Consent to participate in a clinical research study
"Investigating the sylvatic transmission and reservoir potential of Zika, dengue, and chikungunya viruses of co-located humans and long-tailed macaques in Thailand and Cambodia"

Participant Name _____ Age _____

Study Identification Number _____

KEY INFORMATION

This consent form describes a research study and is designed to help you decide if you would like to be a part of the research study.

You are being asked to take part in a research study. This study is sponsored by the National Center for Parasitology, Entomology and Malaria Control (CNM), Ministry of Health, Phnom Penh, Cambodia, and the National Institutes of Health (NIH), United States of America. This section provides the information we believe is most helpful and important to you in making your decision about participating in this study. Additional information that may help you make a decision can be found in other sections of the document. Participation in this study is entirely voluntary, and there is no penalty or loss of benefits for deciding not to participate.

We are doing this study to better understand if animals such as monkeys might carry and help spread germs to people that cause common illnesses in Cambodia and Thailand, like Zika, dengue, and chikungunya. We hope that in the future, we can use this information to improve public health programs and conduct more research studies about these diseases.

In this study, we are collecting blood samples from people who live or work near Wat Amphae Phnom in Chbar Mon town, and from monkeys in the same area. If you agree to join this study, we will do research tests on your blood sample to look for evidence of germs that cause common illnesses. The research tests will tell us if you have had these germs at some point in the past, but they will not tell us if you have the germs right now. We will not share test results with you. We will compare the results of the research tests from humans to those from monkeys to see if they get the same kinds of germs.

All of these test results will be protected and stored. Your name or any other personal information will be removed before these results are shared with other researchers.

You will not directly benefit by participating in this study. The results will help us better understand if monkeys help spread germs in Cambodia. Collecting blood may hurt or cause a little bleeding or bruising, but that should go away within a day.

The remaining document will now describe more about the research study. This information should be considered before you make your choice. Members of the study team will talk with you about the information described in this document. Take the time needed to ask any questions and discuss this study with a member of the study team, and with your family, friends, and personal healthcare providers.

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BACKGROUND/PURPOSE OF STUDY

The purpose of this study is to better understand if animals such as monkeys might carry and help spread germs to people that cause common illnesses in Cambodia, like Zika, dengue, and chikungunya. We hope that in the future, we can use this information to improve public health programs and conduct more research studies about these diseases.

IDENTIFICATION OF STUDY POPULATION

We will enroll adults who live or work near Wat Amphae Phnom in Chbar Mon town. We anticipate enrolling approximately 300 people into this study.

PROCEDURES EXPLAINED

If you agree to join this study, then we will first ask you some questions about yourself, like your age, sex, where you live and work, your health, and your everyday activities.

We will collect a small amount (about 2 teaspoons) of blood from a vein in your arm using a needle. We will use this blood for research tests to look for evidence of germs that cause common illnesses in people in Cambodia. We will compare the results of the research tests from humans to those from monkeys to see if they get the same kinds of germs.

Your participation in this study ends after we collect your blood sample.

ALTERNATIVE TO PARTICIPATION

The alternative to participating in this study is to not participate. Not participating in this study will not prevent you from participating in a future study.

STORED BLOOD AND DATA

Your stored sample and data will be marked with a code and not with your name or any other personal information that would identify you. Only researchers linked to this study can get the codes.

We will store your blood sample and data (information) for a very long time to use for future research on diseases that cause illnesses in Cambodia. Your coded study information will be placed in secure electronic systems, called databases, for use by other researchers. Researchers must request permission to look at information in this system. They may then use the information for future research on any topic. This allows the information to be shared broadly for research purposes. You will not get any information about future research.

Your coded sample and data might be sent to other scientists that we work with for research. We must get approval from the Cambodian and US ethics boards that review this study to share samples and data with other researchers. Other information, such as your sex or age, might also be shared, but your name will not. Your sample will not be sold. You will not be paid for any products that result from this

research. The only risk of allowing us to store your sample or information would be an accidental release of your identity.

If you change your mind and decide you do not want us to store your sample or data, then please let us know. We will do our best to follow your wishes but cannot promise that we will always be able to destroy the sample or data.

DISCUSSION OF FINDINGS

The research tests we do in this study may take many weeks or longer to complete, so we will not return the results to you. However, we will share the general results of the study with the provincial and national health authorities, but not your name or individual results, so they can better predict what diseases they may have to treat in the future.

RISKS/DISCOMFORTS EXPLAINED

Drawing blood may cause discomfort, bleeding, and occasional bruising at the site; rarely, fainting or infection may occur. We will clean the arm before taking blood and will use new needles to obtain the blood.

EXPLANATION OF POTENTIAL BENEFITS

You will not directly benefit by being in this study.

The study team may gain new knowledge about whether animals like monkeys might carry and help spread germs that cause common illnesses in Cambodia. We may be able to use this knowledge to improve public health programs and help future research about these diseases.

COMPENSATION

To compensate you for the inconvenience of providing a blood sample, you will receive $5 in cash.

CONFIDENTIALITY

We will keep the study information private. All files with information that could identify you will be kept in locked cabinets at our laboratory or offices in Phnom Penh. The sample of blood that is collected from you will be marked with a number that tells the study team that it is your blood. The sample will not be marked with your name or other identifying information. Only researchers in this study will have access to information that could identify you. People responsible for making sure that the research is done properly may look at your study records. These might include people or their representatives from the Cambodian National Ethics Committee for Health Research, CNM, or NIH. These people will also keep your identity private. However, we cannot guarantee absolute confidentiality.

STATEMENT THAT NEW FINDINGS WILL BE PUBLISHED

The findings of this study may be reported at meetings or in medical journals, but your name will not be used in the reports.

LIST OF CONTACTS

If you have questions or concerns about this study now or at a later date, you may speak with one of our staff at the Kampong Speu District Referral Hospital or contact Dr. Suon Seila (Tel: 855 23 996 202), Project Manager for the CNM, or Professor Eng Huot (Tel: 012 950 122), Chairman of the National Ethics Committee for Health Research, in Phnom Penh. The doctors at the Referral Hospital can help you contact Dr. Seila or Professor Huot.

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If you agree to participate in this study, then please put your signature or thumbprint below.

_____	_____	_____
Thumbprint - or -	Signature of Participant	Date
_____	_____	_____
Investigator Name	Investigator Signature	Date
_____	_____	_____
Witness Name	Witness Signature	Date

National Institute of Allergy and Infectious Diseases (NIAID)
National Institutes of Health (NIH)

Protocol Face Sheet

NIH Principal Investigator (PI): Institute/Branch: Address: Phone: Email:	Jessica Manning, MD, MSc NIAID/Laboratory of Malaria and Vector Research (LMVR) United States (US) Embassy Phnom Penh Unit 4540, Box 10 DPO, AE 09975 +855 61 202 473 Jessica.manning@nih.gov
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Protocol Number:	20-I-N054
Version Description:	Version 1.0
Version Date:	22 May 2020
Protocol Title:	Investigating the sylvatic transmission and reservoir potential of Zika, dengue, and chikungunya viruses of co-located humans and long-tailed macaques in Thailand and Cambodia
Abbreviated Title:	eASIA
Institute Name:	NIAID
Accrual Period:	24 months
Proposed Dates:	15 May 2020 – 15 May 2022
Total Subjects to be Accrued:	350
Ionizing Radiation Use:	None
Is Tissue Being Collected for Research Purposes?	Yes (sera only)
Location of the Study:	Kampong Speu community, Chbar Mon, Kampong Speu, Cambodia
Investigational New Drug/Device:	None
Coordinating Center:	NIAID

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) and the following:

- US Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46)

NIH-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form (ICF), recruitment materials, and all participant materials will be submitted to the NIH Intramural Institutional Review Board (IRB) and Cambodian National Ethics Committee for Health Research (NECHR) for review and approval. For Cambodia, approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the NIH IRB and NECHR before the changes are implemented to the study. In addition, all changes to the consent form will be approved by the NIH IRB and NECHR; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

For activities in Thailand, a separate IRB application, protocol, and consent form per Thai standards was submitted and approved by the Mahidol University IRB. The macaque sampling in Thailand was performed in 2018 and human sampling was performed from September 2019 through March 2020. A copy of the Thai IRB approval will be included with this protocol for NECHR. A Materials Transfer Agreement will be in place prior to sharing of data and serological materials between Cambodia and Thailand portions of the study.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Investigating the sylvatic transmission and reservoir potential of Zika, dengue, and chikungunya viruses of co-located humans and long-tailed macaques of Thailand and Cambodia
Study Description:	This is a biospecimen procurement protocol at one site in Cambodia. For Cambodia, specimens will be taken from individuals aged 18 to 55 years living or working within 10 km of Wat Amphae Phnom in Kampong Speu, Cambodia. Please see **Schema and Schedule of Activities, Sections 1.2 and 1.3**.

Objectives:	The primary objectives of the study are to: 1. Describe the seroprevalence of Zika virus (ZIKV), dengue virus (DENV), and chikungunya virus (CHIKV) in humans living in close proximity to long-tailed macaques in Thailand and Cambodia. 2. Compare the serology patterns against ZIKV, DENV, and CHIKV between nonhuman primates (NHPs) and humans living in the same areas. The secondary objectives are to: 1. Compare serology patterns against ZIKV, DENV, and CHIKV in macaques and humans between Thailand and Cambodia. 2. Compare serology patterns against *Aedes aegypti* salivary gland homogenate proteins in humans and monkeys between Thailand and Cambodia.
Endpoints:	The primary endpoints are: 1. Assessment of seroprevalence via screening enzyme-linked immunosorbent assay (ELISA) and/or plaque reduction neutralization assay (PRNT) 50 titers for ZIKV, DENV, and CHIKV in Cambodian adults. 2. Assessment of seroprevalence via screening ELISA and/or PRNT50 titers for ZIKV, DENV, and CHIKV in Cambodian macaques as compared to humans. The secondary endpoints are: 1. Comparative assessment of seroprevalence via PRNT50 titers for ZIKV, DENV, and CHIKV in Cambodian adults and macaques to that of Thai adults and macaques. 2. Assessment of reactivity to salivary gland homogenate of *Aedes aegypti* as detected by ELISA or western blot in human and macaque sera.
Study Population:	Healthy adults aged 18 to 55 years (at study entry)
Total Enrollment:	300 subjects
Description of Sites/Facilities Enrolling Participants:	Chbar Mon town, Kampong Speu, Cambodia
Study Duration:	24 months
Participant Duration:	60 minutes for informed consent, exam, baseline assessments, and blood sampling

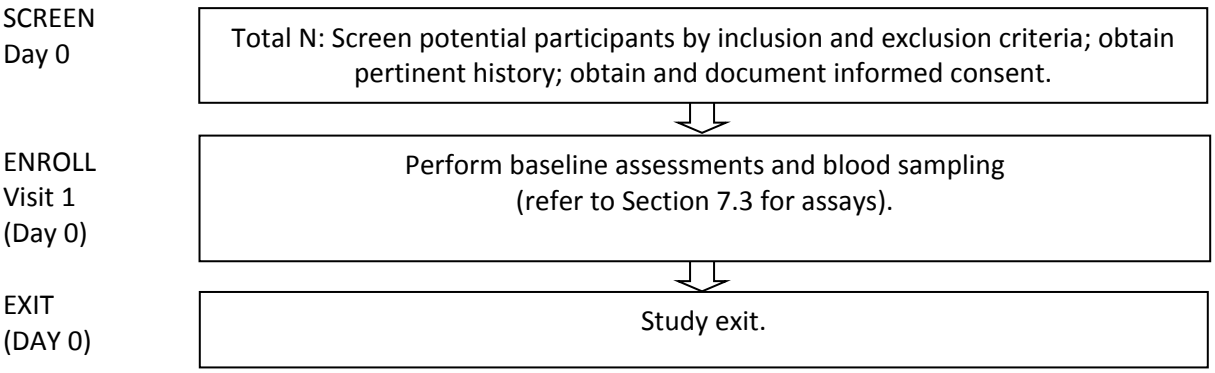
1.2 PRECIS

Arboviral epidemics continue to emerge suddenly and spread of disease is unpredictable. The 2015-16 Zika epidemic resulted in a high case number in Thailand, but not in neighboring Cambodia. It is known that nonhuman primates (NHPs) are important reservoirs of arboviruses, but the importance of their

epidemiological role in the transmission of arboviruses is not clearly understood. While transmission dynamics are complex and require consideration of many variables, primate reservoirs are not routinely sampled, particularly in Southeast Asia, because of the level of operational complexity and skill required.

Here, we propose a serological survey for evidence of Zika virus (ZIKV), dengue virus (DENV), and chikungunya virus (CHIKV) exposure in long-tailed macaques and human adults who live or work in close proximity to these monkeys in Thailand and Cambodia. We hypothesize that ZIKV seroprevalence in both humans and macaques will be higher in Thailand than Cambodia. With the current rise of arboviral diseases around the world, we hope the results of this study contribute to better understanding of the epidemiology and burden of arboviral diseases in this region.

Schema 1: Study Flow Design



1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Screen (Day 0)	Enroll (Day 0)
Informed Consent	X	
Review of Eligibility Criteria and Screening Medical History	X	
Baseline Assessments and Risk Factors		X
Blood Draw for Sera		X

2 INTRODUCTION

2.1 STUDY RATIONALE

Mosquito-borne diseases such as DENV, CHIKV, and ZIKV have a significant impact on public health, particularly in Southeast Asia.[1–3] It has been reported that NHPs play an essential role in mosquito-borne diseases transmission via the sylvatic cycle of these viruses.[4–6] Likewise, recent studies from Africa have described the probable role of NHPs as the reservoir and/or amplification hosts of these arboviruses.[6,7] The purpose of this study is to further understand the relationship between humans and NHPs in the maintenance and amplification of mosquito-borne viruses in Southeast Asia. The study hypothesis is that long-tailed macaques, ubiquitous in populated areas in the Greater Mekong Subregion with

substantial human interaction, play a critical role in mosquito-borne disease transmission in Thailand and Cambodia. To test this hypothesis, we will sample humans and macaques co-existing in the same areas, typically pagodas, in Thailand and Cambodia.

2.2 BACKGROUND

While there continue to be reports of DENV and CHIKV circulation in many Southeast Asian countries, there is no clear evidence to explain the relatively low reported prevalence of ZIKV infection in some parts of Asia, despite recent expansion and explosive outbreaks seen in the Pacific and the Americas.[8–10] Though historical evidence has indicated that ZIKV epidemiology includes a sylvatic cycle involving NHPs, recent outbreaks have been centered in urban areas where the primary vector implicated is *Aedes aegypti*. Contrastingly, there is evidence that NHPs play a role in the maintenance of two other *Aedes*-transmitted viruses, DENV and CHIKV.[11–13] The role of NHPs in the epidemiology of anthropogenic arboviruses is variable, as they may or may not play an important role as reservoir for human infections.[14] Characterizations of the sylvatic cycle and the transmission trajectories of these arboviruses are critical for not only understanding the risk ecology of these pathogens, but for developing educational and health policy to control transmission as vaccines for these viruses currently are not available. The sylvatic cycle of DENV is complex, as evidence suggests it can go from NHP to human populations, as well as from human to NHP populations, increasing the complexity of its transmission.[11,14] CHIKV and ZIKV were both originally isolated from NHPs in Africa, and recent studies from Africa describe the probable role of NHPs as reservoir and/or amplification hosts of these arboviruses.[4,15–17] However, the current transmission ecology of these viruses in Southeast Asia remains under-characterized, meaning there is a gap in our understanding of the fundamental biology of these arboviruses in Southeast Asia. Such a gap confounds our ability to respond to and control infections, and to dedicate resources and target education programs for interruption of transmission and infection control. Though our protocol will evaluate all three of these viruses in NHP and human cohorts, the rationale and approach is described below using ZIKV as the prime example.

During the 2015-16 ZIKV epidemic, the Thai Ministry of Public Health reported approximately 700 cases of ZIKV (Thai Ministry of Public Health, personal communication), while nearby Cambodia detected fewer than 10 cases via reverse transcription polymerase chain reaction in retrospective surveys of over 13,000 febrile patients by the Naval Medical Research Unit Two (NAMRU-2) and Institut Pasteur from 2007 to 2016, suggesting transmission is low (personal communication).[8,18] This raises the questions: Aside from differences in surveillance capacity, why did two neighboring countries with similar ecological characteristics experience different transmission intensities of ZIKV? Is there a difference in disease prevalence in the NHPs as well as co-existing humans in each country?

To answer these questions, we propose to compare the serology of humans with the most ubiquitous NHPs, long-tailed macaques (typically *Macaca fascicularis*), in Thailand and Cambodia. The arboviral serologies of these macaques and nearby human populations from central Thailand (where ZIKV human cases were reported during April-August 2017) will be compared with those of macaques from Cambodia living near villages where the presence of the *Aedes* vector(s) is suitable for ZIKV transmission to occur. Collection of sera from the macaques from Thailand is complete, and the ongoing human sampling is set to be completed in March 2020. As we are interested in the immunoglobulin (Ig) G antibody response as an endpoint, the temporal distance is not relevant.

We aim to conduct a similar prospective serosurvey in Cambodia, sampling both human and macaque populations living in proximity to one another within the same season. Macaques were chosen because they are ubiquitous in moderately to heavily human-populated areas around Asia and tend to have heavy human interaction in tourist areas. This means that the possibility for transmission is likely, as they overlap with the human population. This is relevant not only because the macaque could act as a spill-over vector into the human population, but also if there is no ZIKV in Cambodia, it is likely that it will be introduced in heavily touristed areas, meaning there is the potential to establish both anthroponotic and sylvatic cycles. Cambodian macaque serum samples will be collected separately by Thai and Cambodian veterinarians as approved by the Cambodian Ministry of Agriculture, Forestry, and Fisheries, specifically the Director General of Animal Health, within a year of this human study in Cambodia.

An arboviral pediatric cohort study (NIH protocol 18-I-N100, "Investigating vector-borne determinants of *Aedes*-transmitted arboviral infections in Cambodia: an observational longitudinal cohort study in children") is already established by NIAID in a peri-urban area of Kampong Speu province, known for 2 other *Aedes*-transmitted viruses, DENV and CHIKV (Figure 1). Therefore, we propose to prospectively collect sera from long-tailed macaques at Wat Amphae Phnom in Kampong Speu, Cambodia and nearby adults for comparison with sera sampling performed in Thai macaques and nearby villagers.

Figure 1. Cambodia Sampling Map
Left: Blue circle represents Chbar Mon town in Kampong Speu province (in red).
Right: Red circle represents Wat Amphae Phnom and H designates hospital in Chbar Mon town.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

The risk of enrollment in the study is the effect of venipuncture.

Risks of blood draw include pain, bruising, bleeding, local discomfort, lightheadedness, dizziness or possibly fainting and rarely infection or blood clot. The amount of blood drawn will be within the limits allowed for adult participants by the local Cambodian authority NECHR, which is 21 mL in a single visit.

2.3.2 KNOWN POTENTIAL BENEFITS

There is no direct benefit to the participant. Results of this study may contribute to better understanding of the epidemiology and burden of arboviral diseases in this region and inform public health programs in Cambodia.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Given the very low risk of a single venipuncture and the indirect benefit of generalizable knowledge, the risks and benefits of the study have been appropriately weighted.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Describe the seroprevalence of ZIKV, DENV, and CHIKV in humans living in close proximity to long-tailed macaques in Thailand and Cambodia.	Assessment of seroprevalence via screening ELISA and/or PRNT50 titers for ZIKV, DENV, and CHIKV in Cambodian adults.	PRNT assays are the gold standard to detect virus-specific antibodies given multiple circulating flaviviruses in Southeast Asia. Understanding the prevalence of these *Aedes*-transmitted viruses, and how they differ across the Mekong region, is critical to understanding the risk to Cambodians of future epidemics.
Compare the serology patterns against ZIKV, DENV, and CHIKV between NHPs and humans living in the same areas.	Assessment of seroprevalence via screening ELISA and/or PRNT50 titers for ZIKV, DENV, and CHIKV in Cambodian macaques as compared to humans.	Because primates can be amplifying hosts of these viruses, it is critical to understand the prevalence of these *Aedes*-transmitted viruses in macaques as well.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Secondary		
Compare serology patterns against ZIKV, DENV, and CHIKV in macaques and humans between Thailand and Cambodia.	Comparative assessment of seroprevalence via PRNT50 titers for ZIKV, DENV, and CHIKV in Cambodian adults and macaques to that of Thai adults and macaques.	Understanding how the prevalence of these *Aedes*-transmitted viruses differs across the Mekong region is critical for cross-border disease detection and management, particularly given the high level of human migration in the Greater Mekong Subregion.
Compare serology patterns against *Aedes aegypti* salivary gland homogenate proteins in humans and monkeys between Thailand and Cambodia.	Assessment of reactivity to salivary gland homogenate of *Aedes aegypti* as detected by ELISA or western blot in human and macaque sera.	Characterizing vector salivary protein reactivity profiles (mosquitos, ticks, fleas) in Cambodians with vector-borne disease is the first step to better understanding transmission patterns, responsible vectors, and Cambodians' risk of exposure to these vectors.

4 STUDY DESIGN

4.1 SITE DESCRIPTION

Located in Cambodia, the main town of Chbar Mon (estimated population approximately 55,000-60,000 over 10 km^2) is located 44 km from Phnom Penh city center. Chbar Mon is the largest operational district in Kampong Speu province and is the site of the Kampong Speu District Referral Hospital, a hospital of approximately 120 beds with 130 staff of medical doctors, nurses, and lab technicians. NIAID has a field house behind the hospital that serves as an office and temporary clinic when needed.

Wat Amphae Phnom is a pagoda next to a river tributary of the Mekong with 3 different macaque troops, numbering over 75 to 100 per troop. Similar to the sites in Thailand, the pagoda is a popular tourist destination where locals purchase food from vendors to feed the monkeys and also hosts a local marketplace and restaurants (called Resort Amphae Phnom). Over 100 monks also live at the pagoda. The closest residences are in the section of town called Taing Toun Le, where NIAID already has many children enrolled in a healthy pediatric cohort.

4.2 OVERALL DESIGN

This is a multisite, cross-sectional study to procure biospecimens from individuals aged 18 to 55 years who live or work near the monkeys. The NIAID is the data coordinating center and is responsible for overall planning, document collection, monitoring, communication, and data management and analysis

among all involved institutions. All enrollment and human subjects research activities will take place in Cambodia.

4.3 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed the Day 0 assessments and blood draw in the Schedule of Activities (SoA), Section 1.3.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated ICF
2. Able to provide informed consent
3. Stated willingness to comply with study procedures
4. Male or female, aged 18-55 years
5. Live/work within approximately 10 km of the Wat Amphae Phnom monkey habitat for minimum of 2 years
6. In good general health as evidenced by screening medical history
7. Willing to allow biological samples to be stored for future research

5.2 EXCLUSION CRITERIA

1. Any underlying, chronic, or current medical condition that, in the opinion of the investigator, would interfere with participation in the study (e.g., inability or great difficulty in drawing blood)

5.3 INCLUSION OF VULNERABLE PARTICIPANTS

Pregnant women are eligible to screen because excluding pregnant women could potentially bias study results. Their exclusion could omit a portion of the study population who may bear a significant burden of infection, confounding study results and limiting the effectiveness of epidemiological understanding and public health campaigns based on this data. Particularly given the disproportionate impact that ZIKV had on women of child-bearing age in the 2015 epidemic, it is important to comprehensively include this population. Moreover, excluding this population would significantly limit the number of women eligible, leaving them underserved and preventing women from benefiting from the important generalizable knowledge this protocol will provide. Thus, the inclusion of pregnant women is essential to the development of important biomedical knowledge which cannot be obtained by any other means. Additionally, the study procedures (baseline assessment and one-time venipuncture of a small volume of blood) do not have any additional risks to pregnant women or fetuses, and the risk to the woman and fetus is not greater than minimal.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently entered into the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Advertising and recruitment in this province will mainly be via word-of-mouth using provincial health staff and the local village chiefs to communicate to people living and working near Wat Amphae Phnom.

For this biospecimen procurement protocol, we anticipate having to screen no more than 350 to enroll our goal number of 300 participants over the study period. We have no pre-set male:female ratios or age structures.

The anticipated length of individual study participation is limited solely to that time of blood draw and baseline assessments.

Lastly, the typical cost of reimbursement from a blood draw in Cambodia is $5 US (to be paid in Cambodian Riel). We will adhere to this guideline for recruitment and reimburse participants in cash.

6 PARTICIPANT DISCONTINUATION/WITHDRAWAL

6.1 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study procedure non-compliance (e.g., refuses phlebotomy)
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

The reason for participant discontinuation or withdrawal from the study will be recorded on the case report form (CRF).

6.2 LOST TO FOLLOW-UP

This is a cross-sectional study for biospecimen procurement at a single timepoint, so there is no follow-up in the study.

7 STUDY SCHEDULE

This study will primarily take place in the community space at Wat Amphae Phnom or a nearby pagoda, but the Kampong Speu District Referral Hospital is nearby for referral and treatment for any adverse events (AEs) related to blood draw. The study schedule is described below, in the study schema (Section 1.1), and the SoA table (Section 1.3).

7.1 ENROLLMENT

The research team will thoroughly discuss the ICF with each eligible individual and obtain consent prior to performing any study procedures.

7.2 PROCEDURES

Screening (Day 0):
The enrollment will take place in or near the pagoda or other appropriate location as has been done for prior NIAID studies in Kampong Speu using privacy screens, tables, and chairs that are typically set up within the main rooms of the pagoda. The following procedures will be performed:

- Informed consent
- Review of inclusion/exclusion criteria

Enrollment (Day 0):

- Baseline assessments and risk factors
- Blood draw

Any participant who experiences complications due to phlebotomy will be examined by the physician onsite or referred to the hospital and followed until complications have resolved and/or referral to the necessary medical care has been made.

7.3 UNEXPECTED OR INCIDENTAL MEDICAL CONDITIONS OF THE PARTICIPANT

If unexpected or incidental medical conditions are diagnosed during the medical evaluation in this protocol, the participant will be referred to an appropriate physician and/or hospital and encouraged to follow up for treatment of their condition. Standard-of-care treatment may be offered by the study team if necessary while the participant is being referred to appropriate outside medical care.

7.4 RETURN OF RESEARCH RESULTS

Research testing will not occur at the same time as the participant is sampled, and results will not be returned to the participant. This study investigates past exposure to the viruses under study (as evidenced by the presence or absence of antibodies in serum), and individual serostatus would not inform the participant's clinical care at any point in the present or future. Moreover, PRNT assays are not a clinically validated test.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 CLINICAL EVALUATIONS

- **Screening (Day 0):** Prior to conducting any study-related procedures, informed consent in Khmer will be obtained from the participant via in-person interview with an investigator. If illiterate, a literate witness will read and sign the informed consent on behalf of the participant, and the participant will provide a thumbprint. The participant will be queried regarding a history of significant medical disorders and other eligibility criteria. A screening log will be kept to document eligibility of all participants.

- **Enrollment/Visit 1/Exit (Day 0): Baseline Assessments**
    - Baseline demographics will be collected such as sex, age, and behaviors related to vector-borne disease development (e.g., number of domestic water containers), including recent travel and extent of interactions with the macaques.

8.2 STUDY PROCEDURES

8.2.1 PHLEBOTOMY

Venipuncture will be performed for approximately up to 10 mL of blood into a serum-separating tube (SST).

8.3 LABORATORY EVALUATIONS

Specific laboratory methods for each assay and instructions for handling and storage of samples will be maintained in a manual of procedures (MOP) that includes all the standard operating procedures (SOPs) for the study.

8.3.1 SPECIMEN COLLECTION, PREPARATION, HANDLING, AND SHIPPING

All human and NHP biological samples will be received, processed, aliquoted, and stored at the central laboratories of CNM-NIH or Kampong Speu District Referral Hospital according to standard lab procedures.

Given that the purpose of the protocol is to build research capacity in Thailand and Cambodia, CNM-NIH lab personnel may travel between labs with de-identified samples to learn how to optimally prepare their samples in order to bring that knowledge and training back to Cambodia. Similarly, if cross-reactivity is determined to be a problem in the PRNT assays, certain exploratory assays (e.g., blockade of binding or BOB assays) may be carried out in one lab and not the other, necessitating transport of de-identified samples between labs. Samples requiring transport from the clinical collection site to the designated central repository or to the main NIH labs in the US will follow designated study sample shipping procedures, which will include shipment of samples with no participant identifiers.

8.4 SAFETY AND OTHER ASSESSMENTS

8.4.1 ADVERSE EVENT DEFINITIONS

Adverse Event: Any untoward medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

Serious Adverse Event: Any AE that

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Unanticipated Problem Involving Risks to Subjects or Others (UP)**:** Any incident, experience, or outcome that meets all of the following criteria:

1. **Unexpected** (in terms of nature, severity, or frequency) given
    a. the research procedures that are described in protocol-related documents, such as the IRB-approved research protocol and informed consent document; and
    b. the characteristics of the subject population being studied; and
2. **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
3. Suggests that the research places subjects or others (which may include research staff, family members, or other individuals not directly participating in the research) at a **greater risk of harm** (including physical, psychological, economic, or social harm) related to the research than was previously known or expected.

Protocol Deviation: Any change, divergence, or departure from the IRB approved research protocol.

- *Major Deviations:* Deviations from the IRB-approved protocol that have or may have the potential to negatively impact the rights, welfare, or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- *Minor Deviations:* Deviations that do not have the potential to negatively impact the rights, safety, or welfare of subjects or others, or the scientific integrity or validity of the study.

Non-compliance: Failure of investigator(s) to follow the applicable laws, regulations, or institutional policies governing the protection of human subjects in research, or the requirements or determinations of the IRB, whether intentional or not.

- *Serious non-compliance:* Non-compliance, whether intentional or not, that results in harm or otherwise materially compromises the rights, welfare and/or safety of the subject.

Non-compliance that materially affects the scientific integrity or validity of the research may be considered serious non-compliance, even if it does not result in direct harm to research subjects.

- *Continuing non-compliance:* A pattern of recurring non-compliance that either has resulted, or, if continued, may result in harm to subjects or otherwise materially compromise the rights, welfare and/or safety of subjects, affect the scientific integrity of the study or validity of the results. The pattern may comprise repetition of the same non-compliant action(s), or different noncompliant events. Such non-compliance may be unintentional (e.g., due to lack of understanding, knowledge, or commitment), or intentional (e.g., due to deliberate choice to ignore or compromise the requirements of any applicable regulation, organizational policy, or determination of the IRB).

8.4.2 ADVERSE EVENT MANAGEMENT

Subjects entered onto this protocol will only be subject to limited blood sampling. There will be expected events as a result of these procedures. The only AEs that will be recorded on this protocol will be those related to phlebotomy, except for the expected events, as described below.

Expected Events
The following are mild (Grade 1) to moderate (Grade 2) signs or symptoms that are induced by or associated with phlebotomy. If deemed related to phlebotomy by the PI, they will **not** be recorded as AEs in the Research Electronic Data Capture (REDCap) system per protocol, unless deemed by the PI to be abnormal or greater than Grade 2.

- Bleeding or bruising at venipuncture site

Reactions should be graded in the following manner:

Grade 1	Mild transient reaction; interruption not indicated; intervention not indicated.
Grade 2	Interruption indicated but responds promptly to symptomatic treatment (e.g., nonsteroidal anti-inflammatory drugs, narcotics, intravenous fluids); prophylactic medications indicated for less than 24 hours.
Grade 3	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death.

Information on AEs related to research procedures developing after the procedure will be collected by the investigators and entered into a computerized database (REDCap, Section 10.1.6).

Table 1. Attribution of Adverse Events (AEs)

Relationship	Attribution	Description
Unrelated intervention	Unrelated	The AE *is clearly* ***NOT*** *related* to the intervention
	Unlikely	The AE *is* ***doubtfully related*** to the intervention
Related intervention	Possibly	The AE ***may be related*** to the intervention
	Probably	The AE ***is likely related*** to the intervention
	Definitely	The AE ***is clearly related*** to the intervention

8.4.3 REPORTING

The PI is responsible for reporting per NIH IRB and NECHR reporting requirements. Reportable events will be tracked and submitted to the NIH IRB and NECHR as outlined in NIH Human Research Protections Program Policy 801.

According to Policy 801, all UPs, major protocol deviations, all non-compliance, new information that might affect the willingness of a subject to enroll or remain in the study, and any suspension or termination of research activity will be reported within 7 calendar days of investigator awareness. Any death of a research subject that is possibly, probably, or definitely related to the research will be reported within 24 hours of investigator awareness.

The following events will be reported to the NIH IRB and NECHR in summary at the time of continuing review:

- AEs and SAEs that are not UPs, as a narrative summary indicating whether these events were within the expected range.
- Minor and major protocol deviations.
- UPs reported to the NIH IRB/NECHR.
- Non-compliance reported to the NIH IRB/NECHR that is not related to a protocol deviation.

The PI will report UPs, major protocol deviations, and deaths to the NIAID clinical director according to institutional timelines.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

We hypothesize that seroprevalence as measured via ZIKV PRNT50 titers will be lower in Cambodian human adults and macaques compared to Thai human adults and macaques.

9.2 SAMPLE SIZE JUSTIFICATION AND ANALYSIS PLAN

Though we will also be assessing DENV and CHIKV in humans and monkeys, the following statistical plan is primarily guided by the anticipated ZIKV seroprevalence for two reasons. First, DENV has hyperendemic transmission in both Thailand and Cambodia. Therefore, we expect that sample sizes that are adequate for ZIKV will also be adequate for dengue. Second, CHIKV is known to have sporadic and highly focal transmission in both countries, but there is not enough information to develop reasonable assumptions about the expected prevalence. All sites in Thailand and Cambodia have had documented ZIKV transmission in the past.[3,18]

As there are limited data available for the seroprevalence of these arboviruses in macaques in Southeast Asia, we based our assumptions about seroprevalence on a field study in Senegal that identified seroprevalence of CHIKV in three species of monkeys.[19] This study found that the seroprevalence across all three species was high, 72% (479/667). There are no studies evaluating ZIKV seroprevalence in macaques in Southeast Asia, only scattered reports from Malaysia, Brazil, and Africa that focused detection on active viremia, though an African study noted 16% seroprevalence of ZIKV.[6,20,21] Preliminary analyses from our Thai collaborators show that an initial screen for pan-flavivirus antibodies in the Thai macaques is nearly 100% positive (Kob Boonak, Mahidol University, personal communication). Treating sampled macaques as mutually independent simple random samples of binary responses for seropositivity to a given arbovirus, a sample of 100 monkeys per site will yield an average 95% confidence interval (CI) width of less than or equal to 0.2 for seroprevalence at each site, and we will have 80% power to detect a 25% relative reduction in seroprevalence relative to a site where seroprevalence is 75% using a binomial test of proportions with a Type-1 error rate of 0.05.

With regards to the sample size in humans, Thailand and Cambodia have both been endemic for dengue for many years. Only recently has ZIKV been actively detected in both countries.[18,22–24] In Thailand, a serosurvey in a sample of 21 humans estimated ZIKV seroprevalence to be 76% (95% CI: 52%, 91%).[25] Another survey of 600 migrant workers from four Southeast Asian countries, 150 of whom were from Thailand, estimated overall seroprevalence to be 38.8% and seroprevalence among the Thai cohort to be 50.7%.[26] However, in Cambodia, the estimated seroprevalence was lower, estimated to be <1% definitively with 37.3% equivocal results.[8] These equivocal results were based on a potentially cross-reactive IgM assay and indicated a recent flavivirus infection with either Japanese encephalitis virus (JEV), DENV, or ZIKV. Treating sampled humans as mutually independent simple random samples of binary responses for seropositivity, a sample of 300 humans in Cambodia will yield an average 95% CI width of less than or equal to 0.052 for presumed seroprevalence centered at 0.05 (Figure 2).

Our secondary objective is to compare human ZIKV seroprevalence in Cambodia with ZIKV seroprevalence in Thailand. Conservatively taking a value of 50% seroprevalence for ZIKV in Thailand and 37% seroprevalence in Cambodia, we calculate that a sample size of N=300 Cambodian participants and N=300 Thai participants will be sufficient to attain 90% power to reject a null hypothesis of no difference in seroprevalence using a binomial test of proportions with a Type-1 error rate of 0.05.

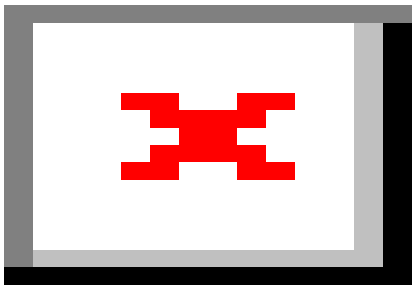


Figure 2. Expected precision of seroprevalence estimate versus sample size and true seroprevalence

9.3 STATISTICAL ANALYSIS

9.3.1 ANALYSIS OF THE PRIMARY ENDPOINT

The primary endpoint will be defined as:

- Assessment of seroprevalence via screening ELISA and/or PRNT50 titers for ZIKV, DENV, and CHIKV in Cambodian adults.
- Assessment of seroprevalence via screening ELISA and/or PRNT50 titers for ZIKV, DENV, and CHIKV in Cambodian macaques as compared to humans.

PRNT50 titer is a continuous variable (e.g., 0 to 40960) as measured from human and macaque sera at a single timepoint. For the purpose of classifying an individual as positive for a particular arbovirus, a PRNT50 titer of 50 will be taken to indicate that an individual has seroconverted (based on distribution of assay, we can consider <10 as seronegative, 10-50 as indeterminate, and >50 as seroconverted). We will estimate seroprevalence rates for humans and macaques, and report summary statistics of seroprevalence and titer distributions overall and within meaningful subgroups (e.g., males vs. females, by decade of age, by occupation, by bed net use, by intensity of self-reported interaction and duration with macaques).

9.3.2 ANALYSIS OF THE SECONDARY ENDPOINT

The secondary endpoints will be defined as

- Comparative assessment of seroprevalence via PRNT50 titers for ZIKV, DENV, and CHIKV in Cambodian adults and macaques to that of Thai adults and macaques.
- Assessment of reactivity to salivary gland homogenate of *Aedes aegypti* as detected by ELISA or western blot in human and macaque sera.

Differences in seroprevalence will be assessed via a two-sample test of binomial proportions, treating participants and each study sample as mutually independent of one another. Pairwise comparisons of PRNT50 titers as continuous variables will be assessed via Wilcoxon rank sum tests as follows: Thai adults versus Cambodian adults, Thai macaques versus Cambodian macaques, and Thai adults and macaques versus Cambodian adults and macaques.

For salivary gland reactivity, arbitrary ELISA units as a continuous variable will be assessed and categories will be made for high, medium, low, and negligible reactivity to salivary gland homogenate. These categories will be compared as: Thai adults versus Cambodian adults, Thai macaques versus Cambodian macaques, and Thai adults and macaques versus Cambodian adults and macaques. Regression models fit via generalized estimating equations will be used to assess if higher antibody reactivity is associated with PRNT50 titer and positive/negative seroprevalence of ZIKV, DENV, or CHIKV.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

ICFs describing in detail the study procedures and risks are given to the participants, and written documentation of informed consent is required prior to starting research procedures and evaluations. The ICFs in English (to be translated into Khmer for submission for Cambodian ethics review and for use in study) are submitted with this protocol.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an ongoing conversation between the human research subject and the researchers that begins before consent is given and continues until the end of the subject's involvement in the research. Discussions about the research will provide essential information about the study in Khmer and include at the very least the purpose, duration, procedures, alternatives, and risks and benefits. Potential participants will be given the opportunity to ask questions and have them answered. Those who are literate will sign and date the ICF (written in Khmer) prior to undergoing any research procedures. In this case, a witness will not sign and date the ICF. If the potential participant is illiterate, a thumbprint will be obtained and the date will be documented by the investigator. In this case, an impartial witness who is literate and not involved with the study will then sign and date the ICF.

Participants may withdraw consent at any time throughout the course of the study. A signed copy of the ICF will be given to them for their records. The investigator will document the signing of the ICF in the participant’s study record. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended (paused) or prematurely terminated (halted) if there is sufficient reasonable cause. If the PI prematurely terminates or suspends then study, then the PI will promptly inform study participants (who have not exited study) and the NIH IRB/NECHR in writing, and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule. The study may resume once concerns about safety, protocol compliance, and data quality are addressed.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators and their staff. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible. In the community, privacy screens will be used for interviews, exams, and phlebotomy.

The study monitor, the Office of Clinical Research Policy and Regulatory Operations (OCRPRO) or their authorized representatives , representatives of the IRB, NECHR, or regulatory agencies may inspect all documents and records required to be maintained by the investigator. The clinical study site staff will permit access to all study records.

The study participant's contact information will be securely stored at the NIH-CNM laboratory in Phnom Penh for internal use during the study. ICFs and enrollment logs will be the only links between the unique study identification numbers and participant names. These will be maintained as paper documents at the NIH-CNM lab in locked cabinets. Access will be restricted to those investigators authorized to access identifiable information. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRBs, institutional policies, or sponsor requirements. CRFs, ICFs, and source documents will eventually be transported from the study site to our offices in Phnom Penh, where they will be retained under lock and key by the investigators listed on this protocol and made available for clinical monitoring.

Study participant research data, which are captured on tablets for purposes of statistical analysis and scientific reporting, will be transmitted to and stored on a central server in Phnom Penh and/or LMVR at NIH main campus. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by research staff will be secured and password protected. At the end of the study, the study database will be archived at LMVR in Rockville, MD.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other

proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Intended Use: Samples, specimens, and data collected under this protocol may be used to study the following:

- Development of new diagnostic tests to enable more sensitive and specific tests for vector-borne diseases.
- Determination of immune response to vector saliva and vector-borne diseases.
- Determination of incidence and pathogenesis of co-circulating infections (e.g., dengue, JEV, chikungunya, Zika, influenza), as they contribute to this community.
- Development of vaccines and therapeutic interventions to prevent or treat vector-borne infections.
- Isolation or sequencing of viruses (e.g., dengue, chikungunya, Zika), parasites, or bacteria.
- Isolation or sequencing related to host immune response or pharmacogenomics.

Storage: All of the stored study research samples are labeled by a code that only the investigators can link to the subject. Samples are stored at the Malaria Vector and Research Laboratory (CNM-NIH laboratory) at the National Center for Parasitology, Entomology, and Malaria Control, a secure facility with limited access, in Phnom Penh, Cambodia. During the high-volume collection, samples may be temporarily stored in the Kampong Speu District Referral Hospital main laboratory, which is also a secure facility with limited access. In the future, it may be necessary to ship a limited number of samples to Mahidol University when Cambodians go to train and learn the immunoassays in the Thai laboratories. As with previous CNM-NIH collaborative studies, it may be necessary to ship a limited number of samples to NIH for analyses that cannot be performed in Cambodia, and these would be kept in a secure facility at the LMVR in Rockville, MD. However, the purpose is to build laboratory capacity in Cambodia and a priority will be to process as many samples as possible here in the CNM-NIH laboratory. Data will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data.

Tracking: Samples and data acquired under this protocol will be tracked using either Microsoft Excel (for input into the Biological Specimen Inventory) or REDCap.

Disposition:

- In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data for research purposes. If the planned research falls within the category of “human subjects research,” appropriate NIH IRB and NECHR review and approval will be obtained. This includes the NIH or CNM researchers sending out coded and linked samples or data and getting results that they can link back to their subjects.

Loss or Destruction:

- Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that meets the definition of a reportable event will be reported to the NIH IRB and the NECHR according to NIH Policy 801.

- Additionally, subjects may decide at any point not to have their samples stored. In this case, the PI will destroy all known remaining samples and report what was done to both the subject and to the NIH IRB and NECHR. This decision will not affect the individual's participation in this protocol or any other protocols at NIH.
- However, withdrawal of consent with regard to bio-sample storage may not be possible after the study is completed. If a subject had previously consented to future use and some of their blood has already been used for research purposes, then the information from that research may still be used even after consent is revoked.

10.1.5 CLINICAL AND SAFETY MONITORING

Clinical site monitoring may be conducted to ensure that the rights and wellbeing of participants giving samples are protected, that the reported data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with ICH GCP, and with applicable regulatory requirement(s).

According to ICH GCP section 5.18, clinical protocols are required to be adequately monitored. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to NIAID/OCRPRO will visit the research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: (1) to verify the existence of signed ICFs and documentation of the informed consent process for each monitored subject; (2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; (3) to compare abstracted information with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and (4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections), and applicable guidelines (ICH GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

During monitoring visits, the PI (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit. The PI will be provided copies of monitoring follow-up letters within 20 days of a visit.

The investigator (and/or designee) will make study documents (e.g., ICF) and pertinent hospital or clinical records readily available for inspection by the NIH IRB, NECHR, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the PI and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status, and regulatory obligations.

The data gathered during this study will be monitored by the PI for safety and compliance with protocol-specified requirements.

10.1.6 QUALITY ASSURANCE AND QUALITY CONTROL

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the PI for clarification/resolution.

REDCap will serve as the core data collection component of the data management system. REDCap, which has been deployed in approximately 100 countries in support of 244,000 studies, is already in use at the site in an ongoing cohort study. It has an extensive range of features, the most important of which are the following:

- The ability to operate from a web browser on a server or laptop and from Android and Apple tablets in disconnected mode and to synchronize data between any of these devices;
- The ability to interface with and receive data from external data management systems and adjudicate data imports prior to incorporation into the database;
- A lightweight application that requires minimal processing power or memory;
- The ability to define and run data quality rules over the duration of the study that can be run on both a real-time and ad hoc basis;
- Support for updates through multiple mechanisms, including programming directly in the system at the site and transmission of updates from the Data Coordinating Center;
- Support for multiple languages, both in the user interface and data collection instruments; and
- Real-time reports, data summaries, and data exports, including frequencies for outlier detection.

Precision and accuracy of actual data collected will be checked by internal procedures at random (5%). Data editing and error resolution will be performed monthly.

Following written SOPs, the monitors will verify that the clinical trial is conducted, data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements.

The study site will provide direct access to source data/documents, reports for the purpose of monitoring and auditing, and inspection by local and regulatory authorities.

10.1.7 DATA HANDLING AND RECORD KEEPING

10.1.7.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Upon enrollment in the protocol, all subjects will be assigned a unique identifying combination code of four to seven numbers depending on assigned protocol number followed by four-digit identification number (e.g., 001-0001) as it is a single site study. The identifying codes will serve to link specimens and data in CRFs and study databases with individual subjects without subject names. Study personnel involved in data acquisition, entry, and analysis will therefore not have access to names or any other identifiers. Standard CRFs containing clinical and laboratory data will be completed by authorized individuals on tablets using the aforementioned REDCap that will be locked with a code and uploaded to password-protected servers. Relevant subject data will be entered into a hybrid system (REDCap plus Datafax as needed, which is already established in the NIAID sites in Cambodia). Electronically captured

data will be checked by study investigators and verified by a data manager. The PI is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

Access to subject records and databases for demographic data analysis will be restricted to the investigators listed on this protocol and those working under their direct supervision. Access to ICFs, which link identifying numbers to names, will be restricted to those investigators authorized to obtain consent.

10.1.7.2 STUDY RECORDS RETENTION

The PI will retain all study records for a minimum of 7 years in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever is longest. The PI will keep all stored records confidential to the extent required by federal, state, and local law. Data captured electronically via tablets will be backed up nightly to the site's central server and transmitted on a weekly basis. All data will be archived at the end of the study and retained for a period of time consistent with IRB requirements.

Should the PI wish to assign the study records to another party and/or move them to another location, the PI will provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. NIAID will be notified in writing and written OCRPRO/NIAID permission shall be obtained by the site prior to destruction or relocation of research records.

10.1.8 PROTOCOL DEVIATIONS

As a result of a protocol deviation (as defined in Section 8.4.1), corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3.
- 5.1 Quality Assurance and Quality Control, section 5.1.1.
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

Protocol deviations must be sent to the NIH IRB and NECHR per their policies (Section 8.4.3). The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.1.9 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

The NIH Public Access Policy 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.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As

such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 5 years after the completion of the primary endpoint by contacting Jessica Manning (NIAID).

Any microbial or human data generated in this study will be shared for future research as follows:

- De-identified data in an NIH-funded or approved public repository.
- De-identified data in another public repository.
- De-identified or identified data with approved outside collaborators under appropriate agreements.
- Data sharing may be complicated or limited in certain cases by contractual obligations or agreements with outside collaborators.

10.2 ABBREVIATIONS

AE	Adverse Event
CFR	Code of Federal Regulations
CHIKV	Chikungunya Virus
CI	Confidence Interval
CNM	National Malaria Center
CRF	Case Report Form
DENV	Dengue Virus
ELISA	Enzyme-linked Immunosorbent Assay
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
Ig	Immunoglobulin
IRB	Institutional Review Board
JEV	Japanese Encephalitis Virus
LMVR	Laboratory of Malaria and Vector Research
MOP	Manual of Procedures
NECHR	National Ethics Committee for Health Research
NHP	Nonhuman Primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
PI	Principal Investigator
PRNT	Plaque Reduction Neutralization (Assay)
QC	Quality Control
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SoA	Schedule of Activities
SOP	Standard Operating Procedure
UP	Unanticipated Problem Involving Risks to Subjects or Others
US	United States

ZIKV	Zika Virus

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Intramural Research Program
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INITIAL APPROVAL

DATE: June 12, 2020

TO: Jessica E Manning
NIAID - I - Laboratory of Malaria and Vector Research

FROM: National Institutes of Health (NIH)
Institutional Review Board (IRB)

RE: **IRB #:** 20IN054
iRIS Reference #: 542312
Investigating the sylvatic transmission and reservoir potential of Zika, dengue, and chikungunya viruses of co-located humans and long-tailed macaques of Thailand and Cambodia

Approval Date: 06/12/2020
Expiration Date: N/A
Risk Level: Minimal Risk under 45 CFR 46
Review Level: Expedited **(Category 2a, 2b, 7)**
Subjects Approved: 350

This study was reviewed and Approved under the National Institutes of Health's Federalwide Assurance (FWA00005897), in accordance with Federal regulations 45 CFR

The response to stipulations was reviewed and accepted on 06/12/2020

The following documents are approved with this submission:

Study Application	Version 1.1	Approved

Submission Components Approved		
Document Type	Version	Date Approved
Submission-Initial Review Submission Form	Version 1.3	06/12/2020
Consent Form-NIH_20IN054_consentclean_22May2020	Version 1.2	06/12/2020

Document-NIH_20-I-N054_studypersonnelpageclean_10Apr2020	Version 1.1	06/12/2020
Document-NIH_20IN054_protocolclean_22May2020	Version 1.3	06/12/2020

Regulatory Determinations:

Expedited Review Category:

Category 2(a): Collection of blood samples by finger stick, heel stick, ear stick, or venipuncture from healthy, nonpregnant adults who weigh at least 110 pounds. For these subjects, the amounts drawn may not exceed 550 mL in an 8 week period and collection may not occur more frequently than 2 times per week

Category 2(b): Collection of blood samples by finger stick, heel stick, ear stick, or venipuncture from other adults and children, considering the age, weight and health of the subjects, the collection procedure, the amount of blood to be collected, and the frequency with which it will be collected. For these subjects, the amount drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period and collection may not occur more frequently than 2 times per week

Category 7: Research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies

Pregnant Women/Fetuses/Neonates:

Inclusion of pregnant women approved under 45 CFR 46.204

Documentation of Informed Consent:

Written consent in accordance with 45 CFR 46.117

Federal regulations and NIH policy require prompt reporting of any unanticipated problems involving risks to subjects or others, or serious harm involving subjects, to the IRB. In addition, changes in research activities may not be initiated without prior review and approval by the IRB, except when necessary to eliminate apparent immediate hazards to subjects.

If you have any questions, contact the IRB Office at 301-402-3713 / email: IRB@od.nih.gov.