Natural History and Immunological Response to Emerging Infectious Disease "X"

DMID Protocol Number: xx-xxxx

Sponsored by:

National Institute of Allergy and Infectious Diseases (NIAID)
Division of Microbiology and Infectious Diseases (DMID)

DMID Funding Mechanism:

Co-Principal Investigators:

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STATEMENT OF COMPLIANCE

Each institution engaged in this research will hold a current Federal wide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research. The Institutional Review Board (IRB)/Independent or Institutional Ethics Committee (IEC) must be registered with OHRP as applicable to the research. The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11 (Electronic Records and Electronic Signatures), 21 CFR Part 312 (Investigational New Drug Application), and 21 CFR 812 (Investigational Device Exemptions)
- The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E6(R2) GCP; 62 Federal Register 25691 (1997); and future revisions
- The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- The policies and procedures of National Institutes of Health (NIH) Office of Extramural Research and Division of Microbiology and Infectious Diseases (DMID)
- The National Institute of Allergy and Infectious Diseases (NIAID) Terms of Award
- Any additional Federal, State, and Local Regulations and Guidance

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signatures below constitute the approval of this protocol and attachments and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements, applicable US federal regulations and ICH guidelines.

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LIST OF ABBREVIATIONS

ADCC	Antibody-dependent cytotoxicity response
ASC	Antibody secreting cell
BAL	Bronchoalveolar lavage
BCR	B-cell receptor
CAPA	Corrective and Preventative Action Plan
CDC	Centers for Disease Control and Prevention
CEIRS	Centers of Excellence for Influenza Research and Surveillance
CFR	Code of Federal Regulations
COV	Coronavirus
COVID-19	Coronavirus Disease 2019 (aka SARS-CoV-2)
CQMP	Clinical Quality Management Plan
CRF	Case Report Form
DHHS	Department of Health and Human Services
DNA	Deoxyribonucleic acid
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DMP	Data Management Plan
DPO	Days post-illness onset
ELISA	enzyme-linked immunosorbent assay
ELISpot	Enzyme-linked immune absorbent spot
FDA	Food and Drug Administration
FDR	False discovery rate
FRNT	Focus Reduction Neutralization Test
FWA	Federalwide Assurance
FWER	Family-wise error rate
GCP	Good Clinical Practice
GEE	Generalized estimating equations
Hgb	Hemoglobin (blood test)
HIPAA	Health Insurance Portability and Accountability Act
HIPC	Human Immunology Project Consortium
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICS	Intracellular Cytokine Staining
ICU	Intensive Care Unit
IDCRC	Infectious Diseases Clinical Research Consortium
IEC	Independent Ethics Committee
IFN	Interferon
IRB	Institutional Review Board
IVIG	Intravenous immunoglobulin

kg	kilogram
LAR	Legally authorized representative
MAR	Missing at random
MCAR	Missing completely at random
MERS-CoV	Middle Eastern Respiratory Syndrome virus
mg	milligram
mL	Milliliter
mmHg	Millimeter of Mercury
MOP	Manual of Procedures
N	Number (typically refers to subjects)
nAbs	Neutralizing antibodies
NETEC	National Emerging Special Pathogen Training and Education Center
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
NK cells	Natural killer cells
NSAID	Nonsteroidal Anti-Inflammatory Drug
OHRP	Office for Human Research Protections
PBMC	Peripheral Blood Mononuclear Cell
PaO2/FiO2	Partial pressure oxygen/fractional inspired oxygen
PCR	Polymerase chain reaction
PD	Protocol Deviation
PI	Principal investigator
PRNT	Plaque reduction neutralization test
QA	Quality Assurance
QC	Quality Control
R	Correlation coefficient
RNA	Ribonucleic acid
ROC	Receiver operating characteristics
SARS-CoV-1	Severe Acute Respiratory Syndrome coronavirus 1
SARS-CoV-2	Severe Acute Respiratory Syndrome coronavirus 2
SBP	Systolic Blood Pressure
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SCID	Severe combined immunodeficiency
SDSU	Statistical and Data Science Unit
SOE	Schedule of Events
SOP	Standard Operating Procedure
TCR	T-cell receptor
US	United States
VTEU	Vaccine Treatment and Evaluation Unit
WGS	Whole genome sequencing

PROTOCOL SUMMARY

Title: Natural History and Immunological Response to Emerging Infectious Disease "X"

Population: Up to 1,000 participants[1] (male and female adults ≥18 years of age and male and female children <18 years of age) with possible or definitive infection and/or close contacts to patients with possible or definitive infection with Pathogen “X” will be enrolled into one of two patient groups. Suspected cases may be based on appropriate exposure history and/or clinical presentation. Enrollment will be stratified by disease severity or other major categories of disease presentation as applicable.

Group 1: Adults or children with confirmed or suspected Pathogen “X” infection representative of the disease spectrum

Group 2: Adult or child close contacts of Pathogen “X” confirmed and/or suspected cases enrolled in Group 1

Number of Sites: Up to ten VTEU sites

Study Duration: Approximately 2 years from implementation of field activity through closure and analysis

Participant Duration: 12 months

Objectives: Primary:

- To characterize Pathogen “X” binding and neutralizing antibody responses by age and disease severity in study subjects.
- To characterize T cell responses by age and disease severity.
- To characterize B cell responses by age and disease severity.
- To describe the clinical presentation and outcomes of Pathogen “X” infection in study subjects.

[1] Number of participants may be adjusted based on statistical considerations of Pathogen “X” and clinical manifestation.

Secondary:

- To describe innate immune responses to Pathogen "X”.
- To collect specimens for isolation of Pathogen “X” from body compartments pertinent to the pathogen.
- To determine rates of Pathogen “X” infection among close contacts of infected patients enrolled in Group 1.
- To determine the persistence of Pathogen “X” in whole blood, serum, urine, saliva, stool, or other body fluids as available (e.g., semen, vaginal secretions, breast milk) and characterize modes of pathogen shedding to support the development of diagnostic assays and public health prevention policy around transmission risk.

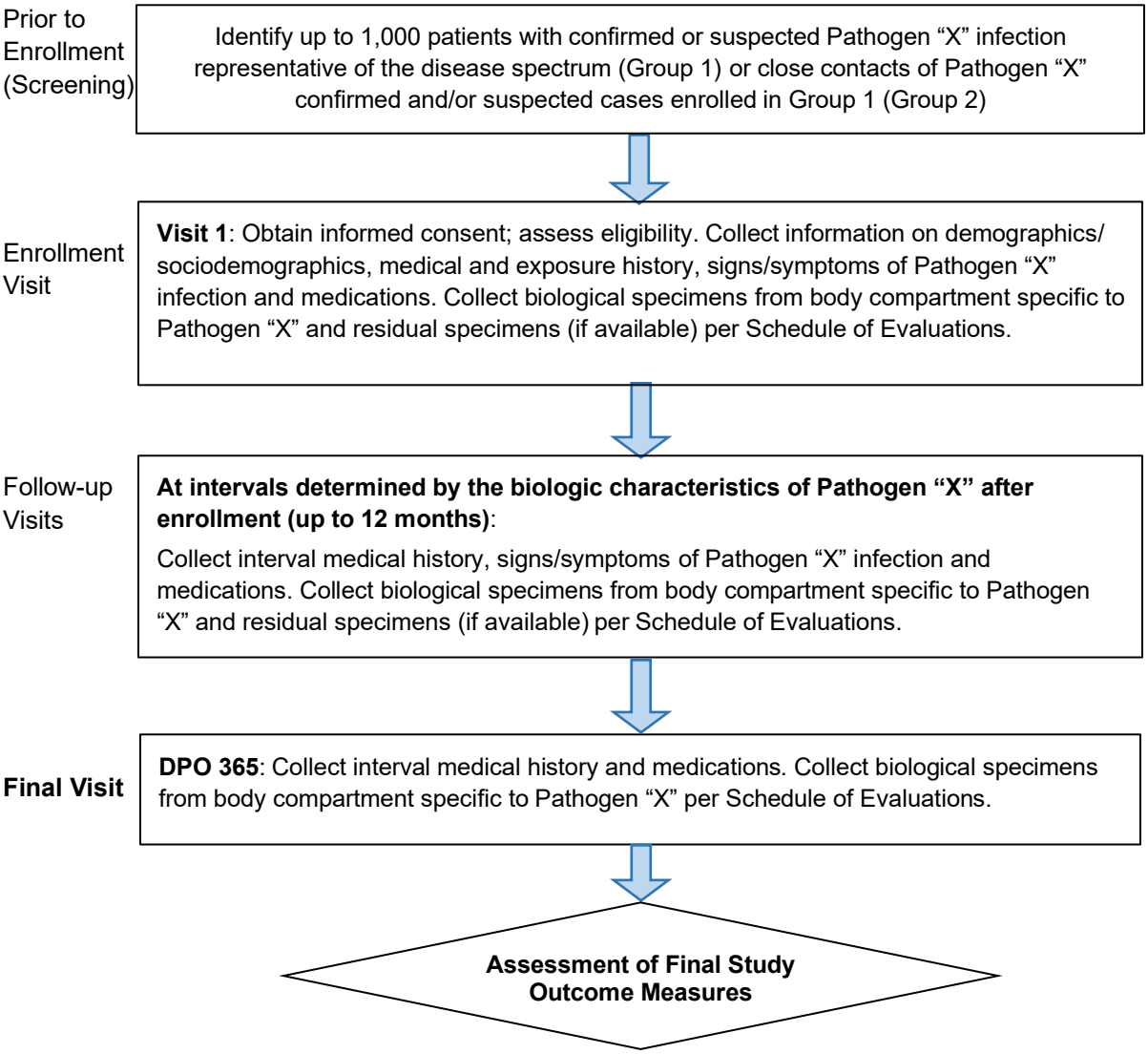
Exploratory:

- To characterize Pathogen “X”-specific immune responses other than classical antibody and T cell responses following Pathogen “X” infection to support vaccine development.
- To make available to the scientific community well-characterized specimens from Pathogen “X”-infected study subjects to facilitate development of diagnostic tests, vaccines, and therapeutics for use in other Pathogen “X” research.
- To collect large numbers of blood immune cells by leukapheresis from selected participants to establish a specimen bank for future detailed Pathogen “X” immunological studies.
- To evaluate the associations of Pathogen “X”-specific humoral and cellular immune responses with Pathogen “X” in whole blood, serum, exosomes, urine, saliva, stool and/or other body fluids as available (e.g., semen, vaginal secretions, breast milk).
- To sequence Pathogen “X” from collected specimens (blood, body compartments specific to Pathogen “X”, etc.) in a subset of participants for the study of within-host pathogen evolution.

Description of Study Design:	This is a prospective, observational, noninterventional cohort study designed to collect clinical information and specimens to evaluate innate and adaptive immune responses of patients with confirmed Pathogen “X” and their close contacts.
Estimated Time to Complete Enrollment:	2 years. This protocol will be shelf ready for specimen collection from potentially emerging pathogens over the life cycle of the current IDCRC.

SCHEMATIC OF STUDY DESIGN

Total N: Up to 1,000 participants enrolled into one of two patient groups



1. KEY ROLES

For questions regarding this protocol, contact: *(insert name of DMID CPM or other appropriate DMID staff)* at NIAID/DMID *(insert contact information)*

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2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

The world has observed the emergence of a number of novel pathogens with geographic spread beyond original restrictions, and pandemics over the past 100+ years. Emergence of these novel pathogens has often occurred across the animal-human interface. Influenza has had a number of antigenic shifts occur including the 1918 influenza pandemic, H2N2, H3N2, H1N1, and p2009H1N1. Other influenza strains have also leapt into humans from animal populations including H5N1, H5N8, H7N9, and H3N2v. Human immunodeficiency virus (HIV) spread into the human population with initial descriptions of AIDS occurring in 1981. Outbreaks of Ebola continue to threaten various communities in Africa. Coronaviruses have spread into the human population with person-to-person transmission, including severe acute respiratory syndrome (SARS), Middle Eastern respiratory syndrome (MERS), and severe acute respiratory syndrome 2 (SARS-CoV-2) which causes coronavirus disease-19 (COVID-19). Geographic spread of viral pathogens has occurred well beyond their original borders including West Nile Virus, Zika, Chikungunya, dengue, and others. Both the 1918 influenza and the COVID-19 pandemics resulted in hundreds of thousands of deaths in the US and millions of deaths worldwide.

2.2 Scientific Rationale

With increasing world population, climate change, and globalization, it is likely that emergence and spread of new infectious threats will continue to occur and could result in potential pandemics. The National Institutes of Allergy and Infectious Diseases (NIAID) and the Vaccine Treatment and Evaluation Units (VTEUs) need to be ready to respond to such emerging threats through efforts to develop assays, understand the immunological response to infection, and develop new vaccines for these emerging pathogens. The proposed study aims to be ready to respond to the emergence or reemergence of a pathogen into the US (Pathogen “X”).

To determine the best approaches to advance vaccines and therapeutics, an understanding of the pathogenesis and immune responses to the infection in different populations is important. Collection of samples of the pathogen can be crucial to facilitating laboratory assay work, including development of pathogen diagnostics (e.g., PCR). Genome sequencing samples of the pathogen can also be used to study within-host pathogen evolution. Pathogen samples can also provide insight into persistence and transmissibility. Sampling close contacts (e.g., household) can provide additional insight into transmission patterns and provide adequate experimental controls. Detecting and describing the innate and adaptive (T and B-cell, antibodies, memory) responses to natural infection is needed to understand the kinetics and durability of such responses. In addition to the kinetics, the quality of the immune response may also be relevant, such as the neutralizing, non-neutralizing, and overall binding antibody

responses. Differences in the immunological response of various populations (e.g., age) and by disease severity could provide insights into markers of an effective immune response. This can ultimately help to identify potential correlates of protection against infection. For example, non-human primates infected with a live SARS-COV-2 or injected with either an RNA or DNA vaccine expressing the S protein developed neutralizing antibodies and were protected from subsequent live SARS-CoV-2 challenge.[1,2] Description of these immunological responses from samples collected longitudinally from patients with specific disease severity and in different age groups is needed. It can also identify potential host differences due to genetic factors. Identifying these immunological responses can result in rapid advancement of new vaccines to address a new emerging pathogen.

For example, global mobilization occurred with the COVID-19 pandemic. The response to the challenge in the US was facilitated by rapid implementation of studies, communication of information, and combined efforts of the federal government, industry, academia, and many other institutions. This allowed the FDA to issue Emergency Use Approval for 2 vaccines about 11 months after the initial sequences from China were published, an unprecedented feat.[3,4]

NIAID supports research critical to advancing human health and prevention of disease. VTEUs are a critical component of response (see Section 2.2.1). This protocol is written to stand 'at the ready' to be activated for the emergence of Pathogen “X” that could threaten national and/or global health.

2.2.1 VTEU Expertise

The VTEUs comprise 10 US sites with capacity to perform a wide variety of clinical studies and collect clinical information and specimens. A pilot study of Zika was conducted at Emory University, as well as a natural history study of Zika virus infection (DMID 16-0017 at Baylor, Emory and Saint Louis University VTEUs) for a rapid research response to Zika virus infections in US residents. Together these studies generated ground-breaking findings, resulting in publication of multiple manuscripts including case reports, viral shedding kinetics, innate and adaptive responses, and development of a Zika antibody dependent cellular cytotoxicity (ADCC) assay.[5-11] Although we are aware that other efforts are underway through other NIAID networks currently (e.g., NETEC, CEIRS, HIPC), we believe the VTEUs have a unique capacity to respond to the Pathogen “X” pandemic, given the VTEU’s demonstrated ability to enroll and follow patients both in and out of the hospital and given the prior experience of DMID 16-0017.

Development of this study by the Emory, Baylor, and Saint Louis University VTEUs (a joint effort combining efforts from several independently submitted concepts), based upon the framework of the prior DMID 16-0017, will provide the opportunity for standardized sample collection and testing and facilitate the development of vaccines and therapeutics. It could also provide important data about potential correlates of protection.

2.2.2 Importance and Impact

Unique aspects of this study include: 1) enrollment of patients representative of the disease spectrum with serial specimen sampling (including early time points) and long-term follow-up (e.g., memory immune response); 2) comparisons of different age cohorts spanning from childhood to the elderly; 3) investigations of close contacts ensuring earlier studies of innate and adaptive immunity; and 4) the collection of specimens for development of immunological assays and assessment of responses over time.

We believe that this study is critical for several reasons:

- It provides prospectively collected specimens from patients with well-defined time points that will enable the advancement of our understanding of the innate and adaptive responses to Pathogen “X”.
- The study includes both adults and children with Pathogen “X”. Data from children were by-in-large missing from the COVID-19 pandemic resulting in deprioritization of vaccine development in this population. Children have different immune responses to infection compared to adults and are critically important in the transmission of many different pathogens and could be relevant for Pathogen “X”.
- It has high value in collecting samples from close contacts, exposed subjects and/or suspected cases before Pathogen “X” infection occurs along with serial sampling to detect potential secondary transmission. Enrollment of close contacts is additionally valuable for identifying and studying cases of asymptomatic infection, which can be otherwise challenging. Samples from uninfected close contacts will also serve as controls.
- This study promises an opportunity to collect specimens that could be critical to future efforts to develop vaccines, assays and validation specimens.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The potential risks of participating in this trial are those associated with having blood drawn, collection of other body fluid specimens, and leukapheresis (only for those adult volunteers consented for leukapheresis, see Sec 2.3.1.1).

Drawing blood may cause temporary discomfort and fainting. Fainting is usually transient and managed by having the subject lie down and elevate his/her legs. Bruising at the blood draw site may occur but can be prevented or lessened by applying pressure to the blood draw site for a few minutes after the blood is taken. Drawing blood may cause infection. The use of aseptic (sterile) technique will make infection at the site where blood will be drawn extremely unlikely.

A urinalysis is considered a safe and non-invasive form of testing. The only risk it may pose is for those who require catheterization to obtain a urine sample. The risks of urinary

catheterization include infection, bleeding, pain, and bladder damage. There are no known risks for collection of urine by routine clean catch methods.

Risks are minimal with a mouth swab. One may experience a dry area in the mouth at the site of swabbing for a short period of time (less than a few minutes). This dryness is transient and will resolve on its own.

The risks of collecting specimens from the upper respiratory tract using oropharyngeal and nasopharyngeal swabs include gagging, feeling uncomfortable and possible minor nosebleed afterwards. Complications of oropharyngeal and nasopharyngeal suction include hypoxia and bradycardia.

The risks of collecting specimens from the lower respiratory tract using bronchoalveolar lavage (BAL) (in which a bronchoscope is passed through the mouth or nose into an appropriate airway in the lungs, with a measured amount of fluid introduced and then collected for examination) include transient hypoxemia, post-BAL fever (seen in up to 30% of patients), bronchospasm, and very rarely pneumothorax.

Risks are minimal with a vaginal swab. There may be slight transient irritation or discomfort when the swab is placed in the vaginal vault at the time of sampling. This is an optional sample in the study. The sample will be collected by a vaginal swab and not by intracervical swabs. The sample will be collected either through self-swab (by the subject) or collected during a clinically indicated vaginal exam. For pregnant women, if there are concerns about rupture of membranes the vaginal swab will not be collected.

There are no known additional risks for a lactating woman who is already expressing breast milk to donate a small amount of breast milk.

Risks are minimal with self-semen collection. This is an optional sample in the study. One may feel embarrassment. To try to minimize this, subjects who agree to provide a semen sample will have access to a private room to obtain the sample or will be able to obtain the sample at home.

Participation in research may involve a loss of privacy. Subject records will be kept as confidential as possible under the law. Individual identity will not be used in any reports or publications resulting from this trial.

2.3.1.1 Risks of Leukapheresis (only for those adult volunteers consented for leukapheresis)

Leukapheresis may be associated with pain, bruising, and discomfort in the arms at the site of needle placement. Vasovagal episodes, characterized by transient hypotension, dizziness, nausea, and rarely syncope, are seen in less than 5% of procedures. Additional risks include increased pulse, seizures, and blood loss. Anticoagulants added to prevent the blood from clotting may lead to a sour taste in the mouth, mild muscle cramps and/or tingling sensation around the mouth, feet, or hands. Mild reactions may be seen in 30-50% of leukapheresis procedures and can usually be relieved by slowing or temporarily interrupting the procedure or administering calcium carbonate tablets.

A temporary decrease (1-2 days) in red blood cell count is common. Rarely, machine malfunction may result in the loss of a half pint to a pint of blood. Leukapheresis does not affect the blood’s ability to form clots in the event of subsequent cuts or injuries.

2.3.2 Known Potential Benefits

There is no direct benefit to the subjects. There is potential benefit to society resulting from insights gained from participation in this study due to the emerging threat of the Pathogen “X” outbreak.

3. OBJECTIVES

3.1 Study Objectives Endpoints (Outcome Measures)

Table 1: Objectives and Endpoints (Outcome Measures)

Objectives	Endpoints
Primary	
• To characterize Pathogen “X” binding and neutralizing antibody responses by age and disease severity in study subjects	• Pathogen “X” neutralization assay (e.g., PRNT, FRNT) • Pathogen “X” pseudoneutralization assay
• To characterize T cell responses by age and disease severity	• T cell ELISpot and/or ICS assays
• To characterize B cell responses by age and disease severity	• Memory B cell testing • Plasmablast antibody secreting cells (ASCs) responses
• To describe the clinical presentation and outcomes of Pathogen “X” infection in study subjects	• Clinical symptoms, laboratory abnormalities, days of illness, residual clinical problems, survival/death
Secondary	
• To describe innate immune responses to Pathogen "X”	• Transcriptomics, flow cytometry, serum and/or culture supernatant cytokine ELISA
• To collect specimens for isolation of Pathogen “X” from body compartments pertinent to the pathogen	• PCR and/or cultures
• To determine rates of Pathogen “X” infection among close contacts of infected patients enrolled in Group 1	• Rates of Pathogen “X” detection among close contacts of infected enrolled subjects at enrollment and over follow-up
• To determine the persistence of Pathogen “X” in whole blood, serum, urine, saliva, stool, or other body fluids as available (e.g., semen, vaginal secretions, breast milk) and characterize modes of pathogen shedding to support the development of diagnostic assays and public health prevention policy around transmission risk	• Repeated PCR and/or cultures

Objectives	Endpoints
Exploratory	
• To characterize other Pathogen “X”-specific immune responses other than classical antibody and T cell responses following Pathogen “X” infection to support vaccine development	• Antibody Dependent Cellular Cytotoxicity, BCR repertoire, TCR repertoire, single cell phenotyping, targeted host genetic changes, immune response gene expression, and/or other endpoints relevant for Pathogen “X”
• To make available to the scientific community well-characterized specimens from Pathogen “X”-infected study subjects to facilitate development of diagnostic tests, vaccines, and therapeutics for use in other Pathogen “X” research	• Collection and storage of samples
• To collect large numbers of blood immune cells by leukapheresis from selected participants to establish a specimen bank for future detailed Pathogen “X” immunological studies	• Leukapheresis on selected subjects to obtain large numbers of blood immune cells for more detailed immunologic future studies
• To evaluate the associations of Pathogen “X”-specific humoral and cellular immune responses with Pathogen “X” in whole blood, serum, exosomes, urine, saliva, stool and/or other body fluids as available (e.g., semen, vaginal secretions, breast milk)	• PCR culture of specific samples or products derived from specific samples
• To sequence Pathogen “X” from collected specimens (blood, body compartments specific to Pathogen “X”, etc.) in a subset of participants for the study of within-host pathogen evolution.	• Whole genome sequencing (WGS) of Pathogen “X” from specimens collected in a subset of participants at enrollment and over time post-enrollment.

4. STUDY DESIGN

This is a prospective, observational, noninterventional cohort study designed to collect clinical information and specimens to evaluate the innate and adaptive immune responses from up to 1,000 study participants. Both males and females will be screened for study interest and eligibility and enrolled, adults ≥18 years of age and children <18 years of age. Two types of participants will be recruited:

- Group 1: Patients with confirmed or suspected Pathogen “X” infection, and
- Group 2: Close contacts of patients with confirmed and/or suspected Pathogen “X” infection enrolled in Group 1.

The study will ensure enrollment of patients that have clinical symptoms representative of the disease spectrum.

Group 1

Baseline data will be collected from participants with confirmed or suspected Pathogen “X” infection including demographics/sociodemographics, medical history, exposure history, medications, signs/symptoms and symptom onset date to determine the days post-illness onset (DPO). Specimens will be collected from body compartment specific to Pathogen “X”. Specimens may include whole blood, serum, urine, saliva, stool or other body fluids as available (e.g., semen, vaginal secretions, breast milk) depending on the specifics of Pathogen “X”. Specimens may be salvaged if available from standard of care testing. Clinical information and specimens will be collected at enrollment and then at an interval determined by the biologic characteristics after enrollment (up to 12 months). See Section 6 Study Procedures/Evaluations.

The association between age, severity of illness, and duration of illness on Pathogen “X” immune responses will also be explored.

An optional study visit will be conducted between DPO 30 and 90 in adult participants from Group 1 who volunteer to undergo leukapheresis. Leukocytes will be selectively harvested; red cells and other blood components will be returned to the patient. Participation will be offered to all patients and volunteers will be accepted until the spots are filled. In a typical 1½ to 3-hour leukapheresis procedure, approximately $5x10^8$ to $1x10^{10}$ cells can be isolated with only minimal loss of red blood cells. No sedation is required. The procedure will be done by trained staff at the study site and will be done using devices and procedures that conform to standard guidelines and SOPs.

Identification of close contacts will take place as soon as possible after Group 1 participants are enrolled.

Group 2

Close contacts, both symptomatic and asymptomatic, will be systematically identified, approached, consented, and enrolled into Group 2 (both children and adults) as soon as possible after their confirmed and/or suspected Pathogen “X” infected contact was enrolled.

Baseline data will be collected from Group 2 participants including demographics/ sociodemographics, medical history, exposure history, medications, and signs/symptoms Specimens will be collected from body compartment specific to Pathogen “X”. Specimens may include whole blood, serum, urine, saliva, stool or other body fluids as available (e.g., semen, vaginal secretions, breast milk), depending on the specifics of Pathogen “X”. Clinical information and specimens will be collected at enrollment and then at an interval determined by the biologic characteristics after enrollment (up to 12 months). See Section 6 Study Procedures/Evaluations.

5. STUDY POPULATION

5.1 Selection of the Study Population

Up to 1,000 male and female participants, adults ≥18 years of age and children <18 years of age, will be screened to enroll participants who meet all eligibility criteria and agree to participate.

Patients will be recruited from 10 VTEU sites and/or their sub-sites. Sites can enroll from either outpatient or inpatient sites, or both. Enrollment will be stratified by disease severity or other major categories of disease presentation as applicable. Pregnant women will not be excluded from enrollment. Blood collection volumes will be adjusted accordingly for these participants. There will not be a required sample size for this population. As this study is a descriptive study, sample size is based on feasibility instead of power for hypothesis testing.

- Group 1: Patients with confirmed or suspected Pathogen “X” infection, and
- Group 2: Close contacts of patients with confirmed and/or suspected Pathogen “X” infection enrolled in Group 1.

A subset of 10-20 adult volunteers from Group 1 will undergo leukapheresis during follow-up to compare immunological responses in those with variable severity and also to allow future comparisons with those receiving Pathogen “X” vaccines (enrolled in other studies).Study staff will inform potential participants/legally authorized representatives (LAR) of the study, obtain informed consent, and determine study eligibility. Individuals who meet all study eligibility criteria and agree to participate will be enrolled.

Usual clinical management at VTEU outpatient or inpatient sites, including standard medical care and standard therapy, will not be modified because of the study. All participants will receive appropriate treatment for Pathogen “X” that is accepted by medical experts as standard of care for the specific institution and is widely used by healthcare professionals.

5.2 Inclusion/Exclusion Criteria

5.2.1 Subject Inclusion Criteria

Subjects must meet all the following inclusion criteria to be eligible to participate in the study:

- Must provide informed consent or have a LAR who provides informed consent.
- Must be available and willing to participate for the duration of this trial.
- Possible or definitive Pathogen “X” infection, or close contacts of patients with possible or definitive Pathogen “X” infection.

5.2.2 Leukapheresis Inclusion Criteria

A subject must meet all the following criteria to be eligible for leukapheresis:

- Written informed consent for leukapheresis is provided.
- Age ≥18 years old.
- Weight ≥110 pounds.
- Screening laboratory (e.g., Hgb) and clinical (e.g., pulse, blood pressure) evaluations are within acceptable normal reference ranges at the site where the leukapheresis procedure will be performed.
- Negative urine or serum pregnancy test within 48 hours of the leukapheresis procedure for women of childbearing potential.
- Adequate bilateral antecubital venous access.
- No use of blood thinners, aspirin or NSAIDs at least 5 days before the leukapheresis procedure.
- Must be an outpatient at time of leukapheresis procedure.
- The investigator deems the participant is medically stable to undergo the leukapheresis procedure.

5.2.3 Subject Exclusion Criteria

Subjects meeting any of the following criteria at baseline will be excluded from study participation:

- The investigator feels, for any reason, that participation in the study is unsafe for the patient or inappropriate for achievement of the study objectives.
- Anticipated mortality within 24 hours of enrollment.
- Receipt of intravenous immunoglobulin (IVIG), plasma, or monoclonal antibody directed against Pathogen “X”.
- Receipt of an investigational immunomodulator or enrollment in a treatment trial that includes potential randomization to receive an investigational immunomodulator (e.g., baricitinib, anakinra, sarilumab, siltuximab, tocilizumab, interferon).[2]
- Prior or current participation in a Pathogen “X” vaccine clinical trial.

[2] This does not include steroids (e.g., prednisone, dexamethasone, solumedrol) or antivirals (e.g., remdesivir, lopinivir/ritonavir).

- Patients with immunosuppressive conditions (e.g., uncontrolled HIV defined as stable CD4 <400 and/or viral load >1,000 within 3 months of enrollment, solid organ transplant, active cancer treatment).

5.3 Reasons for Withdrawal

Participants may voluntarily withdraw their consent for further study participation at any time and for any reason without penalty or prejudice to future medical care. Participants may be withdrawn from further study participation for the following reasons:

- Medical disease or condition, or any new clinical finding for which continued participation, in the opinion of the site principal investigator (PI) or appropriate sub-investigator, would compromise the safety of the participant, interfere with the participant’s successful completion of the trial, or interfere with the evaluation of responses.
- As deemed necessary by the site PI or appropriate sub-investigator for noncompliance or other reason.
- Termination of the study.

5.3.1 Handling of Withdrawals

Participants who withdraw their consent from further study participation will not continue any study procedures and will no longer be contacted for follow-up. Participants who are withdrawn from further study participation by the site PI or appropriate sub-investigator for any reason will not continue study procedures.

If participants fail to appear for a follow-up visit, extensive effort (e.g., three documented contact attempts via phone calls or emails, made on separate occasions and followed by a certified letter) will be made to locate or recall them or at least to determine their health status. Subjects who cannot be located after extensive effort will no longer be contacted for follow-up. These efforts will be documented in the subject’s records.

5.3.2 Termination of Study

Although the study sponsor has every intention of completing the trial, the sponsor reserves the right to terminate the trial at any time for clinical or administrative reasons.

6. STUDY PROCEDURES/EVALUATIONS

Study visit information is listed in this section and Appendix A. Further instructions are described in the protocol-specific Manual of Procedures (MOP).

6.1 Group 1: Participants with Confirmed or Suspected Pathogen “X” Study Procedures

6.1.1 Visit 1 – Screening/Enrollment/Baseline Visit, Day 1

Sites will follow their site-specific instructions for approaching and screening potential participants for the enrollment visit. All participants successfully screened, consented, and enrolled in the study will undergo the following procedures:

- Informed consent (written, electronic or verbal) to participate in the study
- Assessment of eligibility
- Enrollment in the study
- Collection of patient information including demographics/sociodemographics, medical history, exposure history, medications, and medical record review if available
    - Sociodemographic information will include the following: age, sex, race, ethnicity, household income category, highest education achieved, previous receipt of assistance in housing or food, employment, number of persons in household, and travel history (as applicable).
- Collection of signs and symptoms of Pathogen “X” infection
- Collection of contact information
- Collection of hospitalization status (Hospitalized ICU, Hospitalized non-ICU, Outpatient)
- Physical exam may be performed to assess the need for advanced care in participants
- Vital signs[3] may be collected as necessary
- Height and weight will be recorded from medical record if available
- Collection of biological specimens including:
    - Specimens from body compartment specific to Pathogen “X” to test for infection
    - 10mL blood for serum from adults
    - 2.5mL blood for serum from children

[3] Temperature, heart rate, respiration rate, pulse oximetry, etc.

- 24mL blood for PBMCs from adults with significant anemia (Hgb<10), compromised cardiac output and/or significant respiratory compromise
- 60mL blood for PBMCs from adults without significant anemia (Hgb≥10) and without compromised cardiopulmonary functions
- Pediatric blood volumes for PBMCs will total ≤1 mL/kg; see MOP for specific details.

- Collection of residuals from standard of care specimens (if available)[4]

6.1.2 Follow-up Visits
At intervals determined by the biologic characteristics of Pathogen “X” after enrollment (up to 12 months)

Participants will undergo the following procedures as necessary:

- Collection of patient information including interval medical history, medications, and medical record review if available
- Collection of signs and symptoms of Pathogen “X” infection
- Review contact information
- Physical exam may be performed to assess the need for advanced care in participants
- Vital signs may be collected as necessary
- Collection of specimens from body compartment specific to Pathogen “X”
- Collection of residuals from standard of care specimens (if available)

Participants will have the following biological specimens collected as necessary:

- Specimens from body compartment specific to Pathogen “X”
- 10mL blood for serum from adults
- 2.5mL blood for serum from children
- 24mL blood for PBMCs from adults with significant anemia (Hgb<10), compromised cardiac output and/or significant respiratory compromise
- 60mL blood for PBMCs from adults without significant anemia (Hgb≥10) and without compromised cardiopulmonary functions
- Pediatric blood volumes for PBMCs will total ≤1 mL/kg; see MOP for specific details.

[4] Including blood, sputum, endotracheal aspirates, bronchoalveolar lavage and saliva

6.1.3 Optional Visit – Leukapheresis (Window: DPO 30 and 90)

To support development of diagnostics, therapeutics and vaccines, a subset of adult volunteers enrolled in Group 1 may undergo leukapheresis to collect additional samples for secondary research.

Leukapheresis is an outpatient procedure during which leukocytes will be selectively harvested; red cells and other blood components will be returned to the subject. In a typical leukapheresis procedure, approximately $5x10^8$ to $1x10^{10}$ cells can be isolated with only minimal loss of red blood cells. No sedation is required. The procedure will be done by trained staff at the study site and will be done using devices and procedures that conform to standard guidelines and SOPs.

For participants consented for leukapheresis, the following screening procedures will be performed locally during the week prior to the leukapheresis procedure; fasting is not required before collection of the following:

- Collection of blood for Hgb and/or other locally required laboratories
- Conduct urine or serum pregnancy test within 48 hours of the leukapheresis procedure for women of childbearing potential. Results must be confirmed as negative prior to leukapheresis.

Refer to the protocol-specific MOP for details on the leukapheresis procedure.

6.1.4 Final Visit, DPO 365 (Window: Day 351-379)

Participants will undergo the following procedures as necessary:

- Collection of patient information including interval medical history, medications, and medical record review if available
- Review contact information
- Physical exam may be performed.
- Collection of biological specimens including:
  - Specimens from body compartment specific to Pathogen “X”
  - Collection of 10mL blood for serum from adults
  - Collection of 60mL blood for PBMCs from adults without significant anemia (Hgb≥10) and without compromised cardiopulmonary functions
  - An optional blood draw for children will occur at this visit. Collection of 2.5 mL blood for serum and blood volumes for PBMCs will total ≤1 mL/kg; see MOP for specific details.

6.2 Group 2: Close Contacts of Pathogen “X” Patients Enrolled in Group 1 Study Procedures

6.2.1 Screening/Enrollment/Baseline Visit, Day 1

Sites will follow their site-specific instructions for approaching and screening potential participants for the enrollment visit. All participants successfully screened, consented, and enrolled in the study will undergo the following procedures.

- Informed consent (written, electronic or verbal) to participate in the study/(and assent for participants who are children) will be obtained.
- Assessment of eligibility
- Enrollment in the study
- Collection of patient information including demographics/sociodemographics, medical history, exposure history, medications, and medical record review if available
    - Sociodemographic information will include the following: age, sex, race, ethnicity, household income category, highest education achieved, previous receipt of assistance in housing or food, employment, number of persons in household, and travel history (as applicable).
- Collection of signs and symptoms of Pathogen “X” infection
- Collection of contact information
- Physical exam may be performed to assess the need for advanced care in participants
- Vital signs[5] may be collected as necessary
- Height and weight may be recorded from medical record if available
- Collection of biological specimens including:
    - Specimens from body compartment specific to Pathogen “X” to test for infection
    - 10mL blood for serum from adults
    - 2.5 mL blood for serum from children
    - 60mL blood for PBMCs from adults
    - Pediatric blood volumes for PBMCs will total ≤1 mL/kg; see MOP for specific details.

[4]Temperature, heart rate, respiration rate, pulse oximetry, etc.

6.2.2 Follow-up Visits and Final Visit
At intervals determined by the biologic characteristics after enrollment (up to 12 months)

Participants will undergo the following procedures as necessary:

- Collection of patient information including interval medical history, medications, and medical record review if available
- Collection of signs and symptoms of Pathogen “X” infection
- Review contact information
- Physical exam may be performed
- Vital signs may be collected as necessary
- Collection of specimens from body compartment specific to Pathogen “X”

Final Visit only

- Collection of biological specimens including, 10mL blood for serum from adults, 2.5 mL blood for serum from children.

6.3 Clinical Evaluations

Medical history/exposure history will be obtained by interview of the participants or by review of medical records. Participants will be queried regarding a history of signs/symptoms of Pathogen “X”. In Group 2, exposure history obtained will include whether the close contact shared a room with the index case, the number of days of exposure from initial symptoms to quarantine of the index patient, and the quarantine measures taken after diagnosis of the index case by the close contact.

At Visit 1, a physical exam may be performed based on participant’s disease severity and hospitalization status. Vital signs (temperature, heart rate, respiration rate, pulse oximetry) may be collected, as necessary. Height and weight may be collected. If not possible to obtain at enrollment, the above information may be collected by medical history.

Administration of any concomitant medications, therapies or vaccines will be documented. Concomitant medications will include all current medications and medications taken within the 30 days prior to enrollment.

Documentation of receipt of IVIG, plasma, or monoclonal antibodies or investigational immunomodulator, as described by exclusion criterion #4, will be collected at enrollment.

Group 1 patients’ disease severity will be evaluated until symptoms resolve. A final disease severity score will be assessed using the highest level of severity achieved commensurate with any scales that are developed (See Appendix B).

6.4 Laboratory Evaluations

6.4.1 Laboratory Evaluations/Assays

Laboratory testing will be conducted at potentially CLIA-certified laboratories and noncertified labs. Blood specimens and volumes will be obtained in accordance with the SOE (Appendix A). Blood specimens that will be collected as part of the standard specimen collection protocol will include serum and anticoagulated plasma both of which will be used for antibody testing and/or for measurement of circulating cytokines. Blood samples for isolation and cryopreservation of peripheral blood mononuclear cells (PBMCs) are collected for use in cellular and innate immune cell responses.

Specimens from body compartment specific to Pathogen “X “will be collected to test for Pathogen “X” detection.

Specimens will be collected for this study as indicated in the SOE and per detailed guidance provided in the MOP. Adult blood collection will not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period. For participants with more severe Pathogen X disease, blood volumes drawn for PBMC harvests will be reduced from 60 mL to 24 mL for visits 1, 5 and 7 if the participant’s hemoglobin is ≤10 gm/dL, or if they have cardiogenic shock (defined as systolic blood pressure <90 for >30 minutes, requiring pharmacological vasopressor or mechanical support to maintain SBP >90, or Cardiac Index ≤2.2 L/min/m^2 and/or Pulmonary Capillary Wedge Pressure ≥15 mmHg), or severe hypoxemia (defined as PaO_2/FiO_2 <100 or clinical evidence of end-organ ischemia). In addition, we will either reduce the blood volume taken to 24 mL or eliminate the PBMC blood draw on visits 1, 5 and 7, if in the opinion of the physician responsible for the participant’s care the planned blood draw would compromise the patient’s clinical status.

In accordance with US NIH recommendations, pediatric blood collection will not exceed 5 mL/kg in a single day or 9.5 mL/kg in any eight-week period.

Endpoint Assays

The specimens collected as part of this study will be available to the VTEU sites, DMID, and other interested parties as part of a specimen biobank. Certain assays may move forward as part of this study through the VTEU laboratories or IDCRC. Many of these assays may be under development across the VTEUs. With the emergence of new data, the protocol leadership and the IDCRC Leadership Group might decide to prioritize or de-prioritize some of these assays. These may include but are not limited to:

- Antibody assays:
  - Pathogen “X” neutralization assay (e.g., PRNT, FRNT)
  - Pathogen “X” pseudoneutralization assay
  - Binding antibody assays
  - Serology for related pathogens

- Pathogen “X” detection assays:
  - Pathogen “X” detection assays (e.g., PCR, culture)
- T cell responses
  - T cell ELISpot and/or ICS assays
- B Cell responses
  - Memory B cell testing
  - Plasmablast antibody secreting cells (ASCs) responses
- Innate immune responses (e.g., transcriptomics)
- Pathogen “X” whole genome sequencing (WGS)

6.4.2 Specimen Collection, Preparation, Handling and Shipping

Biosafety level 2+ will be utilized for specimen processing as recommended by Centers for Disease Control and Prevention (CDC) (e.g., handling of specimens within a biosafety cabinet, masks). Participants will be enrolled, and samples will be collected from participants by staff following current CDC requirements or local requirements (whichever is more stringent).

All specimens collected for this study will be labeled, transported, processed, tested, stored and/or shipped in accordance with site and local laboratory SOPs. The frequency of specimen collection and testing will be directed by the SOE. Additional details can be found in the protocol-specific MOP.

6.4.2.1 Instructions for Specimen Preparation, Handling and Storage

Instructions for specimen preparation, handling, and storage are included in the protocol-specific MOP.

6.4.2.2 Specimen Shipment

Instructions for specimen shipment are included in the protocol-specific MOP.

7. STATISTICAL CONSIDERATIONS

7.1 Study Hypotheses and Design

The overall objective of this prospective observational cohort study is to characterize the natural history and immune responses to Pathogen “X” infection in patients who present varying levels of disease severity and clinical symptoms, and in their close contacts. The study hypothesizes that Pathogen “X” elicits broad immune responses in individual patients, and that these responses vary over time during infection and as disease severity and symptoms progress. The study will determine whether immune responses associated with disease severity, symptoms, and demographic variables. In particular, it will focus on association with age, and consider other known or suspected factors that associate with immune responses (e.g., sex). It will also characterize spread of infection among close contacts of Pathogen “X” infected patients.

To address its objectives with adequate power, the study will target enrolling approximately 1,000 Pathogen “X” infected or suspected children and/or adult patients (Group 1) and children and/or adult close contacts of the Pathogen “X” infected patients who are enrolled in Group 1 (Group 2). Participants in Group 1 will be representative of the Pathogen “X” disease spectrum.

The primary statistical analysis of data from participants enrolled in Group 1 will be conducted using the most severe level of care (hospitalized ICU, hospitalized non-ICU, outpatients not hospitalized) received by each study participant. Secondary statistical analyses will be performed using additional disease severity scales if developed (see Appendix B).

7.2 Sample Size Considerations

The goal of the statistical analysis for the primary study objective is to study Pathogen “X”-specific binding and neutralizing antibody responses, and to determine whether these responses associate with age and disease severity. The primary and secondary analyses are mostly exploratory in nature and will use a range of descriptive statistics (e.g., mean of continuous outcome variables, correlation coefficients, proportions). Additionally, hypothesis tests will be performed to compare groups or determine whether associations between pairs of variables are significant. The following two sections evaluate the power of the study for continuous outcome variables and for response rates separately. Power calculations are also carried out anticipating that 15% of observations may be missing for any given immunological assay.

7.2.1 Power Analysis for Continuous Outcome Variables

Some statistical analyses will characterize immune responses at a given time point based on continuous outcome variables (e.g., response magnitude of Pathogen “X” specific B cells) by estimating the mean response magnitude in different groups of patients. The precision with which a mean response magnitude (more generally, the mean of a continuous outcome

variable) is estimated may be evaluated by constructing an associated two-sided 95% confidence interval. The width of these intervals is independent of the mean response magnitude but depends on the observed standard deviation of the response magnitude measurements. The half-widths of confidence intervals are reported in Table X for multiple values of the standard deviation and several group sizes [provide examples of group sizes]. The calculations assumed independent and identically normally distributed observations. [discuss results presented in table].

Table X: Half-width of two-sided 95% confidence intervals for the mean of a continuous outcome measure based on observing a particular standard deviation as a function of the sample size (n) and the observed standard deviation

std. dev.	sample size (n)					
0.10						
0.25						
0.50						
0.75						
1.0						
2.0						
5.0						
10.0						

We also evaluated the power of the study to detect statistically significant differences when comparing mean response magnitudes between two independent groups. The calculations assumed a nonparametric two-sided Wilcoxon rank sum test with a 5% significance level, and that observations are sampled from two independent and normally distributed populations. The power of the test is presented in Table X as a function of the standardized (i.e., Cohen’s) effect size and of the sample sizes (n1 and n2). [discuss results presented in table]

Table X: Power for comparison of response magnitude between 2 groups of sizes n1 and n2, respectively, as a function of the standardized (Cohen’s) effect size

standardized effect size		n1						
0.25	n2							
0.50	n2							
0.75	n2							
1.00	n2							

One goal of the primary objective of the study is to determine whether continuous measures of immune responses (e.g., magnitude of binding and neutralizing antibody responses to Pathogen “X” measured in blood) associate with age. These analyses may be conducted by computing correlation coefficients between immune responses and age (or any pair of continuous variables

needed to address objectives of the study). Correlation coefficients may be computed on the entire cohort or on subsets of participants (e.g., enrollment groups). The power of the study to detect a significant association between two continuous variables is shown in Table X for several values of Pearson’s linear correlation coefficient (r) and sample sizes (n).

Table X: Statistical power to detect a significant association between two continuous outcome variables (e.g., a continuous measure of an immune response and age) as a function of the sample size (n) for multiple true values of the correlation coefficient (r)

True correlation coefficient (r)	Sample size (n)						
	20	30	40	45	50	100	150
0.3							
0.4							
0.5							
0.6							
0.7							
0.8							
0.9							

7.2.2 Power Analysis for Response Rates

The immune responses will also be analyzed by estimating response rates in Group 1 overall and in subgroups of Group 1 participants defined by baseline subject characteristics such as age and disease severity. Examples of response rates include the proportion of XXX. The study may also estimate the rate of Pathogen “X” infection among Group 2 participants, i.e. close contacts of Pathogen “X”-positive enrolled subjects.

The precision with which the true response rate can be estimated from the observed data depends on the true underlying response rate and the sample size (n). Two-sided 95% confidence intervals for the response rate based on observing a particular rate of response are presented in Table X for a variety of scenarios.

Table X: Two-sided 95% confidence intervals for the true response rate based on observing a particular rate of responses

Sample size	Number of responses	Response rate	95% confidence interval (in %)

7.3 Participant Enrollment and Follow-Up

We anticipate that the study will complete enrollment in Group 1 within approximately X months upon opening. Given that many sites will enroll participants for the study, it is reasonable to expect that recruitment will be completed in fewer than X months. Every patient in Group 1 will be followed for about X years.

Subjects with close contact exposure to patients with confirmed or suspected Pathogen “X” in Group 1 and who meet the inclusion criteria will be enrolled in Group 2. We anticipate that the study will enroll 1000 participants in Group 2 (i.e., an average of 1.5 to 2 close contacts per patient enrolled in Group 1). We expect that the study will complete enrollment in Group 2 within approximately X months upon opening. Every patient in Group 2 will be followed for about X days/weeks/months.

The age of participants will be monitored during recruitment, and every effort will be made to broaden the age distribution across the Pathogen “X” disease severity spectrum.

7.4 Analysis Plan

All data from enrolled patients will be analyzed. Most primary and secondary analyses will be performed using SAS and R statistical software. This section provides an overview of statistical approaches that will be used to perform data analysis.

7.4.1 Analysis Variables

The analysis variables consist of participant characteristics, such as demographics/ sociodemographics and medical history (including pre-existing conditions, new acute or chronic medical conditions, and concomitant medications), presentations Pathogen “X” infection (including the clinical course of disease), immunogenicity for primary, secondary, and exploratory objective analyses, and host genetics.

7.4.2 Analysis of Primary and Secondary Endpoints

The statistical analysis for the primary and secondary objectives of the study will seek to answer key questions related to the immune responses and clinical course of Pathogen “X” infection.

The study will not randomize patients between study arms. Therefore, in order to account for potential confounders, statistical analyses will either be stratified by group or seek to adjust for covariates where deemed appropriate. For example, some analyses will be stratified by study arm (e.g., Groups 1 and 2). Other analyses will be stratified or adjust for age (treated as continuous or categorical variable), sex, for example.

Categorical variables (e.g., disease severity, binary responses, participants’ characteristics at enrollment) will be analyzed by tabulating the relative frequency of each category by group and time point, as appropriate. Crude response rates will be presented with their 95% confidence interval estimates calculated using the score test.[12] Fisher’s exact tests will be used to compare response rates between two groups. Because participants enrolled in distinct groups may differ in terms of demographic variables and comorbidities, many of the statistical analyses will compare response rates between groups using binary logistic regression to adjust for potential confounders (e.g., age, gender, race, comorbidities).

Continuous variables will be analyzed using univariate and bivariate descriptive statistics to summarize data by group, time point, gender, and race, for example. Data will be visualized using graphical techniques, including boxplots, density estimates, scatterplots, heatmaps, and networks. Comparisons of two independent groups will be performed using a nonparametric Wilcoxon rank sum test. To compare more than two groups, we will first use a Kruskal-Wallis or an F-test (depending on the normality assumption) to test for overall differences. Should the overall test reach statistical significance at the 2-sided 0.05 level, individual (post-hoc) pairwise tests will be performed (e.g., using Dunn’s procedure following a Kruskal-Wallis’ test). Comparisons of paired data will be accomplished using the Wilcoxon signed-rank test or paired *t*-test. In addition, since participants are not randomized between groups at enrollment, some of the statistical analyses will use multiple linear regression to compare groups while adjusting for potential confounders (e.g., age, gender, race, comorbidities). Longitudinal data will be analyzed using statistical methods for repeated measures and dependent data (e.g., mixed-effects models,[13] generalized estimating equations (GEE).[14] Similar modeling approaches will be considered to account for potential intra-close contact correlations in analyses that involve data from Group 2. Preliminary data transformations (e.g., logarithmic, square root) may be

performed in regression analyses to better satisfy assumptions of symmetry and homoscedasticity (constancy of variance).

If the frequency of missing data is substantial, statistical analyses may be performed using parametric models fitted using the method of maximum likelihood. This approach is robust when data are missing at random (MAR). MAR assumes that the probability of an observation being missing may depend upon the observed responses and observed covariates, but it does not depend on any unobserved factors. This assumption is less stringent than the missing completely at random (MCAR) assumption. If measurements of immune responses are left- and/or right-censored, we will use Hughes’ linear mixed effects models to accommodate censoring in analyses of repeated measures.[15] Analyses of repeated immunogenicity measurements may be done using weighted GEE methods,[16] which are valid under MAR.

To visualize multivariate immune response variables, we may use dimensionality reduction techniques (e.g., principal component analysis, multidimensional scaling, Uniform Manifold Approximation and Projection), and perform unsupervised learning (e.g., k-means, model-based clustering) to identify subgroups of participants with similar immune profiles.

Where appropriate (e.g., in transcriptional analysis), we will perform formal p-value adjustment for multiple comparisons. We will control either the family-wise error rate (FWER) using the Holm-Bonferroni procedure [17] or the false discovery rate (FDR) using the Benjamini-Hochberg or the Benjamini-Yekutieli procedures.[18,19] The selection of a method for p-value adjustment may depend on the objective of the statistical analysis.

Some of the primary and secondary analyses will seek to identify predictive signatures of categorical outcome variables (e.g., disease severity) using a combination of demographic (e.g., age, race), clinical (e.g., comorbidity), and laboratory (e.g., viral load, immune responses) variables. We will first build candidate predictive models using multiple regression techniques (e.g., binary logistic regression for binary endpoints) modeling the probability of a particular event (e.g., disease progression, hospitalization) as a function of a set of predictors, while adjusting for potential confounding factors, as appropriate). Models may be fitted using regularization techniques (e.g., lasso, elastic net) and cross-validation to identify optimal tuning parameters. Where appropriate, we will assess the predictive power of candidate signatures using doubly (nested) cross-validated receiver operating characteristics (ROC) curves.

8. ETHICS/PROTECTION OF HUMAN SUBJECTS AND OPERATIONAL CONSIDERATIONS

8.1 Informed Consent Process

Federal regulations specify the elements of informed consent that must be conveyed to research participants through the informed consent process (see 21 CFR 50.20). It is the responsibility of the SI and his/her assigned staff to ensure that all required information has been provided to potential research participants. This study will use two informed consent forms (ICFs), one for study participation and a separate consent for leukapheresis. The consent forms for this study will be IRB approved.

Informed consent is a process that is initiated prior to the individual’s agreeing to participate in the study and continuing throughout the individual’s study participation. Participants may withdraw consent at any time during the trial.

Risk to study staff of Pathogen “X” may exist from fomites that the potential participant may handle, including consent documents. Three types of informed consent may be used in this study depending on the individual IRB policies, hospital or clinic policies, and the health of the potential participant.

- Written informed consent

  Study staff will give the potential participant (or LAR) written consent forms (as approved by the IRB) which describe the study procedures and risks in detail and will keep written documentation of informed consent. Staff will ask the potential participant (or LAR) to read and review the document, or have it read to them, and will be available to answer any questions the participant may have. The potential participant (or LAR) will sign the written ICF before any procedures are performed specifically for the study.

- Verbal informed consent

  Study staff will read/explain a verbal version of the written consent form and potential participants (or LAR) will give their verbal consent in place of written consent to participate. Study staff will document verbal consent in lieu of participant (or LAR) signature.

- Electronic informed consent

  Alternatively, study staff can provide an electronic version of the consent form. Potential participants (or LAR) will consent to the research electronically if they wish to participate. The date and time of the electronic signature are automatically recorded, indicating when informed consent was obtained.

Those who administer consent will provide extensive discussion of the study procedures, risks and possible benefits to the potential participant (or LAR). The potential participant (or LAR) will have the opportunity to discuss the study prior to agreeing to participate. A copy of the applicable consent form will be provided to the participant (or LAR). The consent will state that

the administration and quality of their medical care will not be adversely affected if they decline to participate in this study.

The study staff’s approach to study participants will be compliant with HIPAA regulations.

8.1.1 Informed Consent/Assent Process (in Case of a Minor or Others Unable to Consent for Themselves

This study plans to include legally defined minors, which vary from state to state, but which typically include unemancipated children under the age of 18. They will be contacted to participate in this study. The parents (or legal guardian) will sign a consent form and age-appropriate assent will be obtained prior to participation in this study.

8.1.2 Human Genetic Testing

The research staff will seek the participants’ consent for genetic research in this study, and for extra and residual specimens to be stored and used for secondary research evaluating human genomic and phenotypic markers. The rights and privacy of human subjects who participate in genomic or phenotypic research studies will be protected at all times.

The consent process will include an explanation of the potential risks to the individual participants and their families associated with data submitted to an NIH data repository and subsequent sharing. Data that may potentially identify human subjects will not be released in unrestricted databases. Participants will be informed that the evolution of genomic technology and analytical methods raises the risk of re-identification, even when specimens are de-identified. The consent will include whether individual participant data will be shared through an NIH controlled access data repository. Data for genomic or phenotypic research will be submitted to a controlled access data repository; therefore, informed consent permitting the data sharing must be documented, even if the specimens are de-identified.

8.2 Subject Confidentiality

The participating investigators, their staff, and the sponsor(s) and their agents hold participant confidentiality strictly in trust. This confidentiality includes clinical information relating to enrolled participants. All clinical information will be maintained at the sites.

The study protocol, documentation, data and all other information generated during participation in the study will be held in strict confidence. No information concerning the study, or the data generated from the study, will be released to any unauthorized third party without prior written approval of the sponsor. Subject confidentiality will be maintained when study results are published or discussed at conferences. Authorized representatives of the sponsor or governmental regulatory agencies may inspect all documents and records required to be maintained by the investigator. The clinical study site will permit access to such records.

All records will be kept locked and all computer entry and networking programs will be carried out with coded numbers only and with password protected systems. All evaluation forms, reports, and other records that leave the site will be identified only by a coded number.

This research is covered by a Certificate of Confidentiality from the NIH. The researchers with this Certificate may not disclose or use information, documents, or biospecimens that may identify a subject in any federal, state, or local civil, criminal, administrative, legislative, or other action, suit, or proceeding, or be used as evidence, for example, if there is a court subpoena, unless a subject has consented for this use. Information, documents, or biospecimens protected by this Certificate cannot be disclosed to anyone else who is not connected with the research except, if there is a federal, state, or local law that requires disclosure (such as to report child abuse or communicable diseases but not for federal, state, or local civil, criminal, administrative, legislative, or other proceedings); if a subject has consented to the disclosure, including for their medical treatment; or if it is used for other scientific research, as allowed by federal regulations protecting research subjects.

8.3 Future Use of Stored Specimens

The research team intends to store plasma, serum, PBMCs, other specimen types, and residual samples from participants. Specimens may undergo future testing in the context of this trial. Archived specimens will be identified only by the specimen number, which will allow linkage of the samples to study data but not to any personal identifiers. A participant’s specimen will be kept until it is used up or destroyed after completion of this study.

The protocol ICF is written so that the participant is informed that by agreeing to participate in the study they are also agreeing to have samples stored for future additional research studies. Participants will not be contacted with the results of these future research studies. Future testing on specimens will only occur to the extent authorized in each study site’s ICF or as otherwise authorized under applicable law and after review and approval by the DMID and the IRB of the researcher requesting the specimens.

8.4 Publications Prior to Final Study Report

If interim analyses are performed, study personnel working with laboratory samples will remain blinded to prevent any negative impact on the study. The IDCRC SDSU will provide summary data to the study team for publication, presentation and/or informing iterative improvements in study design.

To accelerate the dissemination of scientific findings, the results from these analyses may be published prior to the final study report and/or used to assist in the design of future Pathogen “X” trials. In addition, informative case studies or case series may be published prior to the final study report. Early publication of other data emerging from this study may occur with the concurrence of the study team PIs, the SDSU, and DMID.

8.5 Quality Control (QC) and Quality Assurance (QA)

To ensure the reliability of study data, each site will develop a Clinical Quality Management Plan (CQMP). The CQMP will describe:

- Routine internal QC and QA activities to measure, document and report study conduct, protocol adherence, human subjects’ protections, and reliability of the protocol-driven data collected.
- A process for addressing data quality issues (i.e., collecting, recording) and reporting findings in a timely manner); systemic issues (i.e., protocol conduct, non-compliance, human subject protections) and implementation and evaluation of Corrective and Preventative Action Plan (CAPA) procedures.

9. DATA HANDLING, DATA QUALITY CONTROL AND RECORD KEEPING

For this study the IDCRC SDSU will be responsible for all issues relating to data collection (forms, tools and systems), quality control and management. SDSU will also provide statistical support for this study.

A detailed data management plan (DMP) will be written prior to study initiation.

9.1 Data Collection

Data collection tools will be developed by SCHARP in conjunction with the protocol team. As part of the study activation process, the study site must identify all Case Report Forms (CRFs) to be used as source documents. Study CRF data will be entered and cleaned using the Medidata Rave EDC tool, a data management system compliant with the International Council on Harmonisation (ICH) Good Clinical Practices (GCP) and US CFR guidelines for electronic data capture.

9.1.1 Data Quality Control

All study sites will conduct quality control and quality assurance procedures in accordance with the *DMID Clinical Quality Management Policy* (https://www.niaid.nih.gov/sites/default/files/qualitymgmtplan.pdf).

CRF Completion Guidelines will be provided by the SDSU prior to study initiation. Quality control queries will be routinely generated in the Medidata Rave EDC tool for study site verification and resolution.

9.1.2 Retention of Data

All study sites will maintain source data/documents in accordance with the *Source Documentation Standards for DMID Clinical Studies* (https://www.dmidcroms.com/Shared%20Documents/Source%20Documentation%20Standards_English.pdf).

The study site will maintain, and store securely, complete, accurate and current study records throughout the study. Thereafter, instructions for record storage will be provided by DMID. No study records may be moved to an off-site location or destroyed prior to receiving approval from DMID.

9.1.3 Protocol Deviations

A protocol deviation (PD) is defined as an individual incident or omission in study conduct that results in added risk to the participant, nonadherence to the protocol, or nonadherence to the

International Council on Harmonisation (ICH) Good Clinical Practices (GCP). Except to safeguard the health of study participants, neither the investigator nor site staff may deviate from an approved protocol without prior agreement by IDCRC and DMID.

Because research is complex and requires adherence to a large amount of detail, PDs are expected to be a normal occurrence during the study. Study site will report PDs in the Medidata Rave EDC tool. In some situations, additional documentation or recommended changes in study practices may be requested to resolve the PD and prevent future occurrence.

10. LITERATURE REFERENCES

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APPENDIX A: SCHEDULE OF EVENTS (SOE)

Table 2: Group 1 Pathogen "X" Suspected or Confirmed Cases SOE

Procedures	Screening/ Enrollment/ Baseline Visit 1, Day 1	FUP Visit 2[1] DPO 7	FUP Visit 3[1] DPO 15	FUP Visit 4[1] DPO 30	FUP Visit 5[1] DPO 90	Optional Study Visit (between DPO 30 & 90)	Study Visit 7[1] DPO 180	Final Visit 8[1] DPO 365
Informed consent	X							
Review Inclusion/Exclusion Criteria	X							
Demographics and Sociodemographics	X							
Medical history/Exposure history	X							
Medications	X	X	X	X	X		X	X
Signs/Symptoms of Pathogen "X" infection	X	X	X	X	X			
Contact Information[2]	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Interval History Updates		X	X	X	X		X	X
Review of medical records	(X)	(X)	(X)	(X)	(X)		(X)	(X)
Physical exam	(X)	(X)	(X)	(X)	(X)		(X)	(X)
Vital signs[3]	(X)	(X)	(X)	(X)	(X)			
Height and weight[4]	X							
Collection of specimens[5]	X	(X)	(X)	(X)	(X)		(X)	(X)
Phlebotomy for serum, adults (mL)	10		10	10	10		10	10
Phlebotomy for serum, children (mL)	2.5			2.5	2.5			2.5[6]

Procedures	Screening/ Enrollment/ Baseline Visit 1, Day 1	FUP Visit 2[1] DPO 7	FUP Visit 3[1] DPO 15	FUP Visit 4[1] DPO 30	FUP Visit 5[1] DPO 90	Optional Study Visit (between DPO 30 & 90)	Study Visit 7[1] DPO 180	Final Visit 8[1] DPO 365
Phlebotomy volumes for PBMCs in adults (mL)	24[7] or 60[8]		8 or 16 mL	24[7] or 60[8]	24[7] or 60[8]		24[7] or 60[8]	60
Phlebotomy volumes for PBMCs in children (mL)	<1 mL/kg[9]		<1mL/kg	<1 mL/kg[9]	<1 mL/kg[9]			<1 mL/kg[6,9]
Collect residuals from standard of care[10]	X	X	X	X	X			
Leukapheresis [subset of adult patients][11, 12]						5x10^8 to 1x10^10 cells		
Per Visit Blood Volume Total, adults (mL)	34[7] or 70[8]		10	34[7] or 70[8]	34[7] or 70[8]		34[7] or 70[8]	70
Running Blood Volume Total, adults (mL)	34[7] or 70[8]		44[7] or 80[8]	78[7] or 150[8]	112[7] or 220[8]		146[7] or 290[8]	216[7] or 360[8]
Per Visit Blood Volume Total, children (mL)	2.5 + PBMC			2.5 + PBMC	2.5 + PBMC			2.5 + PBMC
Running Blood Volume Total, children (mL)	2.5 + PBMC			5 + PBMC	7.5 + PBMC			10 + PBMC

DPO – Days post-illness onset

(X) – If needed or available

1) Clinical information and specimens will be collected at enrollment and then at an interval determined by the biologic characteristics of Pathogen "X" after enrollment (up to 12 months). The study days included are subject to change.
2) Collect contact information at enrollment and review at follow-up as necessary.
3) May be collected as necessary; may include temperature, heart rate, respiration rate, pulse oximetry, etc.
4) May be obtained by history if not possible to obtain at enrollment (e.g., outpatients)
5) Specimens will be collected from body compartment specific to Pathogen "X". Specimens may include whole blood, serum, urine, saliva, stool, or other body fluids as available (e.g., semen, vaginal secretions, breast milk) depending on the specifics of Pathogen "X.

6) Optional blood draw at 1 year.
7) PBMC adult blood volumes for adults with significant anemia (Hgb≤10), compromised cardiac function (defined as systolic blood pressure < 90 for > 30 minutes, requiring pharmacological vasopressor or mechanical support to maintain SBP > 90, or Cardiac Index ≤ 2.2 L/min/m2 and/or Pulmonary Capillary Wedge Pressure ≥ 15 mmHg) and/or significant respiratory compromise (defined as PaO2/FiO2 < 100 or clinical evidence of end-organ ischemia).
8) PBMC adult blood volumes for non-ventilated adults without significant anemia (Hgb>10) and without compromised cardiopulmonary functions.
9) See MOP for detailed blood volumes based upon weight.
10) May be salvaged if available from standard of care testing; may include but not limited to blood, cough specimens, endotracheal aspirates, bronchoalveolar lavage, urine, stool, and saliva.
11) Leukapheresis collection is quantified by the number of cells collected, not by blood volume.
12) Phlebotomy for screening/safety labs and urine/serum pregnancy testing will be performed locally prior to the leukapheresis procedure.

Table 3: Group 2 Close Contact Exposures to Patients with Suspected or Confirmed Pathogen "X" Enrolled in Group 1 SOE

Procedures	Screening/ Enrollment Baseline Visit C1, Day 1	Follow-up Visits[1]
Informed consent	X	
Review Inclusion/Exclusion Criteria	X	
Demographics and Sociodemographics	X	
Medical history/Exposure history	X	
Medications	X	X
Signs/Symptoms of Pathogen "X" infection	X	X
Contact Information[2]	X	(X)
Interval medical history		X
Review of medical records	(X)	(X)
Physical exam	(X)	(X)
Vital signs[3]	(X)	(X)
Height and weight[4]	(X)	
Collection of specimens[5]	X	X
Phlebotomy for serum, adults (mL)	10	10 mL (Day 14 only)
Phlebotomy for serum, children (mL)	2.5	2.5 mL (Day 14 only)
Phlebotomy volumes for PBMCs in adults (mL)[6]	60	
Phlebotomy volumes for PBMCs in children (mL)	≤1 mL/kg[7]	
Per Visit Blood Volume Total, adults (mL)	70	10 mL (Day 14 only)
Running Blood Volume Total, adults (mL)	70	80
Per Visit Blood Volume Total, children (mL)	2.5 + PBMC	2.5 mL (Day 14 only)
Running Blood Volume Total, children (mL)	2.5 + PBMC	5 + PBMC

X) – If needed or available

1) Clinical information and specimens will be collected at enrollment and then at an interval determined by the biologic characteristics of Pathogen "X" after enrollment (up to 12 months).
2) Collect contact information at enrollment and review at follow-up as necessary.

3) May be collected as necessary; may include temperature, heart rate, respiration rate, pulse oximetry, etc.
4) May be obtained by history if not possible to obtain at enrollment.
5) Specimens will be collected from body compartment specific to Pathogen “X”. Specimens may include whole blood, serum, urine, saliva, stool, or other body fluids as available (e.g., semen, vaginal secretions, breast milk) depending on the specifics of Pathogen “X “.
6) PBMC adult blood volume listed is target amount for healthy adults.
7) See MOP for detailed blood volumes for children based upon weight.