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Prospective Cohort Study of HIV and Zika in Infants and Pregnancy (HIV ZIP)

Sponsored by:
The *Eunice Kennedy Shriver*
National Institute of Child Health and Human Development (NICHD)

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Signature Page

I, the Principal Investigator, agree to conduct this study in full accordance with the provisions of this protocol and will ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contributions to the study.

My signature below constitutes approval of this protocol (*Prospective Cohort Study of HIV and Zika in Infants and Pregnancy (HIV ZIP*) and the attachments and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable United States (U.S.) federal regulations and International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines. It is understood that no deviations from the protocol may be made without permission of the Sponsor.

Site Name: _____

Site Principal Investigator:*

Signed: _____ Date: _____

Name
Title

* The protocol should be signed by the local investigator of record who is responsible for the study implementation at his or her specific site.

Study Management

Before the recruitment and enrollment of participants, participating research sites must have the protocol and consent forms approved by their local Institutional Review Boards (IRBs)/Institutional Ethics Committees (IECs). In addition, research sites must receive protocol registration approval from the Regulatory Associate at Westat. All original approved documents must be maintained at the research site. A detailed description of site and protocol registration procedures is included in the HIV ZIP Manual of Procedures (MOP).

All queries for this protocol should be sent to the HIV ZIP Protocol Team using the HIV ZIP Query and Notification System (QNS) accessible via the HIV ZIP page on the NICHD Clinical Studies website (www.nichdclinicalstudies.org). The appropriate team member will respond to queries generally within 48 business hours via the HIV ZIP QNS and copy the other team members. The Protocol Manager, with the help of other Westat personnel and/or NICHD, if necessary, will answer general protocol implementation, case report form (CRF) completion, and specimen shipping queries. The Protocol Co-Chairs or their designees will respond to eligibility, study and participant management, exemptions and/or adverse event (AE) queries and notifications.

Queries and replies will automatically be archived at Westat. The Protocol Manager will post those queries deemed relevant to all sites on the NICHD Clinical Studies/HIV ZIP website, where they will be available for future reference to the protocol team and participating sites.

For protocol registration questions, email Regulatory@westat.com or call 1-888-464-5246 (toll-free) or 00 1 240-453-5661 (long distance charges). Protocol registration material can be sent electronically to Regulatory@westat.com or via fax at 1-888-865-1983 or 00 1 240-455-5580 (long distance charges).

List of Abbreviations

AABR	Automated Auditory Brainstem Response
Ab	Antibody
AE	Adverse Event
Ag	Antigen
AIDS	Acquired Immunodeficiency Syndrome
ArboNET	National Arbovirus Surveillance System
ART	Antiretroviral Therapy
ARV	Antiretroviral
ASQ	Ages and Stages Questionnaire
βhCG	Beta Human Chorionic Gonadotropin
BSID	Bayley Scales of Infant and Toddler Development
cART	Combination Antiretroviral Therapy
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	Confidence Interval
CKNV	Chikungunya Virus
CMV	Cytomegalovirus
CNS	Central Nervous System
CRF	Case Report Form
CSF	Cerebrospinal Fluid
CT	Computerized Tomography
DAIDS	Division of AIDS
DENV	Dengue Virus
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Administration
GA	Gestational Age
GBS	Guillain-Barré Syndrome
GCP	Good Clinical Practice
HAART	Highly Active ART
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus

List of Abbreviations (continued)

HEU	HIV-Exposed Uninfected
HHS	The U.S. Department of) Health and Human Services
HINE	Hammersmith Infant Neurological Examination
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HIV ZIP	Prospective Cohort Study of HIV and Zika in Infants and Pregnancy
HPTN	HIV Prevention Trials Network
IATA	International Air Transport Association
IC	Informed Consent
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEC	Independent or Institutional Ethics Committee
IgM	Immunoglobulin M
IRB	Institutional Review Board
LDMS	Laboratory Data Management System
LMP	Last Menstrual Period
LPC	Laboratory Processing Chart
MAC-ELISA	IgM Ab Capture ELISA
MOP	Manual of Procedures
MOU	Memorandum of Understanding
MPIDB	Maternal and Pediatric Infectious Disease Branch
MRI	Magnetic Resonance Imaging
MTCT	Mother-to-Child Transmission
ND	Neurodevelopmental
NIAID	National Institute of Allergy and Infectious Diseases
NICHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
OAE	Otoacoustic Emission

List of Abbreviations (continued)

OHRP	Office for Human Research Protections
OR	Odds Ratio
PACTG	Pediatric AIDS Clinical Trials Group
PAHO	Pan American Health Organization
PCR	Polymerase Chain Reaction
PE	Physical Examination
PHACS	Pediatric HIV/AIDS Cohort Study
PI	Principal Investigator
PID	Participant Identification
PII	Personally Identifying Information
P.R.	Puerto Rico
PRNT	Plaque Reduction Neutralization
QNS	Query and Notification System
RDC	Remote Data Capture
RNA	Ribonucleic Acid
RPR	Rapid Plasma Reagin
RT-PCR	Reverse Transcription PCR
SID	Screening Identification
SMARTT	Surveillance Monitoring for Antiretroviral Therapy Toxicities
TORCH	Toxoplasmosis, Rubella, CMV, Herpes
UNAIDS	The Joint United Nations Programme on HIV/AIDS
US	Ultrasound
U.S.	United States
VL	Viral Load
VS	Vital Signs
WHO	World Health Organization
WNV	West Nile Virus
YFV	Yellow Fever Virus
ZDV	Zidovudine
ZIKV	Zika Virus
ZIP	International Prospective Observational Cohort Study of Zika in Infants and Pregnancy

Study Abstract

Design:	This is a two-phase prospective international cohort study of pregnant women and their infants from those pregnancies whose goals are to compare the incidence of Zika virus (ZIKV) infection among pregnant women with and without Human Immunodeficiency Virus (HIV) infection and to determine the risk of adverse maternal and child outcomes associated with ZIKV/HIV co-infection across clinical sites in the continental United States (U.S.), Puerto Rico (P.R.) and Brazil. Phase I will enroll pregnant women/infant pairs who are: (1) infected with HIV only; (2) infected with ZIKV only; (3) infected with HIV and ZIKV; and (4) not infected with HIV or ZIKV. Phase I will assess the feasibility of enrolling a total of 200 pregnant women/infant pairs within a year, with a target of 150 HIV-infected women, 50 HIV-uninfected women from the continental U.S. sites only, and a minimum of 20 who are co-infected with HIV and ZIKV. Should the feasibility of Phase I prove successful, Phase II will commence by enrolling up to 1,800 additional pregnant women/infant pairs to the 4 groups described above. The comparison group of *HIV-uninfected* pregnant women/infant pairs from P.R and Brazil (ZIKV-infected and uninfected) will be obtained from data collected in the concurrent International Prospective Observational Cohort Study of Zika in Infants and Pregnancy (ZIP study). All HIV-infected and uninfected study participants will be tested for ZIKV. Enrolled women will be followed throughout their pregnancy and up to six weeks postpartum. Infants born to enrolled women will be followed for a full year after birth. Thereafter, the infants born to women at continental U.S. and P.R. sites also implementing the Surveillance Monitoring for Antiretroviral Therapy (ART) Toxicities (SMARTT) study may be followed up yearly until adulthood through SMARTT depending on the availability of funds. The data analysis will be conducted to address the objectives listed below.
Sample size:	**Phase I:** A total of 200 HIV-infected and uninfected pregnant women and their children (mother/infant pairs) from that pregnancy. **Phase II:** Up to 1,800 additional HIV-infected and uninfected pregnant women and their children from that pregnancy.
Duration:	Depending on the success of Phase I, the duration of the study may vary from four to six years. As described below, its duration will be four years if Phase I does not demonstrate feasibility, and six years if Phase I demonstrates feasibility. The duration of participation for pregnant women enrolled in Phase I is from the time of enrollment through approximately six weeks postpartum. The duration of participation for infants born to enrolled women is from birth through approximately one year of age. ■ Year 1- 200 pregnant women at risk for ZIKV and their newborn infants will be recruited and assessments begun. ■ Year 2- Completion of infant enrollments and maternal assessments, and continuation of infant assessments.

- Year 3- Completion of one-year follow-up of all infants.
- Year 4- Completion of all Phase I laboratory assays, reporting of results and data analyses.

At the end of Year 1, we will assess the success of Phase I. If the feasibility phase proves successful, enrollment into Phase II will begin. Irrespective of feasibility, Year 2 through 4 activities (described above) will occur for the 200 women and their infants enrolled in Phase I.

Phase II: The duration of participation for pregnant women and their infants is identical to Phase I.

- Years 2 and 3*- Up to 1,800 pregnant women at risk for ZIKV and their infants will be recruited.
- Year 4- Completion of newborn enrollments and maternal assessments, and continuation of infant assessments.
- Year 5- Completion of one-year follow-up of all infants.
- Year 6- Completion of all Phase II laboratory assays, reporting of results and data analyses.

* Phase II may begin earlier than Year 2 and end earlier than Year 6 if Phase I enrolls 200 eligible women and proves feasible in less than one year. The duration of the Phase II enrollment period may be adjusted depending on the status of the ZIKV epidemic and availability of future funding.

Population:

Maternal participants:

Pregnant women who are at risk for ZIKV are eligible for participation if they meet the following criteria:

- Reside in a geographic area accessible to one of the selected clinical research sites;
- Provide written informed consent (IC) (or assent with parent(s)/legal guardian(s) permission, where required);
- Age 15 years and older at enrollment;
- Confirmation of pregnancy by Beta Human Chorionic Gonadotropin (βhCG) measurement in blood or urine or fetal ultrasound (US) with fetal heart tones present; and
- Confirmation of pregnancy at less than 18 weeks gestational age (GA) based on pregnancy calculator or fetal US; or
- Confirmation of pregnancy at 18 weeks or greater GA based on pregnancy calculator or fetal US and with acute ZIKV symptoms and laboratory-confirmed ZIKV infection by ZIKV ribonucleic acid (RNA) detection.

In addition, at least one of the following three ZIKV-exposure risk categories must be met:

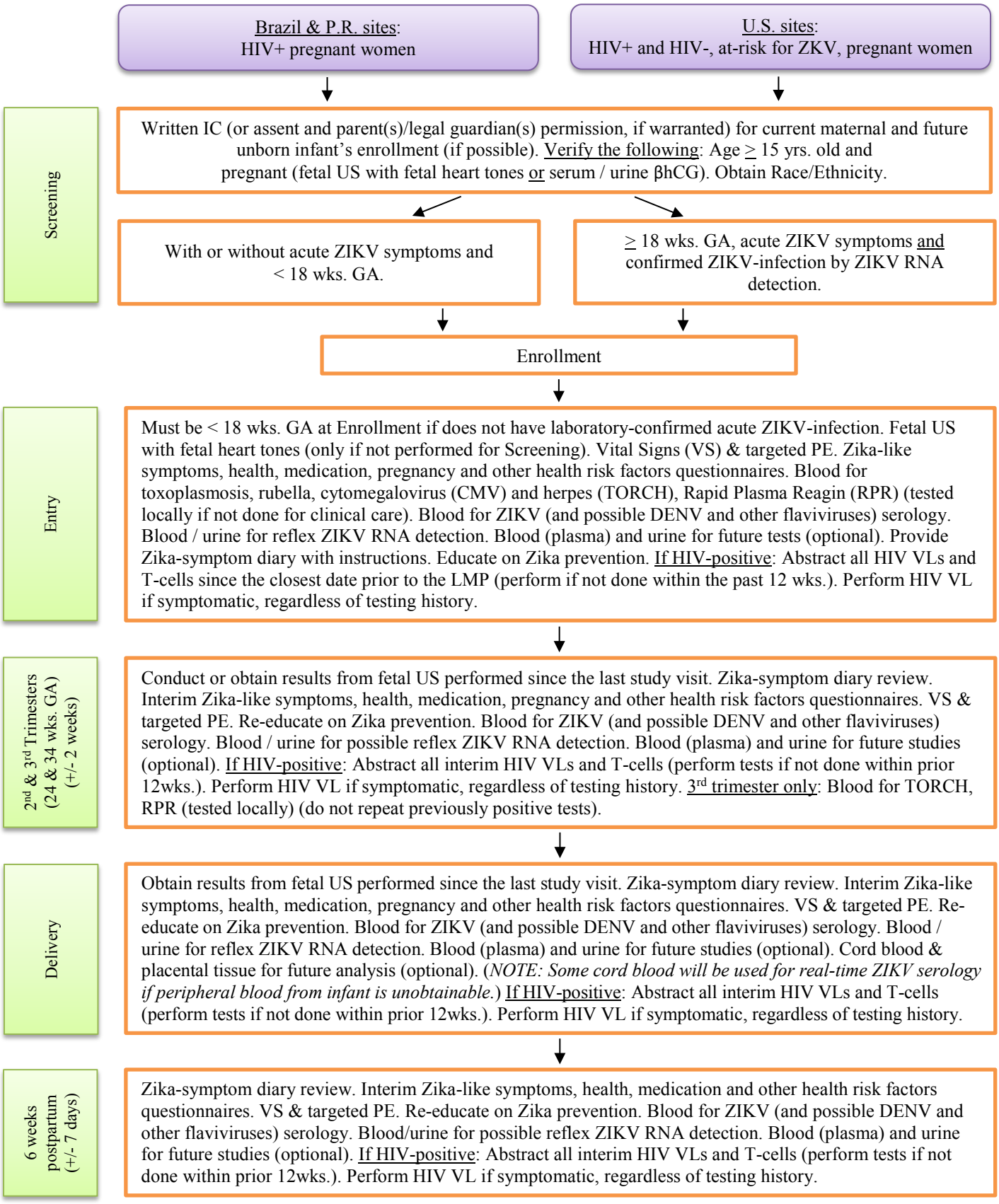
- Has resided in for at least three months or traveled within the last three months to a country or U.S. Territory with active ZIKV transmission. The most up-to-date list of these locations can be found at http://www.cdc.gov/zika/geo/active-countries.html.
- Within the U.S., the list of communities with local active ZIKV transmission can be found at: http://www.cdc.gov/zika/intheus/maps-zika-us.html;

	■ Sexual partner has resided in or traveled within the last six months to a country or U.S. Territory with active ZIKV transmission, or was diagnosed with ZIKV within the previous six months; or ■ Household member has been diagnosed with ZIKV infection or has traveled since the woman's last menstrual period (LMP) to a country or U.S. Territory with active ZIKV transmission. **Infant participants:** All infants born to women while enrolled in this study are eligible to participate if the parent(s)/legal guardian(s) provides written consent for his or her child's participation.
Primary objectives:	The primary objectives of the HIV ZIP study are: 1. To determine the feasibility of enrolling pregnant women with ZIKV/HIV co-infection, HIV infection alone, ZIKV infection alone as well as doubly uninfected women into a prospective cohort study at selected sites in the continental U.S., P.R. and Brazil. 2. To compare HIV viral suppression in HIV-infected women with and without ZIKV co-infection during pregnancy and the time of delivery. 3. To compare the incidence of ZIKV infection among pregnant women with HIV infection and those without HIV infection. 4. To compare the incidence of adverse pregnancy outcomes between women co-infected with HIV and ZIKV, women infected with either HIV or ZIKV alone, and doubly uninfected women. 5. To compare the incidence of vertical transmission of HIV and ZIKV between women co-infected with HIV and ZIKV, and women infected with either HIV or ZIKV alone. 6. To compare the incidence of congenital malformations and other adverse outcomes (including microcephaly, neonatal death, central nervous system (CNS) malformations, hydrops and ocular abnormalities) among offspring of women co-infected with HIV and ZIKV, women infected with either HIV or ZIKV alone and doubly uninfected women. 7. To compare the long-term effects (on growth, hearing, vision and neurodevelopment) of co-infection among children with *in utero* exposure to HIV and ZIKV, *in utero* exposure to either HIV or ZIKV alone and no *in utero* exposure to either virus.

Secondary objectives:	1. To compare the severity of maternal and infant outcomes of ZIKV infection in symptomatic and asymptomatic women. 2. To determine if co-infections and cofactors (including social and environmental factors) contribute to the presence of CNS malformations and if these factors influence the severity of adverse outcomes in the offspring.
Evaluations:	**Maternal:** Study visits will occur once per trimester during pregnancy, at delivery and approximately six weeks postpartum. At baseline, demographics will be collected and a targeted physical examination (PE) will be conducted. Baseline and interim medical, medication, substance use and other risk factor history will be obtained by interview and medical record abstraction. Medical history will include, but is not limited to, a recent history of symptoms consistent with ZIKV infection, including in their sexual partners and household members. At all visits, an assessment of symptoms suggestive of ZIKV will be targeted (i.e., fever, rash, arthalgias, myalgias, pruritus, headaches, eye pain and conjunctivitis), lymphadenopathy and a targeted PE performed. Fetal US will be performed (or data abstracted from clinically performed US) in the first, second and third trimesters. At each visit, HIV-infected women will have all available HIV viral loads (VLs), CD4+/CD8+ T-cells counts and percentages (T-cells) abstracted from their medical records. If these laboratory results are unavailable, blood samples will be collected for these laboratory assessments. Testing will be performed at each visit for the diagnosis of ZIKV infection. Tests will be performed to evaluate co-infections such as Dengue virus (DENV) and possibly other flaviviruses and other infections. Testing for environmental contaminants associated with morbidity and mortality in the developing fetus and infant at birth, such as lead, may be performed. **Infant Participants:** Data collected will include basic demographics and birth history. PEs will be conducted at birth and each study visit (or information abstracted from medical records if a PE was performed within the prior four weeks) and will consist of temperature, cardiac, respiratory, organ systems (hepatomegaly, splenomegaly), skin (petechial, rash, skin scarring), gastrointestinal (feeding, emesis, diarrhea) and neurologic examinations (seizures, apnea, reflexes/tone, asymmetry, eye opening, basic movement). Growth parameters including weight, length/height and head circumference will be recorded at birth and each study visit (or information abstracted from medical records if performed within the prior four weeks). Hearing assessments and ophthalmologic evaluations to detect chorioretinitis or abnormalities in conjunctiva, lens or retina will be performed at birth and each study visit. If initial exams are abnormal, referral for additional audiology and/or ophthalmology testing will be done and results recorded (if available) from any tests done for clinical reasons.

	Results of any imaging assessments done for clinical care will be recorded. These include any neuroimaging studies (US, computerized tomography (CT), or magnetic resonance imaging (MRI)) to detect neurologic abnormalities including intracranial calcifications and non-neurologic imaging (including but not limited to chest X-rays, abdominal X-rays, abdominal CT scans or MRIs). All ZIKV-exposed infants will have a cranial US within the first three months. General motor development markers will be assessed. Neurodevelopmental (ND) status will be assessed using normal developmental milestones tests according to the standard of care. Testing will be performed at birth and subsequent visits for the diagnosis of ZIKV infection. Testing for other infections, including congenital infections that could lead to adverse infant outcomes may also be performed.
Data analysis:	Epidemiological and statistical data analyses will be conducted to address the primary and secondary objectives described above.

Maternal Study Schema



Maternal Study Schema
(ZIKV-Symptomatic or Asymptomatic with Positive Serology)

Women Experiencing Signs / Symptoms Consistent with Acute ZIKV-like Illness at Any Time While On Study

↓

NOTE: *If ZIKV-like symptoms occur between scheduled study visits, have the participant come in for an unplanned, symptomatic visit assessment.*

Zika-symptom diary review. Interim health and medication history.
Record signs and symptoms of ZIKV-like illness (i.e., fever, rash, arthalgia, myalgia, pruritus, headache, eye pain and conjunctivitis). Also, include presence of lymphadenopathy.
Perform VS & targeted PE
Pregnancy questionnaire
Other risk factors questionnaires: If regularly scheduled study visit. If unplanned symptomatic visit, administer at continental U.S. sites only.
If HIV-positive: Abstract all interim HIV VLs and T-cells.
Collect biological samples: Blood for serum ZIKV & DENV (and other possible) serology; serum / urine for ZIKV RNA detection test.

- If ZIKV serology positive or ZIKV RNA positive, perform (urine/serum) ZIKV RNA test from most recent prior visit.
- If ZIKV serology negative, continue doing ZIKV serology at subsequent visits.
- If ZIKV serology positive, continue doing ZIKV serology at subsequent visits until negative.
- If ZIKV RNA positive, continue doing ZIKV RNA testing at subsequent visits until negative.
- Blood for TORCH (if non-immune) and RPR to local laboratory, if clinically indicated.
- Biorepository (if consented for future use): Blood (plasma) and urine.
- Request other laboratory testing as part of standard of care.
- If HIV-positive: HIV VL.

Record results from interim fetal US performed according to country and local clinical guidelines since the prior study visit.

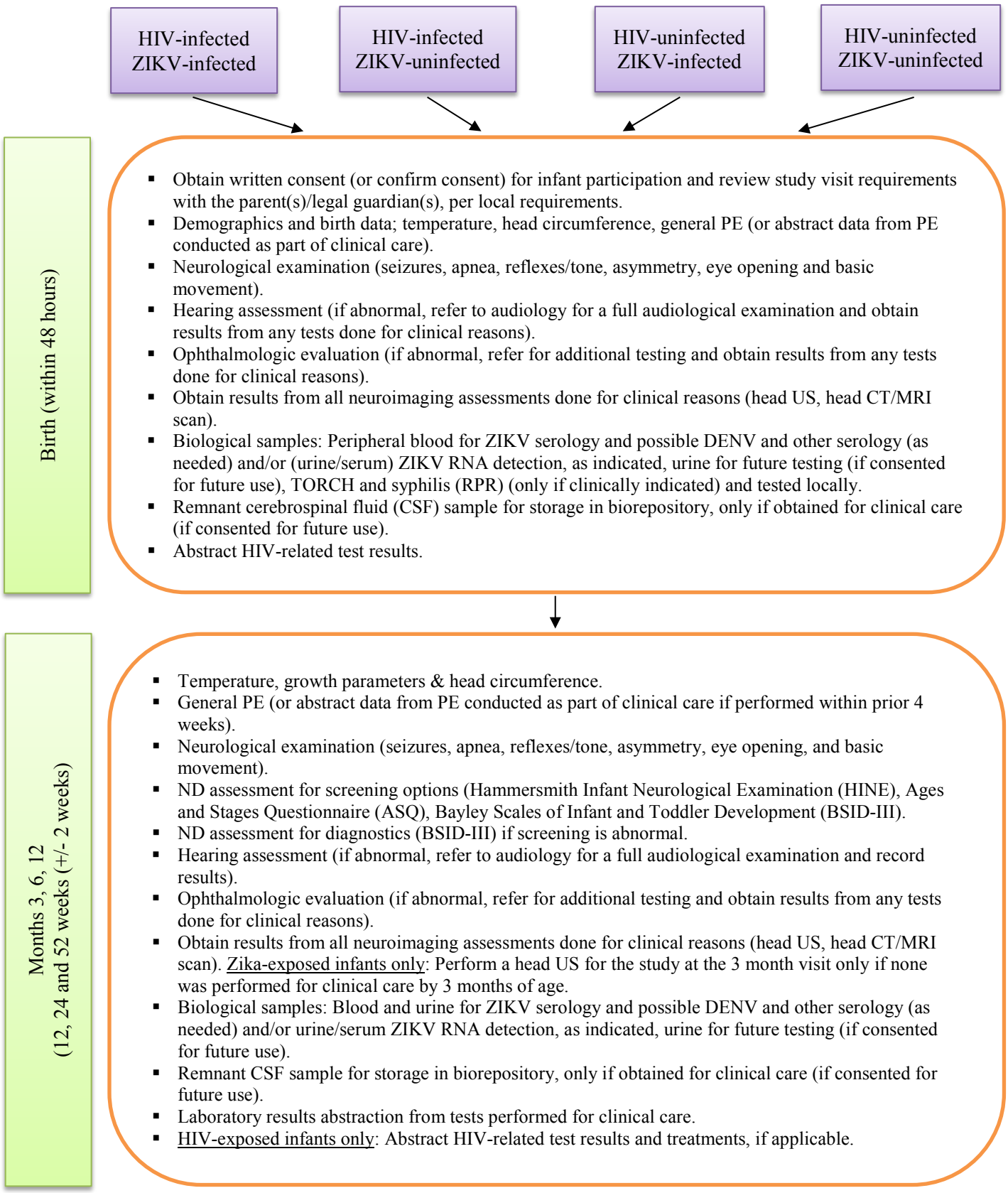
Women with Positive ZIKV Serology at Any Study Visit, But without Clinical Signs / Symptoms of Acute ZIKV-like Illness

↓

Test available blood / urine samples as follows:

- Serum/urine for ZIKV RNA detection on samples from the current visit and from the most recent prior visit, if available.
- If ZIKV RNA negative, perform serum DENV serology from current visit if resides in or recent travel to DENV-endemic region.
- Continue doing ZIKV serology at subsequent visits until negative.
- If ZIKV RNA positive, continue doing ZIKV RNA testing at subsequent visits until negative.

Infant Study Schema



1.0 Introduction

1.1 Background on ZIKV

1.1.1 Virology, History and Epidemiology of ZIKV

ZIKV is a vector-borne single-stranded RNA virus of the genus Flaviviridae.[1] This virus family also includes other human pathogenic viruses such as DENV, West Nile Virus (WNV), Japanese encephalitis virus, Yellow Fever Virus (YFV), and tick-borne encephalitic virus. ZIKV has both Asian and African lineages and is closely related to DENV. While ZIKV has been isolated from many species of Aedes mosquito, Aedes aegypti is thought to be the principal vector spreading ZIKV during the recent outbreaks in Latin America, the Caribbean and the continental U.S.

ZIKV was first identified in the Zika forest near Kampala, Uganda in rhesus macaques in 1947.[2] Between the first isolation in monkeys until 2007, reports of human cases were rare and sporadic.[1] Although the virus had been isolated widely in Africa and Southeast Asia, the infection was largely asymptomatic, and no epidemics were observed. In 2007, an outbreak of the Asian lineage of ZIKV occurred in the Federated States of Micronesia.[3] Reports suggest that 73% of the population was infected within 3 years with 49 confirmed cases based on RNA detection or serologic criteria. In 2013, another outbreak involving 5,895 suspected cases of ZIKV and 294 confirmed cases occurred within a 3-month period in French Polynesia.[4]

In May 2015, the first cases of ZIKV were reported in northeast Brazil where the Asian lineage of ZIKV was confirmed.[1] Because of the lack of immunity in the Brazilian population and the abundance of Aedes aegypti mosquitos, an autochthonous transmission was established with ensuing rapid and large spread of ZIKV. Although initially concentrated in Brazil, the virus spread throughout South America, the Caribbean, and P.R. As of December 15, 2016, 48 countries in the Americas have reported vector-borne transmission of ZIKV.[5]

Until recently, most infections from ZIKV were thought to be self-limiting and mild with resolution of most clinical findings by seven days in the small proportion of patients who were symptomatic.[6] However, during 2013-2014, the occurrence of Guillain-Barré syndrome (GBS) as a post-infectious complication of ZIKV infection was observed during the French Polynesian outbreak.[1] According to the most recent Pan American Health Organization (PAHO) report, 13 countries in the Americas including Brazil, Colombia, El Salvador and Venezuela have reported increases in the number of GBS cases with ZIKV confirmation.[5]

With introduction of ZIKV, Brazil also witnessed an epidemic of microcephaly cases among newborns. According to the most recent PAHO report, 10,441 suspected cases of microcephaly and other CNS malformations were reported in Brazil during 2015-2016, as compared to an annual average of 163 cases of microcephaly from 2001 to 2014.[7] To date, the Brazilian Ministry of Health has confirmed microcephaly in

2,228 of these cases. Of the remainder, 5,040 were discarded because they had non-infectious causes or did not meet the case definition and 3,173 are still under investigation. Microcephaly and other CNS malformations have also been noted in 35% of reported miscarriages and stillbirths in Brazil during this period. To date, 22 countries and territories in the Americas (including the U.S. and P.R.) have reported confirmed cases of birth defects and other abnormalities associated with ZIKV infection.[5] These numbers are expected to increase over the coming year as previously and newly exposed women complete their pregnancies.

In the U.S., 4,809 cases of ZIKV with illness onset from January 1, 2015 through December 28, 2016 were reported to the national arbovirus surveillance system (ArboNET).[8] While these cases come from virtually the entire country, over half have come from only four states: New York (21%), Florida (18%), California (9%) and Texas (6%). The vast majority of cases (95.5%) have been associated with travel to an affected area; another 4.5% were acquired through local mosquito transmission, and < 0.1% have been associated with sexual contact with a traveler to an affected area. To date, local mosquito transmission in the U.S. has occurred in Miami-Dade County, Florida and in Brownsville, Texas.[9, 10] The extent of the ZIKV outbreak is far greater in the U.S. Territory P.R. where 33,865 locally acquired cases (through mosquito and sexual transmission) and 129 travel-associated cases were reported to ArboNET as of December 28, 2016.[8] Furthermore, 1,246 pregnant women in the U.S. and 2,701 pregnant women in U.S. territories (mainly P.R.) have been reported with laboratory evidence of possible ZIKV infection.[11]

1.1.2 Modes of ZIKV Transmission

While ZIKV is spread primarily through bites of infected Aedes mosquitos, other forms of transmission have been described. In particular, several case reports have documented that ZIKV can be sexually transmitted from an infected male or female to his or her sexual partner, even if the infected individual is asymptomatic.[12-14] Supporting these data is evidence of ZIKV in the semen of infected males lasting as long as three months after symptom onset and the presence of infection in vaginal swabs.[15, 16] Other documented routes of ZIKV transmission include blood resulting in transmission through transfusions[17] and perinatal transmission from mother-to-child.[18] While ZIKV has been detected in saliva, urine, breast milk and conjunctival fluid of infected humans,[19-21] there is limited data documenting transmission via these body fluids, though a recent report suggests transmission through contact with highly infectious body fluids in the hospital setting.[22]

1.1.3 U.S. Clinical Testing Guidelines for ZIKV

In July 2016, the Centers for Disease Control and Prevention (CDC) issued guidelines for testing pregnant women who have had possible exposure to Zika and have symptoms consistent with ZIKV infection.[23] Possible exposure is defined as travel to or residence in an area with active ZIKV transmission, or sex with a partner who has traveled to or resides in an area with active ZIKV transmission without using condoms or other barrier methods to prevent infection. The recommended tests follow

a complicated algorithm (see http://www.cdc.gov/mmwr/volumes/65/wr/mm6529e1.htm) that includes real-time RT-PCR testing of serum and urine, a ZIKV Immunoglobulin M (IgM) antibody (Ab) test, and a plaque reduction neutralization (PRNT) test to rule out a false positive IgM result. However, a recent update to CDC laboratory testing guidelines does not recommend the use of PRNT for confirmation of ZIKV infection in P.R.[24]

On the other hand, no testing is currently recommended for women who are contemplating pregnancy.[25] Instead, they are advised to consider avoiding non-essential travel to areas with ZIKV transmission and, if they have traveled to or live in an area with ZIKV, to take steps to prevent mosquito bites and exposure through sex. The latter includes waiting at least eight weeks since last exposure before trying to conceive. The CDC also recommends that men who may have been exposed to ZIKV have protected sex for at least six months after the start of symptoms or last exposure.

1.1.4 Clinical Sequelae of ZIKV Infection During Pregnancy

Microcephaly was not reported in association with ZIKV infection prior to the outbreak in the Americas. However, this association was not examined systematically during previous outbreaks. Recently, Besnard et al. reported a summary of findings from a retrospective review of the data from the 2013-2014 ZIKV outbreak in French Polynesia.[18] They describe 10 cases of congenital cerebral malformations in fetuses and newborns including 8 with major brain lesions and severe microcephaly, 6 with severe cerebral lesions without microcephaly and 5 with brainstem dysfunction without visible malformations. Among amniotic fluid samples that were collected between 20 and 29 weeks gestation from 7 cases, ZIKV RNA or infectious ZIKV isolates were detected in 4 of 5 microcephalic, but not in 2 normocephalic infants with severe brain lesions.

In January 2016, one of the first reports of microcephaly cases in the current epidemic in Brazil was published.[26] All 35 infants met the case definition of a head circumference $\geq$ 2 standard deviations below mean for sex and GA, and 74% of their mothers reported a rash illness during pregnancy. The CSF of affected infants was being tested for ZIKV at the time of publication. In another Brazilian study of ophthalmologic findings associated with ZIKV infection, 29 infants with microcephaly and presumed intrauterine ZIKV infection were examined. Ocular abnormalities were found in 10 children.[27] Over the past year, additional case series have been reported linking prenatal ZIKV infection to an increasing number of abnormalities in infants including joint contractures, clubfoot, and hearing loss.[28, 29]

More recently, formal epidemiological studies have found further evidence of adverse birth and pregnancy outcomes associated with prenatal ZIKV infection. Preliminary findings from an ongoing case-control study in Brazil reported that mothers of 41% newborns with microcephaly vs. 0% of unaffected controls, had laboratory-confirmed ZIKV infection (crude odds ratio (OR): 55.5, 95% confidence interval (CI): 8.6 to infinity).[30] Another Brazilian study of symptomatic pregnant women found that 46%

of ZIKV-exposed pregnancies vs. 11.5% of unexposed pregnancies had adverse pregnancy and infant outcomes, including miscarriage, fetal death, growth retardation, microcephaly and other CNS abnormalities.[31] Lastly, a study from the U.S. Zika Pregnancy Registry found that 6% of women with laboratory evidence of possible recent ZIKV infection had offspring with ZIKV-associated birth defects, primarily microcephaly and brain abnormalities.[32] This proportion rose to 11% among women with symptoms or exposure exclusively during the first trimester. The prevalence of microcephaly among ZIKV-exposed women in this study is substantially higher than the background prevalence (7 per 10,000 live births) in the U.S.[33]

1.2 Background on HIV Infection in Pregnant Women

Since the start of the HIV epidemic, there have been over 78 million people infected with HIV. Currently, more than 36 million people are living with HIV and 2 million new infections occurred in 2015.[34] In 2015, there were approximately 150,000 children infected through mother-to-child transmission (MTCT), substantially fewer than in 2010 due to the fact that approximately 77% of HIV-infected pregnant women have access to ART during pregnancy, which has become standard of care worldwide.[34]

The first cases of Acquired Immunodeficiency Virus (AIDS) in Brazil were reported in 1982, and Brazil was one of the first countries to provide universal access to ART.[35] In 2015, Brazil had a total of 830,000 people with HIV, and set a record for the number of people initiating ART (81,000), resulting in more than 455,000 people with HIV on ART.

In the U.S., approximately 8,500 HIV-infected women give birth each year.[36] The standard of care in the U.S., as well as throughout the world, is to provide ART for both the health of the woman and to reduce the risk of MTCT. With the combination of routine HIV testing of pregnant women and access to ART, the MTCT rate has dropped to well below 1%. In the Pediatric HIV/AIDS Cohort Study (PHACS) - SMARTT, there have been 9 transmissions among more than 2,500 births in the past 10 years.[37] While the use of ART during pregnancy has been a tremendous public health success, concerns still remain regarding both the short-term effects of HIV and ART during pregnancy as well as the longer-term effects among HIV-exposed uninfected (HEU) children.

Given these concerns, the PHACS-SMARTT cohort study was initiated in 2007 to evaluate the effect of HIV infection and ART among HEU children. Many ART medications given to a pregnant woman cross the placenta and can be detected in the amniotic fluid and cord blood resulting in substantial fetal exposure.[38] Therefore, there is concern about toxicity of the drugs in the fetus and infant. It is noteworthy that none of the currently approved ART medications for the prevention of MTCT of HIV are in Food and Drug Administration (FDA) Pregnancy Category A (no fetal risk ascertained in adequately controlled human studies). Thus, there is continued need to examine the toxicity of ART in HIV transmission prevention for the short-

term toxicity of newer agents as well as the unanswered questions of longer-term toxicity and subtle adverse effects.

1.2.1 Short-term Toxicity of ART to the Fetus and Infant

The first major study of ART use to prevent maternal transmission of HIV (the Pediatric AIDS Clinical Trials Group (PACTG) 076 Study) documented little short-term toxicity beyond transient anemia.[39] Since then, a number of increasingly complex regimens were studied for HIV treatment efficacy during pregnancy for the mother as well as for reducing transmission to the infant. These included studies that utilized nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors. The short-term toxicity risks for the fetus and infant were low; therefore, HIV transmission prevention by the use of ART has become standard of care and has been thought to have an acceptable risk profile in the short term. However, PHACS and others have demonstrated an increased risk of preterm birth with exposure to protease inhibitor-based combination antiretroviral therapy (cART) early in pregnancy.[40]

1.2.2 Long-term Toxicity of ART to the Infant

A number of possible toxicities have been reported in ART-exposed children with some of the major areas being growth, neurologic, ND and cardiac. These and other toxicities need examination. The SMARTT study uses an innovative trigger-based surveillance approach for identifying AEs that may be attributed to prenatal and perinatal ART exposure. All children receive periodic clinical and laboratory evaluations and only those meeting certain thresholds or "triggers" receive additional assessments to evaluate whether or not they meet specific AE case criteria. We demonstrated that this approach estimates AE rates more efficiently than evaluating a random sample of the same size, and yields unbiased estimates of AE rates after correcting for the sensitivity of the trigger to identifying AEs.[41] To date, 49% of participants have met a trigger and 25% met case status (an AE) in $\geq$ 1 domains.[42] We identified several signals suggesting specific AEs from ART exposure that warrant further evaluation. For example, zidovudine (ZDV) was associated with higher risk of a metabolic case, characterized as high body mass index and abnormal lipid levels and/or insulin resistance. The combination of didanosine plus stavudine was also associated with higher risk of both neurologic and language cases. In other SMARTT-related research studies, we also found that atazanavir was associated with lower language achievement at one year of age and a two-fold higher risk of congenital anomalies.[43-45]

1.2.3 Neurologic, ND, Behavioral, and Language Dysfunction

French studies[46] have suggested that perinatal exposure to ART nucleoside analogues may lead to persistent mitochondrial dysfunction. Mitochondrial disorders encompass a broad spectrum of clinical disease that include neurologic or ND abnormalities. The French studies specifically reported an increased prevalence of both severe and mild/moderate neurobehavioral abnormalities. Furthermore, they

reported an increased prevalence of febrile seizures associated with perinatal exposure to ARV nucleoside analogues in uninfected but exposed children.[47] In contrast, a study from the U.S. suggested that there is no global effect of ZDV on neurodevelopment up to four years of age.[48, 49] However, there are sporadic reports of mitochondrial-like disease with ragged red fiber abnormalities on muscle biopsy and developmental and visual disturbances,[50] as well as reports of CNS MRI abnormalities in children prenatally exposed to ART.[51]

HIV and HIV-associated treatment exposures during gestation of uninfected infants may also affect neurobehavioral development and result in more subtle abnormalities that may not necessarily be related to mitochondrial function or birth defects.[47] The Women and Infants Transmission Study[52] showed that among HEU children up to 24 months of age, mental and motor development scores were lower among older children, both with and without prenatal exposure to illicit drugs. The extent of this decline and decreased levels of functioning (more than 8% scoring > 2 standard deviations below expected) are significantly greater than were observed in a similar sample of unexposed children of low socioeconomic and minority status.[53] The etiology of this ND decline and impaired level of functioning has not been elucidated, but factors that may play a role are time of exposure to HIV, ART, other HIV-associated medications, illicit drug use and/or other environmental toxin exposure.

PHACS has shown that HEU youth are at increased risk for poor ND outcomes.[44, 54-58] However, no specific ARTs or classes have been clearly implicated. PHACS found a high risk of late language emergence among HEUs at both one (26%) and two (23%) years of age, with risk at one year of age associated with atazanavir exposure.[43, 44] PHACS is currently exploring the hypothesis that atazanavir results in chronic fetal exposure to modest elevations of bilirubin.[59]

Different aspects of the nervous system may be at risk of insult during the successive gestational periods because of the course of prenatal development.[47] A number of studies have shown that insults, such as maternal infection, particularly during the second trimester, are associated with neuropsychiatric abnormalities,[60] while insults during the first trimester seem more associated with abnormalities in global structure of the brain.[61] The effects of illicit drug abuse during pregnancy are well documented and may interact with the effects of HIV infection and ART providing additive negative effects on neurobehavioral development.

1.2.4 Hearing Dysfunction

While the majority of cases of moderate to profound permanent hearing loss with congenital and neonatal onset are identified by direct screening,[62-64] some cases are missed by otoacoustic emissions (OAEs) and/or automated auditory brainstem response (AABR) hearing screening technologies in the newborn period. This is because the hearing impairment is mild in degree,[64, 65] unusual in configuration[66] or late in onset.[67] Infants who fail hearing screening or children who are later identified with risk indicators for hearing loss (e.g., speech and language delay or family

concern), require referral for comprehensive audiometric examination to determine hearing status and the need for follow-up treatment/testing.[68]

A challenge in studying hearing in infants and children who were prenatally exposed to ART is that maternal HIV infection can be associated with conditions that affect hearing in the infant. Infants of mothers living in poverty are at increased risk of prematurity, very low birth weight and other maternally transmitted infections such as syphilis, toxoplasmosis, and CMV. These conditions are known to be associated with increased risk for hearing loss in the newborn period.[68-70] In contrast, other exposures that occur in HIV-infected mothers such as prenatal drug and alcohol exposure have not been associated with sensorineural hearing loss in the newborn.[71, 72]

Overall, PHACS found that 3.1% of infants in SMARTT had an abnormal newborn hearing screen, with a lower risk found with first trimester tenofovir exposure.[73]

1.2.5 Consequences of Prenatal HIV Infection on Immunity and Impacts of Co-Infections on Mother and Fetus

To promote and support pregnancy and the growing fetus, an immunological adaptation during pregnancy is needed. This sanctuary is damaged when this protection is broken by a viral infection and so adverse outcomes may result such as increased maternal morbidity related to the infection, propagation of other microorganisms, fetal demise, preterm labor and MTCT of viral and other agents.[74]

HIV infection is known to have deleterious effects on the immune system, mainly on the CD4+ T-cells compartment, B-lymphocytes and Ab responses to pathogens and vaccines.[75] These alterations are linked to immune activation/dysregulation and chronic inflammation.[76] When used correctly, ART results in rapid control of HIV with VL suppression in blood and partial restoration of immune function, thereby preventing the various complications that define AIDS. However, even after treatment, HIV infection evolves into a chronic condition with the potential to continuously affect the host immune system.[77] These facts are especially important to the pregnant mother and fetus when the pregnancy is threatened by a superimposed latent or acutely acquired infection.

Increased risk of severe illness during pregnancy from viral infections has been reported during pandemics of influenza, Ebola, and Lassa fever, even in HIV-uninfected women.[74, 78] Regarding ZIKV infection in pregnancy, apparently, no enhanced risk of morbidity or mortality in HIV-uninfected women has been described so far.[79] Nevertheless, only two cases of ZIKV infection in HIV-infected individuals have been reported to date: an adult male who had only mild symptoms and recovered well,[80] and an immunosuppressed pregnant woman who was using a combination ARV regimen and presented with a mild disease. While the pregnant case had a rapid and complete recovery, her severely affected fetus died at 20 weeks gestation.[81] Considering the potential of more severe disease in HIV-infected mothers, it is very

relevant to examine pregnant women with both ZIKV and HIV infections to assess the consequences of co-infection on the mother's health.

Moreover, it is possible that ZIKV may act as an HIV facilitator pathogen. That is, it could enhance HIV replication during infection due to the release of cytokines that can activate CD4 cells offering the inflammatory microenvironment, or it could directly bind to HIV proteins that support HIV replication.[82] High maternal HIV load is the strongest risk factor for MTCT of HIV, and reduction of HIV load with ARV significantly reduces risk.[83] Possibly, any infection that increases HIV VL in plasma, the genital tract or breast milk may indirectly raise the risk of MTCT of HIV. In addition, HIV's systemic immunological activation consequences and impairment of the placental barrier could boost the risk of MTCT of the infecting agent, including ZIKV.

The potential facilitation of MTCT of HIV and another virus in the presence of co-infections with several microorganisms during gestation, before and after the era of ARVs, has been investigated in many studies. It has been shown that placental malaria is associated with increased MTCT of HIV, even at low maternal HIV loads.[84] Syphilis, a common co-infection in HIV-infected women, can also facilitate in utero transmission of HIV to infants.[85] Similarly, type 2 Herpes Simplex Virus co-infection has been associated with increased intrapartum HIV transmission.[86] Although the Hepatitis B Virus (HBV) infection does not seem to be independently associated with increased transmission of HIV, maternal HIV/HBV co-infection may increase HBV transmission to the infant.[83] In addition, HIV/Hepatitis C Virus (HCV) co-infected mothers are at increased risk for perinatal transmission of both viruses.[87, 88] In the same direction, the prevalence of congenital CMV infection among infants born to HIV-infected women is higher (2% to 7%) as compared to the general newborn population (0.7%).[89] Although there are data suggesting that highly active antiretroviral therapy (HAART) decreases the risk of congenital CMV infection among HIV-infected mothers,[90] a more recent report shows that congenital CMV infection remains higher among infants born to HIV-infected mothers receiving ARVs.[91]

In contrast to HIV, the correlates of MTCT of ZIKV and the fetal disease caused by ZIKV infection are currently unknown. However, it is likely that the immune system dysfunction of HIV-infected pregnant women potentially enhanced by acute viral infection, even in the presence of ARVs, could increase the risk of MTCT of both HIV and ZIKV and their consequences.

1.3 Rationale for HIV ZIP Study

Remarkable strides have been made in the last decade in the treatment of HIV-infected pregnant women, suppressing HIV RNA levels, which result in immune restoration for their own health and the prevention of MTCT of HIV. The occurrence of ZIKV infection among HIV-infected pregnant women raises serious concerns regarding the ARVs ability to suppress HIV RNA levels and the risk of MTCT of both viruses. There is also a growing number of significant adverse infant outcomes

following prenatal ZIKV infection, and given the fact that HIV is also a neurotropic virus, the ability of an HIV-infected pregnant woman to maintain HIV RNA suppression will be critical in regards to her child's health. Therefore, there is an urgent need to formally investigate both maternal and child effects of HIV and ZIKV co-infection in pregnant women. Delineation of adverse effects would allow formulation of standard-of-care recommendations to minimize adverse effects but enable continuation of preventive therapy.

2.0 Study Objectives

2.1 Primary Objectives

2.1.1 To determine the feasibility of enrolling pregnant women with ZIKV/HIV co-infection, HIV infection alone, ZIKV infection alone as well as doubly uninfected women into a prospective cohort study at selected sites in the continental U.S., P.R. and Brazil.

2.1.2 To compare HIV viral suppression in HIV-infected women with and without ZIKV co-infection during pregnancy and the time of delivery.

2.1.3 To compare the incidence of ZIKV infection among pregnant women with HIV infection and those without HIV infection.

2.1.4 To compare the incidence of adverse pregnancy outcomes between women co-infected with HIV and ZIKV, women infected with either HIV or ZIKV alone and doubly uninfected women.

2.1.5 To compare the incidence of vertical transmission of HIV and ZIKV between women co-infected with HIV and ZIKV, women infected with either HIV or ZIKV alone.

2.1.6 To compare the incidence of congenital malformations and other adverse outcomes (including microcephaly, neonatal death, CNS malformations, hydrops and ocular abnormalities) among offspring of women co-infected with HIV and ZIKV, women infected with either HIV or ZIKV alone and doubly uninfected women.

2.1.7 To compare the long-term effects (on growth, hearing, vision and neurodevelopment) of co-infection among children with *in utero* exposure to HIV and ZIKV, *in utero* exposure to either HIV or ZIKV alone and no *in utero* exposure to either virus.

2.2 Secondary Objectives

2.2.1 To compare the severity of maternal and infant outcomes of ZIKV infection in symptomatic and asymptomatic women.

2.2.2 To determine if co-infections and cofactors (including social and environmental factors) contribute to the presence of CNS malformations and if these factors influence the severity of adverse outcomes in the offspring.

3.0 Study Design

This is a two-phase prospective international cohort study of pregnant women/infant pairs whose goals are to compare the incidence of ZIKV infection among pregnant women with and without HIV infection and to determine the risk of adverse maternal and child outcomes associated with ZIKV/HIV co-infection across clinical sites in the continental U.S., P.R. and Brazil.

Phase I will enroll pregnant women/infant pairs who are: (1) infected with HIV only; (2) infected with ZIKV only; (3) infected with HIV and ZIKV; and (4) not infected with either HIV or ZIKV, and will assess the feasibility of enrolling a total of 200 pregnant women/infant pairs within a year, with a target of 150 HIV-infected women across all sites, 50 HIV-uninfected women from continental U.S. sites only and a minimum of 20 who are co-infected with HIV and ZIKV.

Should the feasibility of Phase I prove successful, Phase II will commence by enrolling up to 1,800 additional pregnant women/infant pairs to the 4 groups described above. The comparison group of *HIV-uninfected* women/infant pairs from P.R and Brazil (ZIKV-infected and uninfected) will be obtained from data collected in the concurrent ZIP study.

All HIV-infected and uninfected participants will be tested for ZIKV. Enrolled women will be followed throughout pregnancy and up to six weeks postpartum. Infants born to enrolled women will be followed for a full year after birth. Thereafter, the infants born to women at continental U.S. and P.R. sites also implementing the SMARTT protocol may be followed up yearly until adulthood through SMARTT, depending on availability of funds.

3.1 Study Population

The study will enroll pregnant women/infant pairs, ages 15 years and older, who reside in a geographic area accessible to one of the selected clinical research sites in Brazil, P.R. and the continental U.S., meet the criteria for ZIKV infection risk, are less than 18 weeks GA and are HIV-infected or uninfected (the latter in the continental U.S. only).

In addition to the above, the study will enroll HIV-infected and uninfected women (the latter in the continental U.S. only) at 18 weeks or greater GA who present with acute ZIKV-like symptoms and have confirmed ZIKV infection by a positive ZIKV RNA detection test at the Screening visit.

Lastly, all infants born to women while enrolled in this study will also be enrolled if the parent(s)/legal guardian(s) consents for the infant to participate. Infants may fall into one of the following groups: (1) HIV-infected only; (2) ZIKV-infected only; (3) HIV- and ZIKV-infected; and (4) HIV- and ZIKV-uninfected.

3.2 Sample Size

Phase I of the study will enroll 200 pregnant women at risk for ZIKV infection and their children born from that pregnancy to determine the feasibility of enrolling a target of 150 HIV-infected and 50 HIV-uninfected women within a year, and a minimum of 20 women who are co-infected with HIV and ZIKV. Should the feasibility of Phase I prove successful, Phase II will commence by enrolling up to 1,800 additional pregnant women at risk for ZIKV infection and their children born from that pregnancy.

4.0 Protocol Registration Procedures

Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol consent form approved by its local IRB/IEC and any other applicable regulatory entity. The site must then register the protocol with the Westat Regulatory Affairs by completing the protocol registration checklist and submitting all required regulatory documents. Westat Regulatory Affairs will review the materials to ensure that the site has the necessary resources to fulfill study requirements and to follow federal and local IRB/IEC regulations. Sites may not begin enrollment prior to receiving registration approval from Westat Regulatory Affairs. All original approved documents must be maintained at the clinical site.

The procedures for registration are outlined in the HIV ZIP MOP, which can be found in the HIV ZIP link on the NICHD Clinical Studies website (www.nichdclinicalstudies.org).

5.0 Selection and Enrollment of Study Participants

5.1 Maternal Inclusion Criteria

Pregnant women who reside in a geographic area accessible to one of the selected clinical research sites are eligible for participation if the following criteria are met:

5.1.1 Provides written IC (or assent and parent(s)/legal guardian(s) permission, where required per state or country regulations).

5.1.2 Age 15 years or older at enrollment.

5.1.3 Confirmation of pregnancy by βhCG measurement in blood or urine or fetal US with fetal heart tones present.

5.1.4 Based on pregnancy calculator or fetal US: Confirmation of being at < 18 weeks GA of pregnancy or at any GA if presents with acute ZIKV-like symptoms (i.e., fever,

rash, arthralgia, myalgia, pruritus, headache, eye pain and conjunctivitis) and has laboratory-confirmed ZIKV infection by ZIKV RNA detection.

5.1.5 Plans on remaining in the area of the current study site or if moving, within an area of any other study site, for the duration of her and her child's participation.

5.1.6 Willingness of parent(s)/legal guardian(s) to provide written consent to enroll the infant from the current pregnancy once delivered.

In addition, at least one of the following three ZIKV-exposure risk categories must be met:

5.1.6.1 Has resided in for at least three months or traveled within the last three months to a country or U.S. Territory with active ZIKV transmission. The most up-to-date list of these locations can be found at http://www.cdc.gov/zika/geo/active-countries.html.

(Within the U.S., the list of communities with local active ZIKV transmission can be found at http://www.cdc.gov/zika/intheus/maps-zika-us.html.)

5.1.6.2 Sexual partner has resided in or traveled within the last six months to a country or U.S. Territory with active ZIKV transmission, or was diagnosed with ZIKV within the previous six months; or

5.1.6.3 Household member has been diagnosed with ZIKV infection or has traveled since the woman's LMP to a country or U.S. Territory with active ZIKV transmission.

5.1.7 HIV-infected women only: Laboratory evidence or clinical criteria for a confirmed case of HIV infection per CDC Surveillance Case Definition for HIV Infection, 2014 (Section 1.11 or Section 1.1.2) http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6303a1.htm.

5.2 Maternal Exclusion Criteria

5.2.1 Incarcerated or placed in detention.

5.2.2 Enrolled in other clinical research (including other ZIKV research) requiring blood collection, which in combination with HIV ZIP evaluations would exceed a total blood draw volume of 50 ml every 8 weeks and/or blood collection would be required more frequently than 2 times per week.

5.3 Infant Inclusion Criteria

5.3.1 Born to an enrolled mother.

5.3.2 Parent(s)/legal guardian(s) provided written IC for his or her child to participate. *(Written IC for participation of the infant(s) born from this pregnancy may be obtained at the same time maternal consent is obtained or any time during the pregnancy and confirmed at delivery per local requirements.)*

5.4 Infant Exclusion Criteria

5.4.1 Enrolled in other clinical research (including other ZIKV research) requiring blood collection, which in combination with HIV ZIP evaluations, would exceed 3 ml per kg in an 8-week period and/or blood collection would be required more frequently than 2 times per week.

5.5 Participant Recruitment

Participants will be recruited at implementing sites on an ongoing basis, whether from within their existing patient population or outside referrals. However, the protocol team will monitor enrollment of the HIV-negative maternal cohort, so as not to exceed the maximum goal of 50 participants during Phase I. A research staff member will contact potential participants (e.g., during a routine prenatal visit) and provide an overview of the study, including objectives, design and procedures.

Each potential participant who has her medical records reviewed to assess potential eligibility, and is approached for recruitment and/or consented for study participation will be assigned a Screening Identification (SID) number. Any links between individuals' names and their SID numbers will be maintained in a locked cabinet accessible to study staff only. When study accrual ends, site staff will obliterate all linked SID numbers belonging to individuals who did not consent to participate in the study.

The following information will be recorded on an *HIV ZIP Screening Form*, which will be maintained in a secure area at the clinical research site and accessible only to study staff: Age, race, ethnicity, pregnancy status, LMP, HIV status, ZIKV-exposure risk and other non-personally identifying information (PII), and inclusion criteria that do not require study procedures to be performed. Individuals assessed as ineligible for enrollment will have the reasons for ineligibility recorded. Individuals who are approached, but do not consent to participate, will be asked if they are willing to supply their reason(s) for declining participation; responses will be recorded. Information collected on this form will be entered in the study database using the SID number. Names, dates of birth and other individually identifying information will not be recorded or entered into the study database.

The protocol team may request tabulated information on individuals who participated in the recruitment process, but did not provide IC and the reasons not consented, as well as those that provided IC, but were deemed ineligible. These data will provide general information on the population that is recruited at the participating sites into the study.

5.6 Informed Consent

Once it is determined that an individual may qualify for the protocol, study details will be discussed and all questions answered during the IC process. Written IC from the individual (or assent with parent(s)/legal guardian(s) permission, if applicable)

will be obtained before any study-related procedures or activities are performed (see attached separate document Appendix IV). Consent must be obtained prior to performing any study screening procedures.

IC will consist of a description of the study's purpose, study visits and procedures and evaluations to be performed, information that will be collected from their and their child's medical records, possible risks and benefits and the voluntary nature of participating. Individuals who express interest in the study will be required to provide written IC (or assent with parent(s)/legal guardian(s) permission, if applicable) before any study-related procedures are performed. Eligibility criteria will be confirmed by study staff before enrolling into the study. A separate written IC will be obtained for the women and infant participants.

5.7 Co-enrollment Guidelines

HIV ZIP sites that are also implementing the PHACS SMARTT study are encouraged to discuss co-enrollment of the women and their newborn infants into SMARTT. Participants that also consent to enroll into the SMARTT study will be advised through the IC process that data between the two studies will be shared. For women co-enrolled in HIV ZIP and SMARTT, sites should query the SMARTT Protocol Team at enrollment for guidance on modifying the SMARTT blood draws so as not to exceed the maximum total allowed.

Enrollment into other studies is first at the discretion of the local Principal Investigator (PI). However, he or she must take into account any issues (such as blood volume and study burden) that enrollment in the additional study may cause, and which may compromise the site's ability to fulfill the requirements of HIV ZIP. The approval of the HIV ZIP Protocol Co-Chairs and the Protocol Team must be obtained prior to co-enrollment into other studies.

6.0 Study Evaluations

See Appendices I and II for the schedule of evaluations described below. Detailed guidelines for specimen collection, processing, storage and shipping can be found in the HIV ZIP MOP.

6.1 Maternal Evaluations

6.1.1 Baseline and Interim Medical and Medication History

Information will be obtained by chart review and interviewing participants. Participants will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, nervous system, blood, lymph glands, endocrine system, musculoskeletal system, skin and genital/reproductive tract. A history of any allergies, recent travel (of sex partner and household members), animal exposure, and

previous DENV, YFV or Chikungunya (CKNV) infections will be solicited. A recent history of symptoms consistent with ZIKV infections will also be solicited. A history of any prior infections with parvovirus, herpes and syphilis, among others, will be obtained. A vaccination history, as well as prescription and non-prescription medications, vitamins and herbal supplements taken since the woman's LMP and throughout her pregnancy will be documented.

6.1.2 Targeted Physical Examination

This examination will be conducted at each study visit beginning with the Entry visit, and will assess the woman's general appearance and include an assessment of signs and symptoms of acute ZIKV infection (fever, rash, arthalgia, myalgia, pruritus, headache, eye pain, conjunctivitis), as well as lymphadenopathy.

6.1.3 Pregnancy Questionnaire

Signs or symptoms of pregnancy complications will be elicited including decrease in fetal movement, rupture of membranes, vaginal bleeding, headaches, scotomata, nausea and vomiting.

6.1.4 Other Health Risk Factors

Questions on cigarette, alcohol and illicit drug use, as well as environmental risk factors, including, but not limited to, residence and occupation, will be administered.

6.1.5 Imaging Assessments

Fetal US will be performed (or data abstracted from clinically performed US) while the woman is pregnant. If US is performed more frequently for clinical management of the pregnancy, data from the US will also be recorded.

6.1.6 Laboratory Assessments

- Confirmation of pregnancy by βhCG measurement in blood or urine will be done if no prior testing was performed or pregnancy not was not already confirmed by fetal US.

- Assays to detect Abs or antigens (Ags) to TORCH will be run per local clinical laboratory procedures. The Rapid Plasma Reagin (RPR) tests will be performed on plasma in accordance with local clinical laboratory procedures. Because false positives may occur, additional testing may be required and conducted as part of the clinical care for the participant.

- Testing will be performed for the diagnosis of ZIKV in samples from urine and blood. The specific laboratories that will be conducting the ZIKV assays as well as instructions for processing, handling, shipping, testing methods and storage of samples are maintained in the HIV ZIP MOP.

- Assays for the detection of Ab (Enzyme-linked immunosorbent assay (ELISA)) may be performed for DENV and in some cases for CKNV or WNV.

- HIV-infected women will have all HIV VLs and T-cells performed since just prior to their LMP and throughout enrollment abstracted from medical records. HIV VL and T-cells will be done if no results from within the 12 weeks prior to a study visit are available. HIV VL testing will be performed if the woman has ZIKV-like symptoms, regardless of the availability of prior results in her medical record.

NOTE: See the HIV ZIP MOP for the study assay details and algorithms, as well as the Laboratory Processing Chart (LPC) for specific laboratory methods for each assay and instructions for handling and storage of samples.

6.1.6.1 Detection of Anti-ZIKV Antibodies

- Starting from the Entry visit, blood will be collected at each visit, to detect serum anti-ZIKV IgM Abs using standardized protocol and reagents for ZIKV. Anti-ZIKV IgM Abs will be detected initially using laboratory tests such as the CDC qualified assay (ZIKV IgM Ab capture ELISA (MAC-ELISA)). As more specific/sensitive assays are developed, those assays may be substituted (the HIV ZIP MOP will be updated).

6.1.6.2 Detection of ZIKV RNA

- If serum tests positive for anti-ZIKV IgM Abs at any study visit, RT-PCR (or other possible later developed ZIKV RNA detection test) to test for ZIKV RNA will be performed on serum and urine from that same collection date, as well as from the most recent prior study visit, if available. If serum tests positive for anti-ZIKV IgM Abs and ZIKV RNA detection test is negative and the woman resides in or she/her sex partner has a recent travel exposure history to a DENV-endemic area, testing for anti-DENV IgM Abs (and possibly other flaviviruses) will be performed (serum) on the current visit's samples, if available.

- Specimens collected from women presenting with ZIKV-like symptoms at any visit will be processed immediately and have anti-ZIKV IgM Abs (serum), anti-DENV IgM Abs (serum) and RNA detection for ZIKV (serum and urine) performed at the same time. If anti-ZIKV IgM Abs or ZIKV RNA test positive, ZIKV RNA detection testing will be performed (serum and urine) on the most recent prior visit (if available).

- All sites will use a standardized protocol for RT-PCR testing (or other possible later developed ZIKV RNA detection test). Any remaining blood (serum) and urine samples will be temporarily stored for possible future testing pending serology results.

6.1.6.3 Biorepository Specimens (if consented for future use)

- At each study visit, plasma will be prepared from collected blood and additional urine will also be collected; both will be stored in the NICHD Biorepository to be used in future assays for assessment of the immune response to ZIKV as assays are developed. (Allowable future use will be documented in the consent for each site.)

- At the time of delivery, cord blood and placental tissue will be collected for storage at the NICHD Biorepository for possible future ZIKV testing, when convenient.

6.1.6.4 Specimen Collection, Preparation, Handling and Shipping

- Blood and urine samples that are not being tested locally will be received, processed, aliquoted and shipped for testing and temporary storage to the site's assigned central laboratory according to standard laboratory procedures outlined in the HIV ZIP MOP.

- Cord blood samples, as well as placental tissue, collected at the time of delivery for possible future testing will be received, processed, aliquoted (if applicable) and shipped to the NICHD Biorepository for temporary storage as instructed in the HIV ZIP MOP. If newborn peripheral blood is unobtainable, cord blood will be prepared and shipped to the site's assigned central laboratory for real-time ZIKV testing, with remnant cord blood shipped for storage to the NICHD Biorepository.

6.2 Infant Evaluations

6.2.1 Baseline Demographics and Birth Information

Data collected will include basic demographics and birth history (e.g., date of birth, sex, race, ethnicity) and delivery characteristics (e.g., GA, type of delivery, complications at delivery).

6.2.2 Physical Examination

The examination will consist of temperature, cardiac, respiratory, organ systems (hepatomegaly, splenomegaly), skin (petechial, rash, skin scarring), gastrointestinal (feeding, emesis, diarrhea) and neurologic examinations (seizures, apnea, reflexes/tone, asymmetry, eye opening, basic movements). An examination does not need to be performed if required data from an examination performed within four weeks prior to the study visit can be abstracted from medical records.

6.2.3 Growth Parameters

Growth parameters (weight, length/height and head circumference) will be recorded at birth and at each study visit. Growth parameters are not required at study visits if

they were obtained within four weeks prior to the study visit and the required data can be abstracted from medical records. A head circumference must be included in the examination.

6.2.4 Hearing and Ophthalmologic Assessments

Hearing assessments (OAE) or AABR, general eye exams and ophthalmologic exams to detect chorioretinitis or abnormalities in the conjunctiva, lens or retina will be conducted. If initial exams are abnormal, referrals for additional audiology and/or ophthalmology testing will be done and results collected (if available) from any tests done for clinical reasons.

6.2.5 Imaging Assessments

Results of any imaging assessments performed for clinical care will be recorded. These include any neuroimaging studies (US, CT or MRI) obtained for clinical reasons to detect neurologic abnormalities including intracranial calcifications. A head US will be performed at the 3 Month visit on ZIKV-exposed infants only if none was performed by the age of 3 months. In addition, data from non-neurologic imaging will also be recorded (including, but not limited to, chest X-rays, abdominal X-rays, abdominal CT scans or MRIs).

6.2.6 Neurodevelopmental Assessment

Assessment of general motor development markers will be performed. Neurodevelopmental (ND) status of study participants will be assessed using normal developmental milestones tests as per standard of care. ND may also be assessed by administering the ASQ and the BSID, if clinically indicated and trained personnel are available.

6.2.7 Laboratory Assessments

- Individual assays to detect Abs/Ags to TORCH and an RPR for syphilis at birth, if indicated, due to fetal and/or neonatal findings or maternal history (see the HIV ZIP MOP for a table on general guidelines for TORCH and syphilis testing for asymptomatic newborn infants according to maternal history) will be performed in accordance with local clinical laboratory procedures. Because false positives may occur, additional testing may be required and conducted as part of the clinical care for the participant.

- Urine and peripheral blood will be obtained to assess for ZIKV at each study visit. (At the delivery visit, cord blood may be used immediately to assess for ZIKV if newborn peripheral blood is unobtainable, with remnant cord blood shipped for storage to the NICHD Biorepository (see Section 6.1.7.4)). The specific laboratories that will be conducting the ZIKV assays as well as instructions for processing, handling, shipping, testing methods and storage of samples are

maintained in the HIV ZIP MOP, as well as the LPC for specific laboratory methods for each assay and instructions for handling and storage of samples.

- Assays for the detection of Ab (ELISA may be performed for DENV, and in some cases, for CKNV and WNV) will be performed.
- HIV-related testing results will be abstracted from medical records.

6.2.8 Biorepository Specimens (if consented for future use)

- Urine for possible future testing will be stored.
- Remnant CSF, if collected at any time for clinical care, will be stored.

7.0 Study Schedule and Procedures

7.1 Maternal Study Visits

See Appendix I for the schedule of evaluations. Detailed guidelines for specimen collection, processing, storage and shipping can be found in the HIV ZIP MOP.

Medical and medication history will be collected via medical record abstraction and participant self-report.

7.1.1 Screening Visit

Study staff will review eligibility criteria and inform individuals about study objectives, design and procedures. Limited, non-PII will be recorded on the *HIV ZIP Screening Form* as described in Section 5.5. If deemed potentially eligible, individuals will be interviewed to evaluate their willingness to participate in the study. If interested, study staff will obtain written IC (or assent with parent(s)/legal guardian(s) permission) and will assign a unique Participant Identification (PID) number prior to performing any study-related procedures or evaluations. After written consent or assent with parent(s)/legal guardian(s) permission is obtained, the following will be completed:

- Pregnancy confirmation by serum or urine βhCG or fetal US with fetal heart tones, if not already performed for clinical care or unable to access the results.
- Date of birth.
- If acute ZIKV-like symptoms are present and GA is ≥ 18 weeks, collect the following biological samples:
  - Blood for ZIKV RNA detection and ZIKV serology.
  - Urine for ZIKV RNA detection.

- Blood for plasma for future studies (if consented for future use).
- Urine for possible later testing (if consented for future use).

7.1.2 Entry Visit

For the purposes of this study, enrollment will occur on the same day as, but prior to conducting the Entry visit. Verify eligibility criteria prior to study enrollment.

- Women who present with acute ZIKV-like symptoms at the Screening visit and are 18 weeks or greater GA must have a positive ZIKV RNA test to be enrolled, if otherwise eligible.
- Women who present with acute ZIKV-like symptoms at the Screening visit and are less than 18 weeks GA will be enrolled and have their Entry visit conducted on the same day as the Screening visit, if otherwise eligible.
- Women less than 18 weeks GA who do not present with acute ZIKV-like symptoms at the Screening visit, ideally should be enrolled and have their Entry visit conducted on the same day as the Screening visit, but they may complete the Entry visit evaluations at any time prior to 18 weeks GA.
- The following will be completed at the Entry visit:
  - Fetal US with fetal heart tones only if not already done for the Screening visit or clinical care.
  - Medical and medication history.
  - Pregnancy questionnaire.
  - Other risk factors questionnaire.
  - Obtain results from clinical laboratory tests conducted for clinical care.
  - HIV-infected participants: Abstraction of all HIV VLs and T-cells starting from the closest, but prior to, the woman's LMP.
  - Height, weight and VS (including blood pressure and temperature).
  - Targeted PE.
- The following biological samples will be collected:
  - Blood for TORCH testing and syphilis (RPR) for clinical care and send to the local laboratory. (*Do not repeat any tests already performed for routine prenatal care*.)

- Blood for ZIKV RNA detection. (*Do not collect if already collected at the Screening visit (i.e., ≥ 18 weeks GA and acute ZIKV-like symptoms)).*
- Blood for ZIKV serology.
- Blood for DENV serology and other possible serology as needed (CKNV or WNV) if resides in or recent travel history to DENV-endemic region. *(Do not collect if already collected at the Screening visit (i.e., ≥ 18 weeks GA and acute ZIKV-like symptoms))*.
- Blood for plasma for future studies (if consented for future use). *(Do not collect if already collected at the Screening visit (i.e., ≥ 18 weeks GA and acute ZIKV-like symptoms))*.
- HIV-infected women only: Blood for HIV VL and T-cells only if not collected within the prior 12 weeks. *(HIV VL testing is required if ZIKV-like symptoms are present, regardless of testing history.)*
- Urine for ZIKV RNA detection. (*Do not collect if already collected at the Screening visit (i.e., ≥ 18 weeks GA and acute ZIKV-like symptoms))*.
- Urine for possible later testing (if consented for future use). *(Do not collect if already collected at the Screening visit (i.e., ≥ 18 weeks GA and acute ZIKV-like symptoms))*.

- Educate about ZIKV prevention and the signs and symptoms of acute ZIKV infection and instruct to notify the clinic immediately if experiencing these symptoms (or if sex partner or household member is experiencing these symptoms).
- Provide diary and instruct on how to record ZIKV-like symptoms between visits.

7.1.3 Second and Third Trimester Visits

- The following will be completed at the Second and Third Trimester visits, which should be conducted at 24 and 34 weeks GA (+/- 2 weeks), respectively:
  - Conduct or obtain results from fetal US performed since prior study visit.
  - Zika-symptom diary review.
  - Interim medical and medication history.
  - Interim pregnancy questionnaire.
  - Other risk factors questionnaire.
  - Obtain results from clinical laboratory tests conducted for clinical care.

- HIV-infected participants: Abstraction of all interim HIV VLs and T-cells results.
- Weight and VS (including blood pressure and temperature).
- Targeted PE.

- The following biological samples will be collected:

NOTE: If a previously ZIKV RNA negative participant has ZIKV-like symptoms at a scheduled study visit, follow the ZIKV/DENV laboratory testing algorithm outlined in Section 7.1.6, Appendix I and the HIV ZIP MOP.

- Third trimester only (Do not repeat previously positive tests): Blood for TORCH testing and syphilis (RPR) for clinical care and send to local laboratory.
- Blood for ZIKV RNA detection.
- Blood for ZIKV serology.
- Blood for DENV serology and other possible serology as needed (CKNV or WNV) if resides in or recent travel history to DENV-endemic region. (*Do not collect if previously tested positive*.)
- Blood for plasma for future studies (if consented for future use).
- HIV-infected women only: Blood for HIV VL and T-cells only if not collected within the prior 12 weeks. (*HIV VL testing is required if ZIKV-like symptoms present, regardless of testing history*.)
- Urine for ZIKV RNA detection.
- Urine for possible later testing (if consented for future use).

- Re-educate about ZIKV prevention and the signs and symptoms of acute ZIKV infection and instruct to notify the clinic immediately if experiencing these symptoms (or if sex partner or household member is experiencing these symptoms).

- Provide reminder to bring Zika-symptom diary to the next study visit (or at time of delivery).

7.1.4 Delivery Visit (Live Birth or Fetal Loss)

- The study team obstetrician and nurse will perform interviews and PEs and request laboratory testing as part of standard of care.

- The following will be completed within 48 hours after delivery:
  - Obtain results from fetal USs performed since the prior study visit.
  - Zika-symptom diary review.
  - Interim medical and medication history.
  - Interim pregnancy/delivery questionnaire.
  - Other risk factors questionnaire.
  - Obtain results from clinical laboratory tests conducted for clinical care.
  - HIV-infected participants: Abstraction of all interim HIV VLs and T-cells results.
  - VS (including blood pressure and temperature).
  - Targeted PE.
- The following biological samples will be collected within 48 hours of delivery:

NOTE: If a previously ZIKV RNA negative participant has ZIKV-like symptoms at a scheduled study visit, follow the ZIKV/DENV laboratory testing algorithm outlined in Section 7.1.6, Appendix I and the HIV ZIP MOP.

  - Blood for ZIKV RNA detection.
  - Blood for ZIKV serology.
  - Blood for DENV serology and other possible serology as needed (CKNV or WNV) if resides in or recent travel history to DENV-endemic region. (*Do not collect if previously tested positive.*)
  - Blood for plasma for future studies (if consented for future use).
  - HIV-infected women only: Blood for HIV VL and T-cells only if not collected within the prior 12 weeks. (*HIV VL testing is required if ZIKV-like symptoms present, regardless of testing history. See Section 7.1.6.*)
  - Urine for ZIKV RNA detection.
  - Urine for possible later testing (if consented for future use).
  - Cord blood for possible future ZIKV testing (if consented for future use). (*Some cord blood will be used for real-time testing if infant peripheral blood is unobtainable.*)

- Placental tissue for possible future analysis (if consented for future use).

- Re-educate about ZIKV prevention and the signs and symptoms of acute ZIKV infection and instruct to notify the clinic immediately if experiencing these symptoms (or if sex partner or household member is experiencing these symptoms).

- Provide reminder to bring Zika-symptom diary to the next study visit.

7.1.5 Six Weeks Postpartum Visit (Post Live Birth or Fetal Loss)

- The following will be completed at the Six Weeks Postpartum visit:
  - Zika-symptom diary review.
  - Interim medical and medication history.
  - Other risk factors questionnaire.
  - Obtain results from clinical laboratory tests conducted for clinical care.
  - HIV-infected participants: Abstraction of all interim HIV VLs and T-cells results.
  - Weight and VS (including blood pressure and temperature).
  - Targeted PE.

- The following biological samples will be collected:
  - Blood for ZIKV RNA detection.
  - Blood for ZIKV serology.
  - Blood for DENV serology and other possible serology as needed (CKNV or WNV) if resides in or recent travel history to DENV-endemic region. (*Do not collect if previously tested positive.*)
  - Blood for plasma for future studies (if consented for future use).
  - HIV-infected women only: Blood for HIV VL and T-cells only if not collected within the prior 12 weeks. (*HIV VL testing is required if ZIKV-like symptoms present, regardless of testing history. See Section 7.1.6.)*
  - Urine for ZIKV RNA detection.
  - Urine for possible later testing (if consented for future use).

7.1.6 Women Experiencing Symptoms Consistent with Acute ZIKV-like Illness at Any Time during Study Participation

- If the study staff become aware of a symptomatic participant between study visits, schedule an "unplanned symptomatic" visit and conduct the following:
  - Abstract results from fetal US performed according to country and local clinical guidelines since the prior study visit.
  - Zika-symptom diary review.
  - Interim pregnancy questionnaire.
  - Continental U.S. sites only: Other risk factors questionnaire.
  - Obtain results from clinical laboratory tests conducted for clinical care.
  - HIV-infected participants: Abstraction of all interim HIV VLs and T-cells results.
  - Weight and VS (including blood pressure and temperature).
  - Targeted PE.
- The following biological samples will be collected:
  - Blood for ZIKV RNA detection.
  - Blood for ZIKV serology.
  - Blood for DENV serology and other possible serology as needed (CKNV or WNV) if resides in or recent travel history to DENV-endemic region. (*Do not collect if previously tested positive*.)
  - Blood for plasma for future studies (if consented for future use).
  - HIV-infected participants: Blood for HIV VL.
  - Urine for ZIKV RNA detection.
  - Urine for possible later testing (if consented for future use).
- Provide reminder to bring Zika-symptom diary to the next study visit (or at time of delivery, if applicable).

7.1.7 Women with Positive ZIKV Serology at Any Study Visit, But without Clinical Signs/Symptoms of ZIKV-like Illness

- An additional study visit will not be scheduled and additional biological samples will not be collected.
- Available biological samples will be tested as follows:
  - Serum and urine from current visit for ZIKV RNA detection.
  - Serum and urine from most recent prior visit for ZIKV RNA detection, if applicable.
  - If ZIKV RNA test is negative only: Serum for DENV serology and other possible serology as needed (CKNV or WNV) if resides in or recent travel history to DENV-endemic region.
- In addition, future specimens collected may be tested for the presence of ZIKV.

7.2 Infant Study Visits

See Appendix II for the schedule of evaluations. Detailed guidelines for specimen collection, processing, storage and shipping can be found in the HIV ZIP MOP.

7.2.1 Birth Visit

- The following will be completed within 48 hours after birth:
  - Confirm/review consent and study visit requirements for the infant with parent(s)/legal guardian(s) prior to conducting any infant procedures.
  - Collect demographic and birth data.
  - Height, weight, head circumference and temperature.
  - General PE or collect data from exam conducted as part of clinical care.
  - Neurological examination (seizures, apnea, reflexes/tone, asymmetry, eye opening, basic movement).
  - Hearing assessment (*if abnormal refer to audiology for a full audiological examination and obtain results from any tests done for clinical reasons*).
  - Ophthalmologic evaluation (*if abnormal refer for additional testing and obtain results from any tests done for clinical reasons*).
  - Obtain results from all neuroimaging assessments done for clinical reasons (head US, head CT and MRI scan).

- Record results from laboratory tests conducted for clinical care, including TORCH testing and HIV-related laboratory testing results and treatments, if applicable.

- The following biological samples will be collected:

NOTE: Peripheral blood will be collected from the infant, but if unobtainable, some of the cord blood will be used for real-time and reflex testing.

- Blood and/or urine for TORCH testing and syphilis (RPR) to local laboratory, if clinically indicated and according to local practice (see the HIV ZIP MOP for a table on general guidelines for TORCH and syphilis testing for asymptomatic newborn infants according to maternal history).

- Blood for ZIKV serology and RNA detection.

- Blood for DENV serology and other possible serology as needed (CKNV or WNV).

- Urine for ZIKV RNA detection.

- Urine for possible later testing (if consented for future use).

- Remnant CSF samples for possible future testing, if obtained for clinical care (if consented for future use).

7.2.2 Months 3, 6 and 12 Visits

- The following will be completed at 12, 24 and 52 weeks after birth (+/- 2 weeks):

  - Height, weight, head circumference and temperature.

  - General PE or collect data from exam conducted as part of clinical care if performed within the previous four weeks.

  - Neurological examination (seizures, apnea, reflexes/tone, asymmetry, eye opening, basic movement).

  - Hearing assessment (if abnormal refer to audiology for a full audiological examination and obtain results from any tests done for clinical reasons).

  - Ophthalmologic evaluation (if abnormal refer for additional testing and obtain results from any tests done for clinical reasons).

  - Obtain results from all neuroimaging assessments done for clinical reasons (head US, head CT and MRI scan).

- 3 Month visit only: Perform head US only if Zika-exposed and no head US was performed by 3 months of age.
- Record results from laboratory tests conducted for clinical care, including TORCH testing and HIV-related laboratory testing results and treatments, if applicable.

- The following biological samples will be collected:
  - Blood for ZIKV serology and RNA detection.
  - Blood for DENV serology and other possible serology as needed (CKNV or WNV).
  - Urine for ZIKV RNA detection.
  - Urine for possible later testing (if consented for future use).
  - Remnant CSF samples for possible future testing, if obtained for clinical care (if consented for future use).

8.0 Specimen Tracking and Storage

8.1 Specimen Tracking

Laboratory specimens processed and shipped to the designated central laboratories for testing or to the NICHD Biorepository for storage will not contain names, addresses, medical record numbers or any other PII that may be linked to participants. Instead, a unique identification number will be used and the types of specimens collected will be recorded on CRFs and the information entered into the study database.

8.2 Biorepository Storage

HIV ZIP will also store specimens in the NICHD Biorepository for future studies that are currently undetermined.

Biorepository samples will be processed immediately after collection, labeled, stored at the sites in a standardized manner, tracked utilizing the Laboratory Data Management System (LDMS) and shipped to the Biorepository on a scheduled basis. The LDMS will have the capability of identifying the precise location of every Biorepository specimen at all times. Refer to the HIV ZIP LPC for specific storage and shipping instructions and schedules.

9.0 Participant and Study Management

9.1 Tracking Participants / Follow-up

After consent (assent with parent(s)/legal guardian(s) permission) is obtained, participants will be asked to provide several means to contact them (e.g., home and cell phone numbers, mailing address, email, text messaging) including family members and friends, if possible and willing to do so. Participants will be asked whether or not messages can be left for each of the phone numbers provided and if messages can contain information regarding the nature of the project.

Participants will be contacted by study staff via their preferred methods of contact and reminded of follow-up visit dates and times. Site staff should review contact information at each study visit with participants to confirm or update information as necessary.

9.2 Retention

Participant retention is considered a high priority. Study-wide targets for retention in HIV ZIP will be 95% of pregnant women through delivery and 95% of infants through 12 months of age, excluding unavoidable causes of loss (e.g., a move out of the area or death). The targets will be periodically reevaluated based on ongoing experience and efforts made to enhance retention. For those participants who move to a new location that has an HIV ZIP site, the originating and receiving site personnel will make every effort to encourage continued participation at the new site. Sites will be required to submit and have approved a detailed retention plan as part of their site implementation plan.

9.3 Study Visit Management

Study visits will be scheduled and conducted as outlined in the schedule of evaluations (see Section 7.0 and Appendices I and II). If a visit cannot be conducted within the assigned study visit window, site staff should query the HIV ZIP Protocol Team using the HIV ZIP QNS, which is accessible through the HIV ZIP link on the NICHD Clinical Studies website (www.nichdclinicalstudies.org), requesting permission to conduct the visit outside the designated study visit window.

9.4 Premature Study Discontinuation

The HIV ZIP Protocol Team will monitor the rate and reason for discontinuing follow-up. Participants will be discontinued from the study if any of the following occurs:

- The participant or parent(s)/legal guardian(s) withdraws consent;
- The participant or parent(s)/legal guardian(s) fails to comply with the study requirements so as to cause harm to the participant or seriously interfere with the

validity of the study results *and* the site investigator believes that compliance is unlikely to improve;

- The site investigator determines that further participation would be detrimental to the participant's health or well-being;
- The study is stopped by a government agency, including the National Institutes of Health (NIH) or U.S. Department of Health and Human Services (HHS);
- The study must be stopped for administrative reasons; or
- The clinical site is terminated for participant safety, study integrity or poor performance reasons.

9.5 Death of a Participant

Sites will make their best attempt to obtain a copy of the autopsy report or death certificate and medical records on any participant who dies.

10.0 Data Collection and Management

10.1 Development of Protocol and Case Report Forms

Westat, in collaboration with the HIV ZIP Protocol Team, is responsible for the development of this protocol as well as the CRFs needed to collect the information required to implement this protocol. All CRFs for this study will be available for download from the HIV ZIP link on the NICHD Clinical Studies website (www.nichdclinicalstudies.org); hard copies of CRFs may be ordered from that website as well.

10.2 Data Records

Participant-related study information will be identified through the PID on all CRFs. Participant names or other PII will not be used on any study documents. A log that links the names of participants to their PID will also be kept under double locks, separate from all other research records. Clinical research records are stored in a manner that ensures privacy, confidentiality, security and limited accessibility when the clinical research is being conducted and after the research is completed. Records can be maintained in hardcopy, electronic or other media form. It is permissible to transfer these documents from paper records to electronic formats and to archive this information on available media.

Data collection is the responsibility of the research staff at the sites under the supervision of the site investigators. During the study, the site investigators must maintain complete and accurate documentation for the study data. Data elements transcribed onto CRFs must have corresponding paper or electronic source documentation to substantiate all submitted data (e.g., an original or certified copy of

a laboratory report, instrument printout, progress notes of the physician, the study participant's hospital chart(s), and nurses' notes). Research site staff should refer to the NIAID Requirements for Source Documentation in the Division of Acquired Immunodeficiency Syndrome (DAIDS) Funded and/or Sponsored Clinical Trials (see the HIV ZIP MOP).

10.3 Data Submission

Data obtained in the HIV ZIP study will be entered and managed in a 21 Code of Federal Regulations (CFR) Part 11-compliant, secure encrypted remote data capture (RDC) system called REDCap Cloud. Access to the REDCap Cloud system is limited to approved individuals; the PIs and Westat will set the level of access for individuals to the database.

Study site staff must follow the guidelines for CRF completion and data entry that are specified in the HIV ZIP MOP. Once the study database is developed, the data screens are available for data entry and protocol training has been completed, study staff at the sites will be responsible for ensuring that CRF data are entered into the study database within the timeframes specified in the HIV ZIP MOP.

10.4 Data Quality Assurance

The site investigators are responsible for ensuring the accuracy, completeness, legibility and timeliness of the data reported. All source documents and laboratory reports must be reviewed by the site's study team, who will ensure that they are accurate and complete. The investigator or designee must review unanticipated problems. Data reported on the CRF that are derived from source documents or chart review should be consistent with the source documents or the discrepancies should be explained in the participant's research record. All CRFs must be reviewed by the research staff for accuracy, clarity, and completion, and by the data entry staff for completion. Data edits through range checks and field inconsistencies will be built into the REDCap Cloud database to enable real-time correction of data entry and/or CRF completion errors.

Investigators receiving federal funding must adhere to the CFR to protect research participants and produce reliable study information. The PI at each study site is responsible for the overall conduct of research activities at the site. Clinical sites participating in research sponsored by NICHD need to have an internal quality assurance plan that will identify problems and correct errors in research study records.

10.5 Clinical Site Monitoring and Record Availability

Site monitors from Westat will visit participating clinical research sites to review select study documents (e.g., consent forms, CRFs), pertinent hospital and clinic records, as applicable, and ensure that regulatory requirements are being followed.

The site investigator will make study documents readily available for inspection by the local IRB/IEC, site monitors, NIH, the Office for Human Research Protections (OHRP) and the site monitors acting on behalf of NICHD to confirm the study data and regulatory compliance.

NOTE: Participating sites are responsible for specifying these individuals and the HIV ZIP investigators as recipients of private health information in the individual's authorization required under the Health Insurance Portability and Accountability Act (HIPAA).

10.6 Retention of Clinical Research Records

Records relating to research and IRB/IEC records are required by the HHS regulations 45 CFR Part 46.115(b) to be retained for at least three years after completion of the research. Participating sites must follow their local institution's policy on record retention if it is for a longer period.

10.7 Protocol Query Management

For the integrity of the study and the welfare of the participants, it is important for the site staff to have immediate access to the research team. Site staff will send all queries for this protocol to the HIV ZIP Protocol Team using the HIV ZIP QNS accessible via the HIV ZIP link on the NICHD Clinical Studies website (www.nichdclinicalstudies.org). It is expected that the Protocol Co-Chairs or designees will respond to queries within two business days of team receipt. Queries and replies will automatically be archived by the HIV ZIP webmaster. Those queries deemed relevant to all sites will be posted on the HIV ZIP website, where they will be available to all sites for future reference. The categories of queries include IRB/Regulatory, Laboratory/Procedures, General Protocol Issues, Participant Management/Eligibility/Deviations and Unanticipated Problems Notification.

10.8 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice (GCP) or HIV ZIP MOP requirements. The noncompliance may be on the part of the participant, the investigator or study staff. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

These practices are consistent with investigator and Sponsor obligations in ICH E6 (R1):

- Compliance with Protocol, Sections 4.5.1, 4.5.2, 4.5.3 and 4.5.4.
- Quality Assurance and Quality Control, Section 5.1.1.
- Noncompliance, Sections 5.20.1 and 5.20.2.

Deviations from the protocol must be addressed in the participant's source documents and those that compromise participant safety must be promptly reported to the HIV ZIP Protocol Team via the HIV ZIP QNS. Deviations should be reported to the local IRB/IEC, according to their requirements.

10.9 Collaboration with Outside Studies

Periodically, it will be useful for the HIV ZIP protocol team to collaborate with outside investigators. For example, data from the ZIP study, conducted in Zika-endemic areas, may be merged with HIV ZIP. In most cases, ZIP will have the exact same infant assessments as HIV ZIP. A memorandum of understanding (MOU) about the extent and nature of the sharing as well as a data use agreement will be executed for all collaborations. The MOU will include an understanding of the control of the use of the data, publication rights and authorship rights, as well as address the human participant's confidentiality issues.

11.0 Monitoring Unanticipated Problems

Since this is an observational, and not a treatment or interventional study, AE reporting of abnormal medical or laboratory observations at the individual participant level are not required.

Safety monitoring for this study will focus on unanticipated problems involving (1) risks to participants; (2) risks to study site staff; and (3) risks to the community. Some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with AEs. In other cases, unanticipated problems place participants or others at increased *risk* of harm, but no harm occurs. Women enrolled in HIV ZIP may develop known conditions associated with pregnancy that require intervention. They may also develop untoward effects possibly associated with ZIKV infection. Infants enrolled in HIV ZIP may develop common pediatric conditions requiring treatment during the course of the study period. They may also experience AEs possibly associated with ZIKV exposure. Although these events will be documented and entered in the study database, they will not be categorized as unanticipated problems.

Reporting of unanticipated problems negatively affecting study participants, staff or the community will facilitate the reexamination of study procedures and allow

changes as necessary to address concerns about participant management, adequacy of training or the need to modify procedures.

11.1 Unanticipated Problems Definition

The OHRP considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB/IEC-approved research protocol and IC document; and (b) the characteristics of the patient population being studied;

- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in the research); and

- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic or social harm) than was previously known or recognized.

11.2 Unanticipated Problems Categories

11.2.1 Study Participants

First, the study will catalogue any unanticipated problem experienced by the maternal or child participant that may be attributable to participation in or the conduct of the study. Reporting is required for occurrences, including social harms, psychological distress and serious life threatening events, such as suicide attempts. These may be immediately apparent to the study staff, such as the participant's emotional upset requiring referral for counseling; or they may be delayed and reported later to study staff, such as physical harm to an individual for having participated in the study.

11.2.2 Site Staff

Second, study staff may encounter unanticipated problems during the study when working with the study participants, significant others or extended family members that may personally affect them (e.g., visible distress or injury to a staff member resulting from the research encounter). Notification to the HIV ZIP Protocol Team will allow the problem to be immediately addressed and evaluated, and for guidance to be modified or expanded to minimize similar risk to other staff.

11.2.3 The Community

Third, a critically important area that will be evaluated by the HIV ZIP Protocol Team is the impact of the study on the community. Community-associated unanticipated problems are events that affect the site, site milieu or the performance

of the study. This may include events that affect the medical center environment or the perception of the study in the community (e.g., the study is portrayed negatively in the media or a community forum). Reporting of these events will facilitate understanding of the impact of the study on the community and provide the opportunity to address community-level concerns and to intervene in a timely manner to correct misinformation or perceptions of practices that may cause community concern.

11.3 Reporting Requirements

Ultimately, the responsibility for reporting unanticipated problems belongs to the site PI who can delegate it to qualified research staff that directly interact with the participants. No unanticipated problem should be reported through the HIV ZIP QNS without the knowledge of the PI or his or her designee during the PI's absence.

Study staff will notify the protocol team of unanticipated problems affecting study participants, study staff and the community as soon as possible, but no later than 48 hours after awareness of the event using the HIV ZIP QNS accessible through the HIV ZIP link on the NICHD Clinical Studies website (www.nichdclinicalstudies.org). Study staff will also report the unanticipated problem on the *HIV ZIP Unanticipated Problems Form* for entry into the study database within three working days after awareness of the event. Lastly, per OHRP, study staff should report unanticipated problems to their local IRB/IEC within one week of awareness of the problem if serious; otherwise, within two weeks, and to OHRP within one month.

11.4 Grading of Impact

The grading system for unanticipated problems of study participants or staff will be as follows:

- Minimal Impact: managed at the time of the event with no consequences.
- Moderate Impact: managed by referral for supplemental care or counseling.
- Major Impact: needed immediate professional intervention with or without hospitalization.

Community events will not be graded but will be addressed as they occur.

NOTE: The distinguishing feature of moderate and major impact events is the need for enlisting additional support outside the research staff and the research encounter.

12.0 Data Analysis Plan

The following data analysis plan has been developed to address the study aims.

12.1 Aim 1: To Determine the Feasibility of Enrolling Pregnant Women with ZIKV/HIV Co-infection, HIV Infection Alone, ZIKV Infection Alone as well as Doubly Uninfected Women into a Prospective Cohort Study at Selected Sites in the Continental U.S., P.R. and Brazil

Feasibility in enrolling a total of 200 pregnant women/infant pairs within a year, with a target of 150 HIV-infected women across all sites, 50 HIV-uninfected women from continental U.S. sites only and a minimum of 20 who are co-infected with HIV and ZIKV will be assessed by monitoring total accrual, accrual by study sites and accrual into the four study groups described above, at bi-weekly or monthly intervals. Monitoring of accrual at consistent study intervals will allow for assessment of trends over time and forecasting of accrual over the remaining first year of enrollment.

If the feasibility phase proves successful, enrollment into Phase II will begin with the goal of enrolling up to 1,800 pregnant women/infant pairs at risk for ZIKV.

12.2 Aim 2: To Compare HIV Viral Suppression in HIV-Infected Women with and without ZIKV Co-infection during Pregnancy and the Time of Delivery

To address this aim, the study population will be restricted to HIV-infected women. Lack of HIV viral suppression (e.g., "unsuppressed VL") will be defined as having a VL > 1,000 copies/ml as this is the VL threshold identified as being associated with HIV transmission. HIV viral suppression will be assessed at four time points – start of first, second and third trimesters (using the closest VL measure to the start of each trimester) and at the time of delivery (closest VL measure prior to or at the time of delivery). The proportion with unsuppressed VL at these time points will be compared by presence of ZIKV co-infection at these same time points using Fisher's Exact tests. Logistic regression models will be used to estimate ORs and 95% CIs for lack of HIV viral suppression at these time points comparing HIV-infected pregnant women with ZIKV co-infection to HIV-infected pregnant women without ZIKV co-infection, adjusting for potential confounders. We hypothesize that HIV-infected pregnant women with ZIKV co-infection will have a higher risk of having unsuppressed VL during pregnancy and at the time of delivery compared to HIV-infected pregnant women without ZIKV infection. For this aim and all subsequent aims for Phase II, a p-value < 0.05 will connote statistical significance.

12.3 Aim 3: To Compare the Incidence of ZIKV Infection among Pregnant Women with HIV Infection and Those without HIV Infection

To address this aim, the study population will be restricted to women without prevalent ZIKV infection at the time of enrollment (i.e., those at risk for incident ZIKV infection during study follow-up). Cumulative incidence of ZIKV infection will be calculated as the number of new confirmed ZIKV infections identified by the

end of pregnancy divided by the total number of completed pregnancies, overall and by HIV status. The relative risk and 95% CI for ZIKV infection comparing HIV-infected women to HIV-uninfected women will be estimated using log-binomial regression, adjusting for potential confounders.

12.4 Aim 4: To Compare the Incidence of Adverse Pregnancy Outcomes between Women Co-infected with HIV and ZIKV, Women Infected with Either HIV or ZIKV Alone and Doubly Uninfected Women

The cumulative incidence of adverse pregnancy outcomes (i.e., miscarriage, stillbirth and preterm birth) will be calculated as the number of adverse outcomes identified by the end of pregnancy divided by the total number of completed pregnancies, overall and by HIV/ZIKV status. The relative risk and 95% CI for adverse pregnancy outcome comparing HIV and ZIKV co-infected, HIV-infected only and ZIKV-infected only women to doubly uninfected women will be estimated using log-binomial regression, adjusting for potential confounders. Potential confounders include demographic and socioeconomic status variables, smoking, alcoholic beverage consumption and substance use during pregnancy and exposure to specific environmental contaminants during pregnancy.

12.5 Aim 5: To Compare the Incidence of Vertical Transmission of HIV and ZIKV between Women Co-infected with HIV and ZIKV, and Women Infected with Either HIV or ZIKV Alone

To address this aim, the study population will be restricted to women who are infected with HIV, ZIKV or both. Among the doubly infected pregnant women, we will estimate the cumulative incidence of perinatal transmission of both HIV and ZIKV, HIV only and ZIKV only. Among HIV-infected only pregnant women, we will estimate the risk of perinatal HIV transmission. Among ZIKV-infected only pregnant women, we will estimate the risk of perinatal ZIKV transmission. We will compare the risk of perinatal HIV transmission among doubly infected women to women only infected with HIV using log-binomial regression, adjusting for potential confounders. Similarly, we will compare the risk of perinatal ZIKV transmission among doubly infected women to women only infected with ZIKV using log-binomial regression, adjusted for potential confounders.

12.6 Aim 6: To Compare the Incidence of Congenital Malformations and Other Adverse Outcomes (Including Microcephaly, Neonatal Death, CNS Malformations, Hydrops and Ocular Abnormalities) among Offspring of Women Co-infected with HIV and ZIKV, Women Infected with Either HIV or ZIKV Alone and Doubly Uninfected Women

The cumulative incidence of congenital malformations and other adverse outcomes among infants will be calculated overall and by perinatal HIV/ZIKV exposure status. The risk ratios and 95% CI for adverse infant outcomes comparing HIV and ZIKV co-infected, HIV-infected only and ZIKV-infected only women to doubly uninfected women will be estimated using log-binomial regression, adjusting for potential

confounders. Potential confounders include demographic and socioeconomic status variables, substance use during pregnancy and exposure to specific environmental contaminants during pregnancy.

12.7 Aim 7: To Compare Long-term Growth, Hearing, Vision and Neurodevelopment Outcomes in Children with In Utero Exposure to HIV and ZIKV, In Utero Exposure to Either HIV or ZIKV Alone and No In Utero Exposure to Either Virus

The incidence rate of long-term outcomes among infants will be calculated as the number of adverse outcomes identified over follow-up divided by the total person-time of follow-up, overall and by perinatal HIV/ZIKV exposure status. The incidence rate ratios and 95% CI for adverse infant outcomes comparing doubly exposed, HIV-exposed only and ZIKV-exposed only women to doubly uninfected women will be estimated using Poisson regression, adjusting for potential confounders. Potential confounders include demographic or socioeconomic status variables, substance use during pregnancy and exposure to specific environmental contaminants during pregnancy.

12.8 Missing, Unused and Spurious Data

Every effort will be made to ensure that the amount of missing data is kept at a minimum. The extent and pattern of missing data will be assessed. If data are missing for only a few cases, then data analysis will be conducted using the standard complete case analyses as outlined above. However, when such a strategy would result in loss of data from a substantial proportion of participants or lead to biased or inaccurate results, then multiple imputation or inverse probability weighting will be considered.

Unused or spurious data will be documented and discussed when disseminating results of this study.

12.9 Power and Sample Size

Although the key goal of Phase I is to assess feasibility of enrolling HIV-infected and HIV-uninfected women with or without ZIKV infection, it should be noted that the proposed sample size of 150 HIV-infected women would provide 80% power to detect a difference in percent with unsuppressed VL of 38.7% vs. 10% between HIV-infected women with ZIKV and HIV-infected women without ZIKV infection, assuming 10% of the HIV-infected women enrolled (and meeting ZIKV risk criteria) have ZIKV infection by delivery.

In the full Phase II study, we anticipate enrolling an additional 1800 pregnant women to bring total enrollment for this study up to 2000 women. This large enrollment allows precise estimates of event rates and sufficient power to compare subgroups defined on the basis of prenatal HIV/ZIKV exposure or other characteristics. We will first present estimates of precision for various event rates for the full cohort.

Assuming an enrollment of 500, 1,000, or 2,000 women, **Table 12-1** below summarizes estimates of precision of event rates for the development of ZIKV by the end of pregnancy, or for other adverse outcomes in their infants. For the target enrollment of 2,000 women, we will be able to estimate an overall event rate of 10% with a precision (i.e., 1.96* s.e.) of ± 1.4%. If 1,000 of these 2,000 women are HIV-infected, then we will have a precision of 1.8% for estimating the percent with ZIKV infection within this subgroup.

Table 12-1. Precision of event rate estimates for various levels of accrual, overall or within subgroups defined by HIV status

Accrual (overall or within HIV+ or HIV- subgroups)	**Percent with ZIKV infection by end of pregnancy, or other adverse outcome in infants**	
	Event rate	**Precision (half-width of 95% CI)**
500	5%	2.0%
	10%	2.8%
	20%	3.6%
1000	5%	1.4%
	10%	1.9%
	20%	2.5%
2000	5%	1.0%
	10%	1.3%
	20%	1.8%

For comparison of the percent with unsuppressed VL in **Aim 2**, our power is based only on the HIV-infected women enrolled. Assuming that a range of 40% to 60% of the total enrollment is HIV-infected, this translates to 200 to 1,200 women with HIV infection for corresponding total enrollments of 500 to 2,000 participants. **Table 12-2** below summarizes the minimum detectable differences in proportions with suppressed VL by the end of pregnancy (and corresponding ORs) between HIV-infected ZIKV-exposed as compared to HIV-infected ZIKV-unexposed women.

Table 12-2. Minimum detectable ORs (and percent with unsuppressed VL among ZIKV-infected) at 80% power comparing HIV+/ZIKV+ vs. HIV+/ZIKV- women

Assumed percent with unsuppressed VL in ZIKV-uninfected	Percent ZIKV-infected (among HIV-infected enrollees)	Minimum detectable ORs (percent with unsuppressed VL in ZIKV+) for sample sizes:		
		N=200	N=500	N=1200
10%	20%	3.54 (28.2%)	2.36 (20.8%)	1.80 (16.7%)
	30%	3.16 (26.0%)	2.17 (19.4%)	1.69 (15.8%)
	40%	3.01 (25.1%)	2.09 (18.9%)	1.65 (15.5%)
	50%	2.99 (25.0%)	2.08 (18.8%)	1.64 (15.4%)
	60%	3.09 (25.6%)	2.12 (19.1%)	1.66 (15.5%)
	70%	3.34 (27.1%)	2.24 (19.9%)	1.72 (16.0%)
20%	20%	2.89 (42.0%)	2.01 (33.5%)	1.60 (28.5%)
	30%	2.58 (39.2%)	1.86 (31.8%)	1.51 (27.4%)
	40%	2.46 (38.1%)	1.80 (31.1%)	1.48 (27.0%)
	50%	2.44 (37.9%)	1.79 (30.9%)	1.47 (26.8%)
	60%	2.49 (38.4%)	1.81 (31.2%)	1.48 (27.0%)
	70%	2.65 (39.9%)	1.89 (32.1%)	1.52 (27.6%)

For example, at the full target sample size of 2,000 women, assuming 60% (N=1200) are HIV-infected, the study design provides 80% power to detect a difference in the percent with unsuppressed VL of 16.7% vs. 10% comparing ZIKV-infected vs. ZIKV-uninfected women, assuming that 20% of the 1200 become ZIKV-infected by the end of their pregnancy, corresponding to an OR equal to 1.80. The range of 10% to 20% assumed for percent with unsuppressed VL was based on data from the PHACS SMARTT study on HIV-infected but presumed ZIKV-uninfected women.[92] If the percent with ZIKV infection increases to 50%, the minimum detectable OR at 80% power decreases to 1.64 reflecting a difference between 15.4% vs. 10%, or an absolute difference of 5.4% in unsuppressed VL. Higher rates of unsuppressed VL (20%) among the ZIKV-uninfected will also translate to smaller detectable ORs, with absolute differences in percent with unsuppressed VL ranging from 7% to 9%.

For **Aim 3**, we provide the power for detecting differences in the percent with ZIKV infection by the end of pregnancy between HIV-infected and HIV-uninfected women, based on interim enrollment of 500, 1,000, and 1,500 women and a final target enrollment of 2,000 women, and assuming the percent with HIV infection among those enrolled ranges from 40% to 60%.

Table 12-3. Minimum detectable ORs (and percent of ZIKV-infected among HIV-infected) at 80% power for various sample sizes and assumed ZIKV infection rates among HIV-uninfected

Percent of enrolled with HIV infection	% with ZIKV infection in the HIV-uninfected group	Minimum detectable ORs (percent ZIKV+ in HIV-infected) for sample sizes			
		N=500	N=1000	N=1500	N=2000
40%	20%	1.80 (31.1%)	1.53 (27.7%)	1.42 (26.2%)	1.36 (25.3%)
	30%	1.71 (42.2%)	1.46 (38.6%)	1.37 (36.9%)	1.31 (36.0%)
	40%	1.67 (52.7%)	1.44 (49.0%)	1.35 (47.3%)	1.30 (46.3%)
	50%	1.68 (62.7%)	1.44 (59.0%)	1.35 (57.4%)	1.29 (56.4%)
	60%	1.72 (72.1%)	1.46 (68.7%)	1.36 (67.1%)	1.30 (66.2%)
	70%	1.83 (81.0%	1.52 (78.0%)	1.40 (76.5%)	1.33 (75.7%)
50%	20%	1.79 (30.9%)	1.52 (27.5%)	1.41 (26.1%)	1.35 (25.2%)
	30%	1.69 (42.0%)	1.45 (38.4%)	1.36 (36.8%)	1.31 (36.0%)
	40%	1.66 (52.5%)	1.43 (48.8%)	1.34 (47.2%)	1.29 (46.2%)
	50%	1.66 (62.4%)	1.43 (58.8%)	1.34 (57.2%)	1.29 (56.2%)
	60%	1.70 (72.8%)	1.45 (68.5%)	1.35 (67.0%)	1.30 (66.0%)
	70%	1.80 (80.8%)	1.50 (77.8%)	1.39 (76.4%)	1.33 (75.6%)
60%	20%	1.81 (31.2%)	1.54 (27.7%)	1.42 (26.2%)	1.36 (25.4%)
	30%	1.71 (42.3%)	1.47 (38.6%)	1.37 (37.0%)	1.31 (36.0%)
	40%	1.67 (52.7%)	1.44 (49.0%)	1.35 (47.3%)	1.30 (46.3%)
	50%	1.68 (62.6%)	1.44 (59.0%)	1.35 (57.4%)	1.29 (56.4%)
	60%	1.72 (72.0%)	1.46 (68.6%)	1.36 (67.1%)	1.30 (66.2%)
	70%	1.82 (80.9%)	1.51 (77.9%)	1.40 (76.5%)	1.33 (75.7%)

For an example of the interpretation of the results in **Table 12-3** above, if 60% of the final target of N=2,000 women are HIV-infected and 40% are uninfected, and assuming 40% of the HIV-uninfected women become ZIKV-infected by the end of pregnancy, then the study design will provide 80% power to detect a difference in ZIKV infection of 46.3% vs. 40% for HIV-infected vs. HIV-uninfected women, or an absolute difference of 6.3% (with corresponding OR equal to 1.30); at an interim enrollment of 1000, the detectable OR would be 1.44 allowing differences in percent with ZIKV infection of 9.0% or greater to be detected.

To address the power for **Aim 4**, a number of scenarios for possible percentages of enrollment within the four different subgroups defined by the cross-classification of HIV infection and ZIKV infection were evaluated and the power for comparing the percent of pregnancies with adverse outcomes for each of the first three subgroups to the doubly uninfected group was evaluated for a range of sample sizes.

Table 12-4. Power for detecting differences in rate of adverse pregnancy outcomes (for Aims 4 and 6) based on assumed proportion in the HIV and ZIKV co-infected, HIV-infected and ZIKV-infected groups as compared to the HIV and ZIKV-uninfected group

Percent of enrolled in HIV+/ZIKV+, HIV+/ZIKV-, HIV-/ZIKV+, & HIV-/ZIKV- groups	**Assumed % with adverse outcomes in HIV-/ZIKV- group**	**Power for detecting 2-fold difference in odds of adverse outcomes for HIV+/ZIKV+, HIV+/ZIKV-, and HIV-/ZIKV+ groups vs. HIV-/ZIKV- group**			
		N=500	**N=1000**	**N=1500**	**N=2000**
12%, 28%, 12%, 48%	5%	22.2%, 32.8%, 22.2%	39.2%, 57.2%, 39.2%	54.2%, 74.6%, 54.2%	66.4%, 85.7%, 66.4%
	10%	36.3%, 54.1%, 36.3%	62.4%, 83.1%, 62.4%	79.6%, 94.7%, 79.6%	89.6%, 98.5%, 89.6%
	20%	54.5%, 77.0%, 54.5%	83.4%, 96.8%, 83.4%	94.8%, 99.7%, 94.8%	98.5%, 100%, 98.5%
20%, 20%, 24%, 36%	5%	25.4%, 25.4%, 27.1%	45.0%, 45.0%, 47.9%	61.3%, 61.3%, 64.7%	73.7%, 73.7%, 77.0%
	10%	42.3%, 42.3%, 45.3%	70.5%, 70.5%, 74.0%	86.4%, 86.4%, 89.1%	94.2%, 94.2%, 95.8%
	20%	63.6%, 63.6%, 67.6%	90.4%, 90.4%, 92.8%	97.9%, 97.9%, 98.7%	99.6%, 99.6%, 99.8%
15%, 35%, 10%, 40%	5%	23.5%, 32.1%, 19.3%	41.6%, 56.1%, 33.7%	57.2%, 73.6%, 47.0%	69.5%, 84.9%, 58.6%
	10%	38.8%, 53.5%, 31.2%	65.9%, 82.5%, 54.7%	82.7%, 94.4%, 72.1%	91.8%, 98.4%, 83.6%
	20%	58.4%, 77.2%, 47.3%	86.7%, 96.9%, 76.3%	96.4%, 99.7%, 90.6%	99.1%, 100%, 96.6%
25%, 25%, 20%, 30%	5%	25.0%, 25.0%, 27.1%	44.3%, 44.3%, 47.9%	60.4%, 60.4%, 64.7%	72.9%, 72.9%, 69.3%
	10%	42.0%, 42.0%, 45.3%	70.0%, 70.0%, 74.0%	86.1%, 86.1%, 89.1%	94.0%, 94.0%, 92.0%
	20%	64.0%, 64.0%, 67.6%	90.6%, 90.6%, 92.8%	98.0%, 98.0%, 98.7%	99.6%, 99.6%, 99.3%
18%, 42%, 8%, 32%	5%	23.2%, 29.3%, 16.4%	41.1%, 51.6%, 28.0%	56.5%, 68.8%, 39.2%	68.9%, 80.8%, 49.5%
	10%	38.6%, 49.4%, 26.0%	65.6%, 78.6%, 46.0%	82.5%, 92.1%, 62.4%	91.6%, 97.3%, 74.8%
	20%	58.9%, 73.7%, 39.4%	87.0%, 95.6%, 66.7%	96.6%, 99.4%, 83.4%	99.2%, 99.9%, 92.3%
30%, 30%, 16%, 24%	5%	23.0%, 23.0%, 19.6%	40.6%, 40.6%, 34.4%	55.9%, 55.9%, 47.9%	68.3%, 68.3%, 59.6%
	10%	38.8%, 38.8%, 32.4%	65.8%, 65.8%, 56.6%	82.6%, 82.6%, 74.0%	91.8%, 91.8%, 85.2%
	20%	60.7%, 60.7%, 50.5%	88.4%, 88.4%, 79.7%	97.1%, 97.1%, 92.8%	99.4%, 99.4%, 97.7%

From **Table 12-4** above, it is evident that the power for detecting an OR of 2 when comparing pairs of subgroups (e.g., HIV+/ZIKV+ vs. HIV-/ZIKV-) is low when the overall study accrual is 500 or 1,000, but becomes reasonable (> 80%) when the background rate of AEs is at least 10% in the doubly uninfected group and the sample size is at least 1500, or when the background rate is 20% and the sample size is at least 1,000. The first two scenarios in **Table 12-4** above reflect an assumption that a total of 40% of the women enrolled will be HIV-infected, the second two scenarios assume 50% will be HIV-infected, and the last two scenarios assume 60% will be HIV-infected.

Aim 5 involves comparing the rate of transmission of either HIV or ZIKV from mother to child. This study is not powered to detect differences in the rate of perinatal HIV transmission, but will provide good precision for the estimation of such rates. Assuming 40% of women enrolled are HIV-infected, the precision (half-width) of CIs will range from 0.055% (for N=2,000) to 0.09% (for N=1,000) under the assumption of a 0.5% HIV transmission rate. If 60% of women are HIV-infected, the precision would increase and CI half widths would correspondingly decrease to 0.045% (N=2,000) to 0.07% (N=1,000).

The power calculations for **Aim 6** are already provided in **Table 12-4** for a range of AE rates in the doubly uninfected group, and under various scenarios for both interim and final study accrual in Phase II, and for percent of total accrual in each of the four subgroups. As an example, if the underlying percent with congenital malformations or other defects is 10% in the doubly infected group, a sample size of 1,500 will generally provide over 80% power for most pairwise comparisons of interest.

Given the lack of information regarding long-term effects of ZIKV infection in any population, and among HIV-infected women in particular, **Aim 7** is considered exploratory and no power calculations are provided for this specific aim. However, the power calculations in **Table 12-4** can serve as guidance regarding the range of power that would be anticipated for the full study accrual.

12.10 Interim Monitoring

12.10.1 Accrual and Administrative Monitoring

During the first year of the study, the enrollment of pregnant women and children into the HIV ZIP study will be monitored closely. Total accrual reports will be prepared by Westat and distributed on a weekly basis. Monthly accrual reports by site will be monitored by the protocol team. Quarterly administrative reports will also be prepared, which will summarize baseline characteristics of the cohort along with completeness of baseline data, particularly key exposure data such as ZIKV IgM and RNA test results.

13.0 Human Subjects

The study will be conducted in compliance with the protocol, in accordance with the ICH guidelines for GCP (ICH E6 (R1)), the CFR on the Protection of Human Subjects (45 CFR Part 46), the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research and the NIH Office of Extramural Research, Research Involving Human Subjects Policies and Guidance.

13.1 45 CFR Part 46.404

It is the judgment of the protocol team that the HIV ZIP protocol belongs in Category One Research under 45 CFR Part 46.404: Research not involving greater than minimal risk. This judgment is premised on the definition of minimal risk found at 45 CFR Part 46.102(i):

> *Minimal risk* means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.

The principal risks in this study result from the disclosure of sensitive maternal information and the risks inherent in the routine examinations that the mother and children will receive. These are discussed in more detail in the following sections but in essence *are* medical and psychological examinations employed in routine clinical evaluation.

13.2 Institutional Review Board / Institutional Ethics Committee and Informed Consent

This protocol, the IC/assent/parent(s)-legal guardian(s) permission documents (see Appendices IV and V), and any subsequent modifications must be reviewed and approved by the IRB or IEC responsible for oversight of the study prior to implementation. Written IC must be obtained from the participant (or parent(s) or legal guardian(s) of participants who cannot consent for themselves, such as those below the legal age per state or country regulations). The participant's assent must also be obtained if below legal age and she is able to understand the nature, significance and risks of the study. The IC will describe the purpose of the study, the procedures to be followed and the risks and benefits of participation. A copy of the consent form will be given to the participant (or parent(s) or legal guardian(s)).

13.3 Prisoner Participation

The NICHD concluded that this protocol does <u>not</u> meet Federal requirements governing recruitment of prisoners for participation in research and should NOT be considered by any local IRB/IEC for this purpose. Participants themselves recruited from the general population, who, subsequent to enrollment, become incarcerated or are placed in detention, may not continue study participation while incarcerated.

13.4 Participant Confidentiality

Only a coded number to maintain participant confidentiality will identify all study-specific laboratory specimens, evaluation forms, reports and other records. All records will be kept in a secured area with limited access. A list linking the participant names with their assigned coded number will be securely stored at the clinical site under double locks, separate from all other research records. All electronic research records will be password protected, securely stored and backed up. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by OHRP, the local IRB or IEC, local or national regulatory agencies, NICHD, study staff, study monitors and other Sponsors, as applicable.

The Protocol Co-Chairs and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study, any data, record or other unpublished confidential information disclosed to those individuals for the purpose of the study. All research staff persons at the clinical sites are required to sign non-disclosure forms pledging to hold research information in confidence. Prior written agreement from NICHD must be obtained for the disclosure of any said confidential information to other parties.

All protocol team investigators and collaborators are required to sign data use agreements pledging not to seek the identity of study participants.

PII will not be released by the sites without written permission of the participant**,** except as necessary for monitoring by Westat or NICHD.

The site investigator will make study documents (e.g., consent forms, CRFs) and pertinent records available for inspection by the local IRB or IEC and site study monitors on behalf of NICHD, NIH, OHRP or the Sponsor's designee for confirmation of the study data.

13.5 Risks and Benefits

The HIV ZIP Protocol Team has determined that participation in this study involves procedures or interventions that are not greater than minimal risk and present the prospect of direct benefit to the individual participants.

The measurements that are involved in this study require venipuncture to collect blood samples. This procedure may cause local discomfort, bleeding or bruising; rarely can small clot or infection occur at the blood draw site. This measurement should not be considered greater than minimal risk in and of itself given its routine use in general health care delivery.

There is some risk that answering questions about some of the topics may be uncomfortable or upsetting, such as substance use behaviors. In the event of

discomfort or upset, site staff will be able to refer study participants to counseling. Participants do not have to answer any question that they do not want to answer. Furthermore, participants will be informed that at any point, they may stop if they do not wish to continue responding to the questionnaire(s). Every effort will be made to keep the participants' personal information private and confidential, but absolute confidentiality cannot be guaranteed.

There is no guarantee of direct benefit to infants who participate in this study. If parents or legal representatives choose, the clinically relevant information obtained in this study can be made available to their children's health care providers and may inform their health care. Parents or legal representatives of study participants will be encouraged to do this in order to maximize the potential for benefits.

There may be direct benefit to the women and their infants who participate in the study by the testing for ZIKV and other infections at multiple times throughout the pregnancy, which may allow for available treatment and management, as well as the ability to make choices about the pregnancy. Infants may benefit from the close monitoring and frequent evaluations, but there is no guarantee. In addition, future pregnant women and their infants may benefit from knowledge that is gained by their participation.

13.5.1 HIV ZIP Abnormality-Triggered Evaluations

Infants followed in HIV ZIP will be screened for abnormalities using the tests described previously. Abnormal values on these screening measures will "trigger" more extensive clinical evaluations, the results of which will be abstracted for the HIV ZIP study.

13.6 Biorepository Policies

Biological specimens will be collected and stored for future testing that is not specified in the protocol (biorepository specimens). IC (or assent with parent(s)/legal guardian(s) permission) will be obtained from individuals as required by 45 CFR Part 46.116 to have their and their infants' specimens included in the biorepository.

Refer to the HIV ZIP MOP for the NICHD Biorepository Policy, April 16, 2010 for additional information on the policy addressing the management of biorepository specimens, i.e., specimens collected for unspecified future testing and long-term storage.

13.7 45 CFR § 160 and 164 Standards for Privacy of Individually Identifiable Health Information ("Privacy Rule" Pursuant to the HIPAA)

Each site is responsible for adherence to their individual institution's HIPAA policies and procedures. Sites should ensure that their institutional HIPAA authorization form permits this release of protected health information to the clinical provider.

13.8 Study Discontinuation

The study may be discontinued at any time by NICHD, OHRP, the local IRB or IEC, other governmental agencies or local or national agencies, as part of their duties to ensure that research participants are protected.

14.0 Publication of Research Findings

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

Publication of results of this trial will be governed by NICHD policies. Any presentation, abstract or manuscript will be made available for review prior to submission.

15.0 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by CDC. These procedures can be found at www.cdc.gov.

All infectious specimens will be transported in compliance with federal regulations and the International Air Transport Association (IATA) Dangerous Goods Regulations-Packing Instruction 602. Refer to individual carrier guidelines (e.g., Federal Express or World Courier) for specific instructions and to the Guidelines for Shipment and Receipt of Category B Biological Substance Shipment and Instruction for Overnight Shipments documents (see HIV ZIP LPC).

All participating sites are also required to follow the specimen management procedures outlined in the HIV ZIP MOP for collecting, processing, shipping and storing biological specimens for the study. In addition, sites are required to follow protocol-specific procedures outlined in the protocol.

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Appendix I
Maternal Schedule of Evaluations for Asymptomatic and Symptomatic Women

Evaluations / Procedures	All Participants	Enrollment	Acute ZIKV-like Symptoms / ≥ 18 weeks gestation	< 18 weeks gestation[7]	Second Trimester 24 weeks gestation (+/- 2 weeks)	Third Trimester 34 weeks gestation (+/- 2 weeks)	Delivery (live birth or fetal loss) (+ 48 hours)	6 weeks postpartum (+/- 7 days)	On Study / Acute ZIKV-like Symptoms [11]
	Screening		Entry	Entry	All participants on study				
IC [1]	X [2]		X	X					
Basic demographics, LMP, eligibility confirmation	X [2]			X					
Fetal US with fetal heartbeat	X [3]		X [3]	X [3]	X [3]	X [3]	X [3]		X [12]
Provide/review Zika-symptom diary			X	X	X	X	X	X	X
Zika-like symptoms, health & medication history			X	X	X	X	X	X	X
Pregnancy questionnaire			X	X	X	X	X		X
Other risk factors questionnaire			X	X	X	X	X	X	X [13]
Targeted PE			X	X	X	X	X	X	X
HIV-infected only: HIV VL, absolute CD4/CD8 T-cells and % abstraction [4]			X	X	X	X	X	X	X
			LABORATORY EVALUATIONS [#] (Blood volumes in parentheses are collected only if indicated.)						
Urine or blood: βhCG – abstract from medical records, if available	(1 ml) [5]			(1 ml) [5]					
Blood: ZIKV IgM Abs, ZIKV RNA detection, DENV IgM Abs	6 ml [6]			6 ml	6 ml	6 ml	6 ml	6 ml	6 ml
Urine: ZIKV RNA detection	X [6]			X	(X)	(X)	(X)	(X)	X
Blood: TORCH & Syphilis (RPR) blood [8]			(3 ml)	(3 ml)		(3 ml)			(3 ml)
Blood (HIV-infected only): HIV VL [9]			(3 ml)	(3 ml)	(3 ml)	(3 ml)	(3 ml)	(3 ml)	3 ml
Blood (HIV-infected only): CD4/CD8 T-cells [9]			(3 ml)	(3 ml)	(3 ml)	(3 ml)	(3 ml)	(3 ml)	
			BIOREPOSITORY SPECIMENS (if consented for future use)						
Urine	X [6]			X	X	X	X	X	X
Blood for plasma	(4 ml) [6]			(4 ml)	(4 ml)	(4 ml)	(4 ml)	(4 ml)	(4 ml)
Cord blood [10]							20 ml		
Placental tissue							X		
TOTAL BLOOD VOLUMES (max. volume per visit = 20 ml)	**Min 0 ml Max 11 ml**		**Min 0 ml Max 9 ml**	**Min 9 ml Max 20 ml**	**Min 6 ml Max 16 ml**	**Min 6 ml Max 19 ml**	**Min 6 ml Max 16 ml**	**Min 6 ml Max 16 ml**	**Min 9 ml Max 16 ml**

Footnotes:

[#]If insufficient blood volume, prioritize as follows: (1) ZIKV IgM Abs and RNA detection; (2) TORCH testing, if not done for clinical care; (3) plasma for Biorepository.

1. Written maternal IC (or assent with parent(s)/legal guardian(s) permission) must be obtained prior to performing any screening evaluations. Written IC for participation of the infant(s) born from this pregnancy may be obtained at the same time or any time during the pregnancy and confirmed at delivery per local requirements.
2. Perform at the Screening visit for all participants.
3. Perform at the Screening or Entry visit ONLY if not done through routine prenatal care. Otherwise, abstract results from all fetal USs performed during prenatal care.
4. At Entry, abstract all available results starting from the date closest, but prior, to the LMP. At all other visits, abstract all available results since the previous visit.
5. Not required if pregnancy already confirmed by fetal US with fetal heartbeat or by βhCG at routine prenatal care.
6. Perform at the Screening visit ONLY for participants 18 weeks or greater GA that present symptomatic for possible acute ZIKV:
    a. If ZIKV RNA test is positive, some remaining serum will be tested for ZIKV IgM Abs, the participant will be enrolled in the study and the remaining Entry visit evaluations will be performed.
        i. After the Entry visit, the next scheduled study visit will be the one closest to, but occurring after the participant's current gestation.
        ii. The participant will then follow the same visit schedule as those enrolled at < 18 weeks gestation. See the HIV ZIP LPC for subsequent study visits' testing algorithms based on testing results.
    b. If ZIKV RNA test is negative, the participant will not be enrolled in the study; no further testing will be performed and the remaining specimens will be destroyed.
7. Participants < 18 weeks gestation that present symptomatic for possible acute ZIKV will be enrolled (if otherwise screened eligible) and have their Entry visit on the same day as the Screening visit. Ideally, asymptomatic participants < 18 weeks gestation should be enrolled and have their Entry visit performed on the same day as the Screening visit, but they may complete the visit any time prior to 18 weeks gestation. See the HIV ZIP MOP for Entry and subsequent study visit testing algorithms.
8. Abstract results of tests performed for routine prenatal care. Any tests already performed for routine prenatal care do not need to be repeated.
9. Perform testing only if no results are available from within the prior 12 weeks. Perform HIV VL testing if the participant presents symptomatic for possible acute ZIKV, regardless of when the last HIV VL was collected.
10. If peripheral blood is unobtainable from the newborn, send required volume of cord blood to the central laboratory following the Appendix II Birth Visit Laboratory Evaluations. Remaining cord blood will go to the Biorepository (if consented for future use).
11. Follow the "On Study / Acute ZIKV-like Symptoms laboratory collection / testing algorithm for any previously ZIKV RNA negative participant that presents with ZIKV-like symptoms while on study. If the study staff become aware of a participant that is newly symptomatic between study visits, schedule an "unplanned symptomatic" visit.
    a. Collect blood for ZIKV IgM Abs, DENV IgM Abs (unless previously DENV IgM positive), other possible serology and blood / urine for ZIKV RNA detection, which will be performed simultaneously.
    b. Collect blood for TORCH testing and syphilis (RPR), if clinically indicated, and send to the local laboratory. (Do not repeat previously positive tests.)
12. Record results from interim fetal USs performed according to country and local clinical guidelines since the prior study visit.
13. Administer at continental U.S. sites only if this is an "unplanned symptomatic" visit.

Appendix II
Infant Schedule of Evaluations

	Birth (+ 48 hours)	3 Months (week 12 +/- 2 weeks)	6 Months (week 24 +/- 2 weeks)	12 Months (week 52 +/- 2 weeks)
IC (Obtain from parent(s)/legal guardian(s) or confirm if previously obtained)	X			
Health history (include results from laboratory tests conducted for clinical care, if applicable)	X	X	X	X
PE [1]	X	X	X	X
Ophthalmologic exam [2]	X	X	X	X
Neurological exam	X	X	X	X
Hearing assessment [3]	X	X	X	X
HIV-related test results and treatments, if applicable [4]	X	X	X	X
NEURODEVELOPMENTAL/NEUROPSYCHOLOGICAL EVALUATIONS				
ND assessment for *screening* options: HINE; ASQ; BSID-III		X	X	X
ND assessment for diagnostics: BSID-III [5]		X	X	X
NEUROIMAGING RESULTS ABSTRACTION/ASSESSMENT				
Head US (abstract results) [6]	X	X [7]	X	X
Head CT/MRI scan (abstract results) [6]	X	X	X	X
LABORATORY EVALUATIONS * **(Blood volumes within parentheses are collected only if indicated.)**				
Blood: ZIKV RNA detection ZIKV IgM Abs DENV IgM Abs (CKNV and WNV, as needed)	4 ml	3ml	3ml	3ml
Blood: TORCH (blood or urine for CMV) & syphilis (RPR) (see the HIV ZIP MOP for asymptomatic newborn testing guidance) [8]	(3 ml)			
Urine: ZIKV RNA detection	X	X	X	X
BIOREPOSITORY SPECIMENS (if consented for future use)				
Urine	X	X	X	X
Remnant CSF [9]	X	X	X	X
TOTAL BLOOD VOLUMES (max. volume per visit = 3 ml per kg of weight)	**Min 4 ml Max 7 ml**	**Min 3 ml Max 3 ml**	**Min 3 ml Max 3 ml**	**Min 3 ml Max 3 ml**

Footnotes:

[*] **In case of low yield and/or low birthweight, prioritize blood specimen collection for ZIKV testing. Maximum of 3 ml per kg of weight (3.8% of total blood volume). Lower limits for sick children are advisable. Refer to the HIV ZIP MOP for additional guidance on maximum blood volumes and laboratory testing algorithms.**

1. Not required if a PE was performed within the past 4 weeks. Head circumference must be included.
2. If ophthalmology exam is abnormal, refer for additional testing and collect results from any tests done for clinical reasons.
3. If hearing assessment is abnormal, refer to audiology and collect results from any tests done for clinical reasons.
4. Abstract HIV diagnostic testing results, and if applicable, interim HIV VL and T-cells results.
5. Perform diagnostic ND assessments if the screening assessment is abnormal.
6. Abstract results of neuroimaging assessments whenever they are performed. Does not need to coincide with the schedule of evaluations timeline. Do not perform for the study, except head US, as indicated below.
7. ZIKV-exposed infants only: Perform a head US for the study only if none was performed by the age of 3 months.
8. Only at birth if any individual tests are indicated.
9. From specimen done for clinical care only.

Appendix III
List of Participating Sites and Investigators

Site Address	Principal Investigator	Telephone / Email
Brazil		
Institute of Pediatrics Martagao Gesteira Federal University of Rio de Janeiro Rua Bruno Lobo, 50 Ilha do Fundão Rio de Janeiro, 21941-912 Brazil Site 5071	Cristina Hofer, MD	Email: cbhofer@hucff.ufrj.br Phone: 55-21-3148-2255
Hospital Federal dos Servidores do Estado Centro de Pesquisa Clinica, DIP – Serviço de Doenças Infecciosas e Parasitarias, Rua Sacadura Cabral 178, anexo IV, 4 andar, Saude Rio de Janeiro, 20221-903 Brazil Site 5072	Esaú Custódio João, MD, PhD Dra. Maria Isabel Gouvea, MD, PhD	Email: esaujoao@gmail.com Phone: 55-21-2233-0018 Email: : bebelsgouvea@uol.com.br Phone: 55-21-2233-0018
Federal University of Minas Gerais School of Medicine Avenida Alfredo Balena 190 Room 161 Belo Horizonte, Minas Gerais, 30130-100 Brazil Site 5073	Jorge Andrade Pinto, MD	Email: jorgeandradepinto@gmail.com mailto:jpinto@medicina.ufmg.br Phone: 55-31-3409-9822
Ribeirão Preto Medical School Department of Pediatrics University of Sao Paulo Avenida Bandeirantes 3900 Ribeirão Preto Sao Paulo, 14049-900 Brazil Site 5074	Marisa M. Mussi-Pinhata, MD	Email: mmmpinha@fmrp.usp.br Phone: 55-16-3602-2807

Site Address	Principal Investigator	Telephone / Email
Hospital Geral De Nova Igaçu Brazil 953 Avenida Henrique Duque Estrada Mayer Alto da Posse Nova Iguaçu, Rio de Janeiro, 26050-210 Brazil Site 5097	Jose da Silva Pilotto, MD, PhD	Email: pilotto@uninet.com.br jhpilotto@gmail.com Phone: 55-21-98182-9797
United States, including Puerto Rico		
San Juan Hospital Research Unit PMB #128, GPO Box 70344 San Juan, Puerto Rico 00936 Site 5031	Midnela Acevedo, MT, MD Rodrigo Díaz-Velasco, MD, FACOG	Email: macevedo@SanJuanciudadPatria.com Phone: 787-765-4186 Email: rodrigodiazvelasco@gmail.com Phone: 787-765-4186
Children's Diagnostic & Treatment Center, Inc. 1401 South Federal Highway Ft. Lauderdale, FL 33316 Site 5055	Ana M. Puga, MD	Email: apuga@browardhealth.org Phone: 954-728-1050
Bronx-Lebanon Hospital Center 1685 Morris Avenue, Suite 1G Bronx, NY 10457 Site 5114	Murli U. Purswani, MD	Email: mpurswan@bronxleb.org Phone: 718-960-1010
University of Miami Pediatric Infectious Diseases and Immunology Batchelor's Children's Research Institute 1580 NW 10th Avenue, Suite 286 Miami, FL 33136 Site 4201	Gwendolyn Scott, MD Charles Mitchell, MD	Email: gscott@med.miami.edu Phone: 305-243-6522 Email: cmitchel@med.miami.edu Phone: 305-243-2755
University of Puerto Rico Pediatric HIV/AIDS Research Program Medical Sciences Campus Main Building, 3rd floor, Room 374 School of Medicine San Juan, Puerto Rico 00935 Site 6601	Zoe Rodriguez, MD	Email: zoe.rodriguez@upr.edu Phone: 787-645-0937

Appendix IV
Sample Maternal Informed Consent

See attached separate document

Appendix V
Sample Consent for Infant Participation

See attached separate document