-7.
13. Petersen, E.E., et al., *Interim Guidelines for Pregnant Women During a Zika Virus Outbreak--United States, 2016.* MMWR Morb Mortal Wkly Rep, 2016. **65**(2): p. 30-3.
14. Estado Libre Asociado De Puerto Rico Departamento de Salud. *Orden Administrativa Núm. 360*. [cited 2016 December 22]; Available from: http://www.salud.gov.pr/Sobre-tu-Salud/Documents/OA%20360.pdf.
15. Russell, K., et al., *Update: Interim Guidance for the Evaluation and Management of Infants with Possible Congenital Zika Virus Infection - United States, August 2016.* MMWR Morb Mortal Wkly Rep, 2016. **65**(33): p. 870-878.
16. Fleming-Dutra, K.E., et al., *Update: Interim Guidelines for Health Care Providers Caring for Infants and Children with Possible Zika Virus Infection--United States, February 2016.* MMWR Morb Mortal Wkly Rep, 2016. **65**(7): p. 182-7.
17. Staples, J.E., et al., *Interim Guidelines for the Evaluation and Testing of Infants with Possible Congenital Zika Virus Infection - United States, 2016.* MMWR Morb Mortal Wkly Rep, 2016. **65**(3): p. 63-7.
18. Lustig, Y., et al., *Detection of Zika virus RNA in whole blood of imported Zika virus disease cases up to 2 months after symptom onset, Israel, December 2015 to April 2016.* Euro Surveill, 2016. **21**(26).
19. Murray, K.O., et al., *Prolonged Detection of Zika Virus in Vaginal Secretions and Whole Blood.* Emerg Infect Dis, 2017. **23**(1).
20. Musso, D. and D.J. Gubler, *Zika Virus.* Clin Microbiol Rev, 2016. **29**(3): p. 487-524.
21. Musso, D., et al., *Detection of Zika virus in saliva.* J Clin Virol, 2015. **68**: p. 53-5.

22. Lessler, J.T., et al., *Times to key events in the course of Zika infection and their implications: a systematic review and pooled analysis.* Bull World Health Organ, 2016.
23. Project Zika Experimental Science Team (ZEST). *Zika Open-Research Portal*. Project Zika experimental science team (ZEST), 2016 April 24, 2016]; Available from: https://zika.labkey.com/project/OConnor/begin.view.
24. Dudley, D.M., et al., *A Rhesus Macaque Model of Asian-lineage Zika Virus Infection.* Nature Communications, 2016.
25. Suy, A., et al., *Prolonged Zika Virus Viremia during Pregnancy.* N Engl J Med, 2016.
26. Driggers, R.W., et al., *Zika Virus Infection with Prolonged Maternal Viremia and Fetal Brain Abnormalities.* N Engl J Med, 2016.
27. Paz-Bailey, Gabriela, et al. "Persistence of Zika Virus in Body Fluids—Preliminary Report." New England Journal of Medicine (2017)..
28. Oliveira, D.B.L., et al., *Prolonged Shedding of Zika Virus Associated with Congenital Infection.* New England Journal of Medicine, 2016. **0**(0): p. null.