Title: Evaluating Modes of Influenza Transmission through the conduct of controlled human influenza virus infection transmission trials (CHIVITTs)

Short Title: EMIT-2 Transmission Protocol

DMID Protocol Number: 21-0041

Sponsored by:
National Institute of Allergy and Infectious Diseases (NIAID)

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U19-AI-162130 Cooperative Agreement, Evaluating Modes of Influenza Transmission (EMIT-2) using Innovative Technologies and Design in Controlled Environments

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Draft or Version Number: v6.0

Day Month Year
01 November 2024

Statement of Compliance

The study will be carried out in accordance with Good Clinical Practice (GCP) and as required by the following:

- United States (US) Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH): Good Clinical Practice (GCP) E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) Guidance for Industry," published in the Federal Register (83 Federal Register 8882 (2018)
- National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

Signature Page

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

Site Investigator:

Signed: _____ Date: _____

Name

Title

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LIST OF ABBREVIATIONS

ABTC	Advanced Bioaerosol Technology Core
ACGIH	American Conference of Governmental Industrial Hygienists
ACH	Air changes per hour
ADCC	Antibody-dependent cellular cytotoxicity
ALC	Absolute lymphocyte count
ALT	Alanine transaminase
BAMA	Binding antibody multiplex assay
BP	Blood pressure
CBC	Clinical & Biostatistics Core
CFR	Code of Federal Regulations
CHIVITT	Controlled human influenza virus infection transmission trial
COPD	Chronic obstructive pulmonary disease
Cr	Creatinine
CR	Control Recipient
CRF	Case Report Form
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
ELLA	Enzyme-linked lectin assay
EMIT	Evaluating Modes of Influenza Transmission
FOCBP	Female of childbearing potential
FRNT	Focus Reduction Neutralization Test
GBS	Guillain-Barré Syndrome
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HA	Hemagglutinin
HAI	Hemagglutination inhibition
Hgb	Hemoglobin
HR	Heart rate
ICF	Informed Consent Form
ICH	International Council on Harmonisation
iEBA	Infectious exhaled breath aerosols
IR	Intervention Recipient
IV	Intravenous
IRB	Institutional Review Board
MN	Microneutralization
MOP	Manual of Procedures
NA	Neuraminidase
N	Number (typically refers to subjects)
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIOSH	National Institute for Occupational Health and Safety, CDC, DHHS
NIH	National Institutes of Health
NSAID	Non-steroidal anti-inflammatory drug
PHI	Protected health information
PI	Principal Investigator
PLT	Platelets
PPE	Personal protective equipment

PPM	Particles per minute
RR	Respiratory Rate
SAB	Scientific Advisory Board
SAR	Secondary Attack Rate
SOP	Standard Operating Procedure
SpO_2	Oxygen saturation
WBC	White blood cells
WHO	World Health Organization
UHC	University Health Center (College Park)
UMB	University of Maryland, Baltimore
UMD or UMCP	University of Maryland, College Park
UMIC	UMaryland Immediate Care (Baltimore, MD)
UMMC	University of Maryland Medical Center (Baltimore, MD)
UV	Ultraviolet light

PROTOCOL SUMMARY

Title: Evaluating Modes of Influenza Transmission through the conduct of controlled human influenza virus infection transmission trials (CHIVITTs)

Population: up to 200 persons, among which 175 may be Recipients aged 18 through 49 and 25 may be Donors aged 18 through 59 years

Number of Sites: at least 2 (University of Maryland, Baltimore and University of Maryland, College Park)

Study Duration: approximately 5 years

Subject Duration: Up to approximately 2 months.

Primary Objective:

- The primary objective of EMIT-2 is to use a randomized controlled trial (RCT) design to implement interventions which are known to reduce inhalation (airborne) transmission, so that the contribution of transmission by route of aerosols for influenza may be identified.

Primary Endpoint:

- Secondary attack rate (SAR) of influenza in Recipients participating in RCTs of air sanitation-ventilation-filtration and contact-fomite interventions, so called controlled human influenza virus infection transmission trials (CHIVITTs).

Secondary Objectives:

- To measure influenza viral parameters in both Donors and Recipients, including the incidence, duration, and quantity of virus shedding
- To measure influenza illness parameters in both Donors and Recipients, in order to assess the impact of aerosol exposure to influenza virus on disease severity
- To evaluate the role of serologic and mucosal antibody levels on influenza transmission, susceptibility, and immunologic response to infection
- To estimate the infective dose of influenza via aerosol inhalation, through measurement and modeling of aerosol size, virus number per aerosol particle, deposition and environmental dispersion characteristics, and rate of shedding

1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

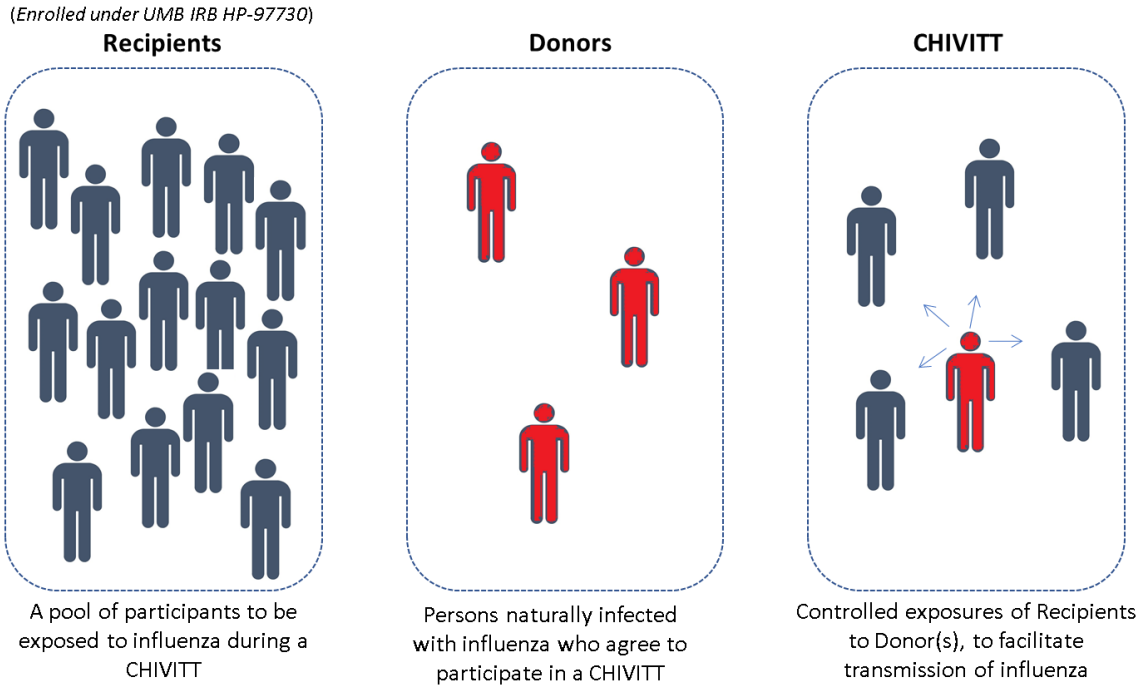
2.1 Background Information

Influenza has a high disease burden to the United States and globally. However, fundamental aspects of influenza virus epidemiology remain poorly understood. Determination of the relative importance of droplet nuclei and droplet sprays in influenza transmission is relevant to the control of both seasonal and pandemic influenza. It will largely settle the debate about the most appropriate personal protective equipment for healthcare workers (respirators or surgical face masks) and pave the way for the development of interventions or control measures specific to the dominant mode of transmission. Although typical modes of influenza transmission between humans may include aerosol, large respiratory droplet, and contact[1,2], the relative importance among these three routes is still under debate.[1] The importance of this question is emphasized in the NIAID strategic plan for a universal influenza vaccine,[3] which identifies as a "key unanswered question the relative contribution of aerosols, droplets, and fomites as modes of transmission."

To answer this critical question the CDC funded a project called "Evaluating Modes of Influenza Transmission using a Human Challenge Model" (hence forth referred to as EMIT-1; PIs: Jonathan Nguyen Van-Tam, Donald Milton, and Werner Bischoff). EMIT-1 was designed as an experimental study of human-human transmission from volunteers deliberately infected with Good Manufacturing Practices (GMP) well-characterized lab-grown influenza viruses. In EMIT-1, "donors" were nasally inoculated with a GMP A/H3N2 virus and susceptible volunteers ("recipients") were exposed to the donors through prescribed close living conditions. EMIT-1 failed to achieve the planned attack rate of influenza transmission, and this was largely hypothesized to be a result of the lack of significant illness and low numbers of shedding of influenza virus with infection.[4] In the wake of EMIT-1, a two-day international workshop "Influenza Transmission and the Built Environment: Understanding Modes of Transmission in a Sustainable Future" was held in March 2014 to review the results and chart a path forward. Workshop attendees proposed an alternative approach for studying the relative contribution of the various modes of influenza transmission and recommended to abandon inoculation with GMP viruses in favor of donors who are naturally infected cases, which could be recruited from a medical clinic.

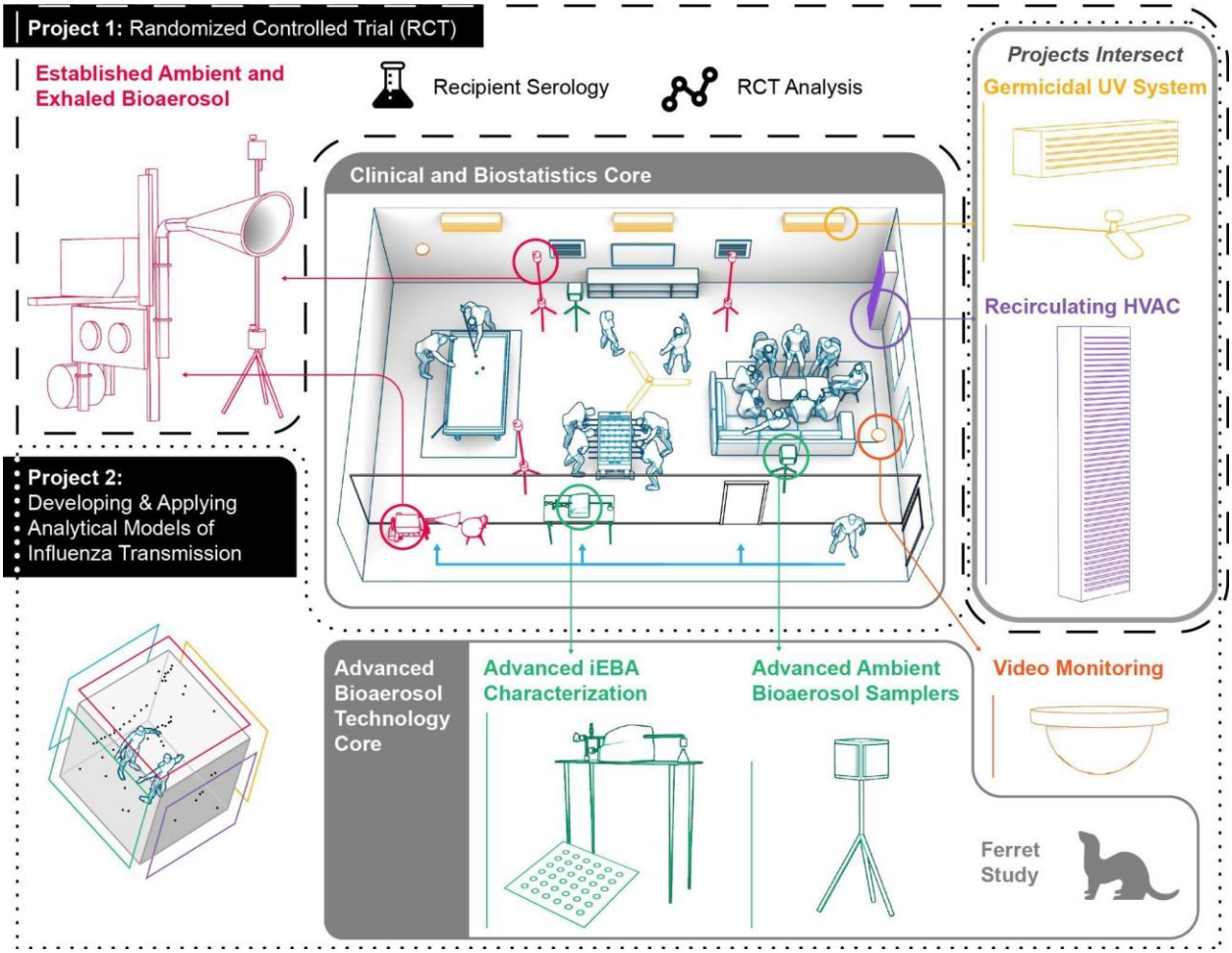
NIAID-funded project "Evaluating Modes of Influenza Transmission using Innovative Technologies and Design in Controlled Environments" (hence forth referred to as EMIT-2; PD: Donald Milton) is in response to NIAID funding opportunity "Multidisciplinary Studies to Improve Understanding of Influenza Transmission" (RFA-AI-20-08). EMIT-2 will perform a series of controlled clinical studies of influenza transmission to comprehensively evaluate viral, host, physical, and environmental factors that facilitate efficient human-to-human influenza transmission. In these studies, healthy participants who are deemed to be susceptible to influenza (recipients) will be in contact with participants naturally infected with influenza virus (donors). These transmission studies are hence forth referred to as controlled human influenza virus infection transmission trial (CHIVITT) studies.

This document represents the EMIT-2 CHIVITT Protocol. The EMIT-2 Donor portion of this document is for the enrollment of persons naturally infected with influenza who will participate in a CHIVITT. Under the CHIVITT Protocol portion of this document, EMIT-2 Recipients (who have already been screened under the EMIT-2 Recipient Protocol, UMB IRB HP-97730) will consent to be "exposed" to influenza from infected Donors. The EMIT-2 Donor enrollment and CHIVITT activities will be conducted during the winter (during the natural annual influenza season) of each designated year.



2.2 High-Level Overview of EMIT-2 Program

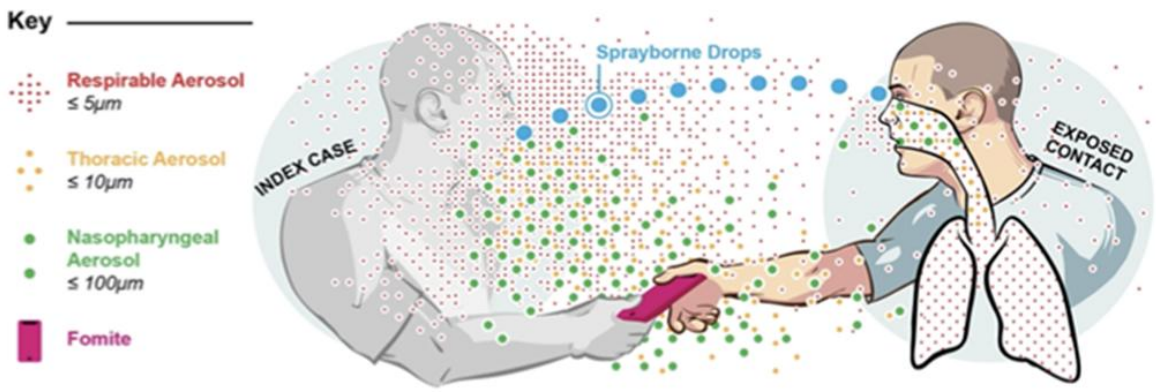
The NIH U19-funded EMIT-2 program is made up of 2 research Projects and 2 scientific Cores. Research **Project 1** consists of the randomized controlled trials which will determine the dominant mode of transmission, the impact of aerosol exposure on disease severity, and the contribution of serological and mucosal susceptibility on infection. Research **Project 2** consists of developing and applying analytical models of transmission, with comparisons to ferret transmission studies. The **Clinical and Biostatistics Core** is responsible for providing the clinical infrastructure, trained personnel, regulatory compliance, quality management, and clinical oversight necessary to conduct the CHIVITT studies. The **Advanced Bioaerosol Technology Core** will be developing and evaluating a novel compact ambient (environmental) sampling and culture device, a next-generation infectious exhaled breath aerosols (iEBA) sampler, an optimized virus collection and cell culture line, and a digital culture microarray. These four components will synergistically be working collaboratively together to achieve the goals of the EMIT-2 program. The hypothesis is that infectious aerosols are a major contributor to influenza transmission and that ventilation, air hygiene, and reduction of aerosol exposure will be associated with a reduction in both secondary influenza attack rates and disease severity.



2.3 Scientific Rationale

The overarching objective of the EMIT-2 program is to evaluate how viral aerosol shedding and ventilation interact to facilitate human-to-human influenza transmission and provide insights into correlates of protection in the setting of absent or low hemagglutination inhibiting (HAI) antibodies using a controlled environment. The central hypothesis of EMIT-2 is that inhalation of infectious aerosols (small size infectious particles suspended in air) is the dominant mode of influenza transmission. EMIT-2 will carefully characterize and quantify exhaled infectious aerosols and the indoor environment while assessing the relative effect of planned interventions. For example, in one planned transmission experiment, under conditions with low ventilation and low air hygiene, half of the cohort of recipients will don face shields and use hand sanitizer every 15 minutes while the remaining half of recipients will not don protection or make frequent use of hand sanitizer; all recipients will be instructed to engage in prescribed close-contact activities (e.g., play a board game for 2 hours) with donors who are infected with influenza. As a result, a secondary attack rate of influenza transmissions of approximately 25% is intended to occur under low air-hygiene conditions with a cumulative total of up to 60 hours of exposure of each recipient. The duration of exposure is chosen to match that used in previous trials (Killingley B et al., J. Infect. Dis. 2012:205:35–43 and Nguyen-Van-Tam JS et al., PLoS Pathog. 2020;16:e1008704).[4,5] Over the course of approximately 5 influenza seasons, a number of controlled transmission experiments are to be conducted with variable air hygiene conditions. The scientific and engineering technologies and aims of the EMIT-2 program are

contingent upon successfully facilitating secondary influenza infections, i.e., influenza transmissions from index cases (Donors) to exposed contacts (Recipients).



Sprayborne
- Ballistic drops (>100 µm)
- Direct hit on eye, nostril or mouth

Inhalation (Aerosol)
- Inhalable aerosols ≤100 µm
- Thoracic aerosols ≤10-15 µm
- Respirable aerosols ≤5 µm

Touch (Contact-Fomite)
- Patient to finger
- Fomite to finger
- Finger to eye, nose, or mouth transfer

2.3.1 EMIT-2 Recipients

The EMIT-2 Recipient Protocol (UMB IRB HP-97730) is a continuous (yearlong with a duration of ~5 years) screening protocol to identify healthy "susceptible" adults, based on lack of potentially protective antibody, to serve as the recipient pool for exposed contacts in CHIVITT. Persons who have consented to the EMIT-2 Recipient Protocol have agreed to be contacted during the winter seasons for whether they would agree to participate in a CHIVITT. Formal separate consent will be performed for recipients that agree to participate in a CHIVITT.

2.3.2 EMIT-2 Donors

This protocol will describe the EMIT-2 Donor recruitment process for the enrollment of persons naturally infected with influenza index cases who agree to participate in a CHIVITT. The recruitment process will be performed from among the community, beginning with case identification on the campuses of the University of Maryland, Baltimore (UMB) in association with the University of Maryland Medical Center (UMMC) and the University of Maryland, College Park (UMCP). From these 2 campuses, investigators may also add additional sites to potentially recruit influenza index cases, including health centers on college and university campuses in the Baltimore area, the Johns Hopkins Medical Institutions emergency and outpatient departments and other local urgent care centers or local primary care practices. Each participating site must have IRB approval.

2.4 CHIVITT

This protocol will describe the general conduct of the CHIVITT, wherein we will estimate the relative contribution of aerosol inhalation (airborne), spray deposition (large droplet), and touch (contact-fomite) transmission on the spread of influenza, by determining the secondary attack rate (SAR) of influenza among uninfected volunteers (Recipients) who closely interact with naturally influenza-infected index

cases (Donors). The close interactions are designed to mimic real-world potential exposures to influenza. To identify the dominant mode of transmission, various interventions will be employed. The exposure room environment can be tuned to a low ventilation minimal filtration environment or a high ventilation, filtration, and air disinfection environment. The residential and common room environment may be supplemented with in-room air filtration and/or germicidal UV air disinfection to achieve high air hygiene conditions to block inhalation of infectious aerosols. Transmission via spray deposition may be blocked by face shield interventions. Touch (contact-fomite) transmission may be blocked through strict hand hygiene and surface cleaning protocols. Aerosol inhalation transmission may be further reduced by the donning of face masks. The determination of SAR in Recipients will be performed through a standardized procedure of collected respiratory specimens and daily symptom surveys. The monitoring and management of influenza illness will be conducted to ensure the welfare of all study participants.

2.5 Potential Risks and Benefits

2.5.1 Potential Risks

Influenza Illness

Common symptoms of common influenza illness includes: body aches or pains, chest congestion, chest tightness, chills, congested or stuffy nose, coughing, diarrhea, difficulty swallowing, eyes sensitive to light, fatigue, feeling dizzy, fever, head congestion, headache, lack of appetite, swollen lymph nodes, nausea, runny or dripping nose, scratchy or itchy throat, shivering, sinus pressure, sleeping more than usual, sneezing, sore or painful eyes, sore or painful throat, stomach ache, sweating, teary or watery eyes.

Moderate complications of influenza, which are unlikely to occur in healthy persons who participate in this study include: sinus infections and ear infections.

Severe complications of influenza, which are also unlikely to occur in healthy persons who participate in this study include: pneumonia, severe dehydration, severe bronchitis, myocarditis, myocardial infarction, pericarditis, Guillain Barré Syndrome/Bell's Palsy, transverse myelitis, encephalitis, worsening of chronic health conditions, admission to hospital, hypoxemia/respiratory failure, arrhythmia/cardiac arrest, and death.

These known risks of complications associated with an influenza infection are to be mitigated by active management by skilled research staff clinicians, including treatment with antiviral therapy (oseltamivir or baloxavir) when indicated. More details of these measures are described in Sections 6.4, 6.5, and 6.6.

Onward Transmission of Influenza Virus

Recipients, through participating in this study, might acquire influenza infection and transmit onwards to contacts outside of the trial, who might include vulnerable individuals. To mitigate this risk, recipients will be isolated and observed for approximately three days after last exposure, which is longer than the 24-48 hour incubation period for influenza, and treated with antiviral therapy prior to discharge if infection is detected.

Blood Draws

Drawing blood may cause transient discomfort and fainting. Lightheadedness or fainting is usually

self-limited and managed by having the subject lie down and elevate his/her legs, until the vasovagal response subsides. 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 technique by designated clinical team members will make infection at the site where blood will be drawn unlikely.

Nose swabs or nose washes

Obtaining nose fluid or a nose swab may cause brief discomfort of the nares, a gag reflex, bleeding of the nose, watery eyes, or coughing during the collection.

Saliva Samples

We will ask participants to stop eating and drinking for about 30 minutes before giving a saliva sample, about ½ teaspoon, in a specimen cup or tube. Giving a saliva sample may be difficult if participants' mouths are dry. To help with that, participants may drink a small amount of water 5 minutes before giving a sample.

Exhaled breath sampling

Exhaled breath sample collection is performed with a specialized apparatus which requires the participant to be seated in a humidified booth or tent with their face at opening (entrance) to the apparatus' large funnel. Participants will be able to breath normally and cough spontaneously. Participants will be asked to vocalize (e.g., say 'whoooooo', sing 'Happy Birthday', or yell a sports team cheer) periodically during sample collection. The existing apparatus is called the Gesundheit-II (G-II, U.S. Patent No. 8250903). As newer devices become available through the Advanced Bioaerosol Technology Core, we may supplement or replace the G-II with newer sampling equipment that may not require a humidified booth. A maximum of two 30-min samples will be collected each day with at least a 10-minute break between samples.

Breath samples may also be collected by breathing humidified air through a spirometer mouthpiece (EasyOne®, NDD Medical Technologies, Inc) for 15 minutes while wearing a nose clip. Participants will be able to rest for 1 minute at 5-minute intervals during sample collecting via the mouthpiece. Participants will be asked to breath slowly and deeply (slow vital capacity maneuver) and may be asked to vocalize (e.g., say 'whoooooo') while breathing through the mouthpiece. A maximum of two 15-minute samples will be collected per day via breathing through a mouthpiece.

Breath samples may additionally be collected with a device that resembles a handheld peak flow meter. Participants will be asked to blow through the device several times, each time taking deep breaths. They may be asked to vocalize (e.g., say 'whoooooo') while breathing through the mouthpiece. These samples will take about 2 minutes to collect.

Total time breathing into a breath sampler will be limited to a total of 1 hour per day for the longer duration samples and 10 min for the 2-minute samples. Sampling will be performed daily for the duration of participant by Donors. Recipients will be asked to provide samples daily for up to four days after onset of symptoms or evidence of infection by laboratory testing.

The G-II presents no breathing resistance. Rarely for some persons, they may feel slightly claustrophobic sitting in front of the G-II cone. Breathing into the spirometer or peak flow meter-

like mouthpiece with a nose clip may cause dry mouth and discomfort from the nose clip. Some people may experience dizziness or lightheadedness due to the slow vital capacity breathing maneuver being employed. The breathing maneuver may also stimulate coughing. There is no noticeable resistance to breathing and the procedure has been well tolerated previously by people infected with influenza and other respiratory viruses. The short duration of the sample collection helps mitigate this discomfort, and the participant will be encouraged to breath slowly to reduce the risk of dizziness.

Loss of Confidentiality

Study participants will be asked to provide personal health information and other identifying information. To preserve protected health information (PHI) and confidentiality, identifying participant data will be kept in a locked, secured facility at the study site. All collected specimens will be coded, removing identifying information, prior to appropriate storage in a locked, secured, monitored facility, accessible only by designated personnel at the study site. The coded specimens will be able to be linked to identifying information which is only accessible to the clinical research staff. Specimens will be shared with EMIT-2 collaborating laboratories; collaborating lab personnel will not have access to identifying data.

Video and Digital Photos

Video footage or digital photos taken during the transmission experiments are to be analyzed and then either de-identified (by blurring or obscuring faces and other identifying marks) or erased by the end of the study. Video footage and digital photos will be recorded onto encrypted, password protected local devices (e.g., USB "thumb" drive) and only be made accessible to pre-designed study team members who will store video files in a password-protected electronic storage device or password-secured campus server drive.

Special Considerations for COVID-19

During the period when the U.S. Public Health Emergency exists for COVID-19, there is a risk of exposure to others with COVID-19 infection. We will minimize this risk by testing for COVID-19. Likewise, all study personnel will follow the most up-to-date CDC guidelines on personal protective equipment throughout the conduct of the Donor enrollment and conduct of the CHIVITT studies. Extensive controls to limit aerosol exposures using ventilation, filtration, and germicidal UV will also be employed throughout the test facility, outside of the exposure room, including individual rooms and hallways.

Risks with oseltamivir phosphate (TAMIFLU®) antiviral

Adverse events reported in at least 1% of adult and adolescent subjects treated with oseltamivir phosphate included nausea (10%), vomiting (9%), diarrhea (7%), bronchitis (2%), abdominal pain (2%), dizziness (2%), headache (2%), cough (1%), insomnia (1%), vertigo (1%), and fatigue (1%). [package insert]

Influenza A virus isolates with reduced susceptibility to oseltamivir carboxylate have been recovered by serial passage of virus in cell culture in the presence of increasing concentrations of oseltamivir carboxylate, from clinical isolates collected during treatment with oseltamivir, and from viral isolates sampled during community surveillance studies. Reduced susceptibility of influenza virus to inhibition by oseltamivir carboxylate may be conferred by amino acid

substitutions in the viral neuraminidase and/or hemagglutinin proteins. Changes in the viral neuraminidase that have been associated with reduced susceptibility to oseltamivir carboxylate. [package insert]

Risks of baloxavir marboxil (XOFLUZA®) antiviral

Adverse events reported in at least 1% of adult and adolescent subjects treated with baloxavir marboxil included diarrhea (3%), bronchitis (2%), nasopharyngitis (1%), headache (1%) and nausea (1%). [package insert]

Influenza A viruses with treatment-emergent amino acid substitutions at positions associated with reduced susceptibility to baloxavir marboxil in cell culture have been observed in clinical studies. It is theoretically possible that baloxavir marboxil treatment can cause treatment-emergent baloxavir marboxil resistant viruses. The overall incidence of treatment-emergent amino acid substitutions associated with reduced susceptibility to baloxavir marboxil in two prior clinical trials was 2.7% (5/182) and 11% (39/370). [package insert]

Rapid flu test

There are some risks associated with rapid flu tests. These tests can sometimes produce false-negative results, meaning that a person who actually has the flu is told that they do not have it. False-positive results are also possible, which can lead to unnecessary treatment and increased healthcare costs. Additionally, rapid flu tests have been known to have a lower sensitivity compared to other flu tests, meaning that they may not be as accurate in detecting the flu virus. Study participants will receive an information sheet that outlines the risks and benefits associated with rapid tests, as well as other instructions. The participants will be encouraged to discuss their test results with their healthcare provider for further guidance and treatment, if necessary.

Unknown Risks

There may be additional risks in this study which are not yet known.

There is also small risk to subjects who report that they are in good health but who have an unknown health problem at the time of screening. This study will screen by medical history through interview, a clinical questionnaire, clinical laboratories, and vital signs. Any clinically significant abnormalities discovered will be discussed with the participant and recommendations for appropriate referrals will be provided.

Potential Risks with Specimens and Data for Secondary Research

Specimens which may be left over after designated assays have been performed or extra specimens collected for secondary research may be indefinitely stored for future research. These specimens will be labeled with a code which will not contain personal identifying information. The risk of loss of confidentiality of a specimen is minimized by maintaining the linking information, to a subject and their personal information, in a secured and locked location with access only available to authorized study staff members.

2.5.2 Known Potential Benefits

There is no direct benefit to the study participant. There is the potential benefit of advancing

scientific understanding of influenza transmission from participation in this study.

3 IDENTIFICATION OF INDEX CASES FOR CHIVITT (DONORS PROTOCOL)

3.1 Donor Protocol – Design

We will enroll persons aged 18 through 59 years of age into this EMIT-2 Donor Protocol. Generally healthy subjects without known risk factors for severe influenza complications who have acute symptomatic influenza infection will be eligible for enrollment. The screening process will include a clinical evaluation for general health status, assessment of drug and alcohol dependence and abuse, and assessment of smoking/vaping history.

Recruitment

We learned from focus groups that many potential donors do not generally go to a clinic when they are sick and may not ever have the cause of their influenza-like-illness (ILI) diagnosed. We also learned that obtaining and paying for a flu test for purposes of determining eligibility for the CHIVITT is a time and cost barrier to participation as a donor. Therefore, to detect cases early enough in the incubation period, we are adding several new options for flu testing to the original strategy of recruiting donors during clinic visits.

Therefor we will take multiple approaches to Donor recruitment.

Clinics and Urgent Care Centers - Donor recruitment will involve identifying influenza case patients at local urgent care centers, emergency rooms, campus health centers, and at local provider offices. Initial recruitment will be focused on the preliminary identification of potential participants being seen within the UMMC Emergency Department (Baltimore, MD), the UMaryland Immediate Care (UMIC, Baltimore, MD), UM Urgent Care - Downtown; UM Urgent Care - Columbia; University of Maryland Faculty Physicians, Inc. at Waterloo Crossing, the University of Maryland Health Center (UHC, College Park, MD), Express Healthcare, LLC (College Park, MD), and Johns Hopkins Medical Institutions (JHMI). However, with ongoing collaborations within the medical community, there will be an expansion of potential urgent care centers, medical practices, and other partners which might provide referrals of influenza patients as donors.

Pop-Up Kiosks. CLIA-waived rapid flu tests will be administered at “pop-up kiosks” on campuses or other locations thought to have a high likelihood of yielding individuals with positive flu tests. Kiosks will be staffed by trained study personnel. Visitors to the kiosk who want to be tested will self-swab, provide their email address, and answer questions about fever, cough, and sore throat. Trained study personnel will perform the rapid flu test and link the test to the visitor’s email address. Study recruitment fliers will be distributed. Individuals who get tested will receive an information sheet describing the risks and benefits of the rapid test, along with study materials. These settings where healthy students, families and/or other adults congregate (such as dormitories, student lounges, barber shops) will allow investigators to raise awareness of the study, provide the means for no-cost tests and improve the chances of enrolling individuals with the influenza very early in the course of their disease.

Lord Baltimore Hotel – We will provide CLIA-waived flu testing kiosk at the Lord Baltimore Hotel during the quarantine CHIVITT to facilitate identifying eligible donor volunteers. These individuals will have heard about the study via social media, fliers, or word of mouth, and have ILI but not been tested.

In these situations, they can obtain a flu test at the Lord Baltimore Hotel during periods when we are recruiting Donors.

Recipient Registry - individuals who are enrolled in the EMIT-2 Recipient Registry (HP-00097730), may be provided FDA approved home influenza rapid test kits during their screening or follow-up clinic visits if test kits are available. (This will be described in the Recipient Registry Protocol.) If they contract influenza-like illness during a CHIVITT donor recruitment, they can test themselves and volunteer as a donor if they are available and otherwise eligible.

For all potential Donors, a review of available electronic medical records may be necessary to assess for the presence of exclusion criteria prior to formal signed informed consent—this would require IRB approval to waive the HIPAA authorization to obtain identifiable private information for this pre-screening process, as it would not be practicable to conduct this research without the waiver.

Potential participant donors who have been identified at external care centers, kiosks, campus/community outreach services, or through self-testing (registry participants only) and have passed a phone screening, may verbally consent to be screened and transported to the research facility.

3.2 Donor – Eligibility Criteria

Inclusion Criteria

1. Provides written informed consent, able to comply with the planned study procedures, available for between 2 and 5 days stay in the research quarantine unit for the CHIVITT, and have the ability to attend the scheduled follow-up visits.

2. Subjects must be able to comprehend the study requirements, as evidenced by a score of ≥70% or better on the comprehension assessment (two attempts permitted).

3. Males and non-pregnant, non-breastfeeding females[1] aged ≥18 and ≤59 years of age, at time of initial consent.
   [1]Pregnancy and breastfeeding status to be determined by self-report

4. Laboratory-confirmed influenza infection[2] within the past 48 hours at time of entry into the exposure event.
   [2]A rapid antigen test in the setting of known local influenza activity and with symptoms suggestive of influenza at that time is acceptable.

5. Within the past 48 hours at time of entry into the exposure event, onset of influenza-like illness, as defined as fever (measured oral temperature of ≥100.2°F or self-reported fever in the absence of a measured temperature) AND cough or sore throat, or onset of less specific symptoms with a positive molecular test for influenza virus infection.

6. No self-reported or known history of alcohol or drug abuse within the past two years and no illicit drug use within the last 30 days.

7. Do not have clinically significant medical, psychiatric, and chronic or intermittent health conditions including those listed in Exclusion Criteria.

8. Does not have an ongoing symptomatic condition[3] for which subject has had or has ongoing medical investigations but has not yet received a diagnosis or treatment plan.
   [3]*e.g., ongoing and debilitating fatigue without a diagnosis for the symptom.*

9. Agrees to the collection of specimens for secondary research.

Exclusion Criteria

1. Female of childbearing potential who is breastfeeding or has positive urine pregnancy test upon admission to the hotel quarantine unit.

2. Presence of self-reported or medically documented significant medical or psychiatric condition(s)[5]
   [5]*Significant medical or psychiatric conditions include but are not limited to:*

   a. *Respiratory disease (e.g., chronic obstructive pulmonary disease [COPD], asthma, cystic fibrosis) requiring daily medications[6] currently or any treatment of respiratory disease exacerbations or hospitalizations for acute respiratory illnesses (e.g., asthma exacerbation) in the last 5 years.*
      [6] *Asthma medications: inhaled, oral, or intravenous (IV) corticosteroids, leukotriene modifiers, long and short acting beta agonists, theophylline, ipratropium, biologics.*

   b. *Significant cardiovascular disease (e.g., congestive heart failure, cardiomyopathy, ischemic heart disease) or history of myocarditis or pericarditis as an adult.*

   c. *Neurological or neurodevelopmental conditions (e.g., epilepsy, stroke, seizures, encephalopathy, focal neurologic deficits, Guillain-Barré syndrome, encephalomyelitis or transverse myelitis).*

   d. *Ongoing malignancy or recent diagnosis of malignancy, including leukemia; treated, non-melanoma skin cancers are permissible.*

   e. *An autoimmune disease.*

   f. *An immunodeficiency of any cause.*

   g. *A blood disorder (e.g., sickle cell disease)*

   h. *Endocrine disorders (e.g., diabetes)*

   i. *Liver, kidney, metabolic disorders*

   j. *BMI ≥40* kg/m^2

   k. *Any other condition or behavior that in the opinion of the PI would affect the ability to participate in the transmission study over the next several days.*

3. Presence of immunosuppression or any medications that may be associated with impaired immune responsiveness[7].
   [7]*Including, but not limited to, corticosteroids exceeding 10 mg/day of prednisone equivalent, immunoglobulin, interferon, immunomodulators, cytotoxic drugs, or systemic corticosteroids or other similar or toxic drugs during the preceding 2-month period. Low dose topical and intranasal steroid preparations used for a discrete period are permitted*.

4. Is a habitual smoker[8] of tobacco, marijuana, or e-cigarettes per self-report.

[8]Habitual smokers are those who smoke or vape more than four cigarettes, other tobacco products, e-cigarettes or marijuana in a week for more than three months or use an inhaled nicotine or marijuana product more than 3 days a week on average. Edible or patch forms of tobacco or marijuana products do not constitute an exclusion.

5. Known allergy or intolerance to treatments for influenza and other respiratory infections (including but not limited to acetaminophen/paracetamol).
6. History of a previous severe allergic reaction to medicines of any kind with generalized urticaria, angioedema, or anaphylaxis.
7. Presence of co-infection with SARS-CoV-2, as detected via a multiplex nucleic acid amplification test (e.g., Biofire).
8. Participating in any other interventional clinical research study that has a scheduled intervention 30 days prior to the CHIVITT or 30 after discharge from the research quarantine unit.
9. Any condition, to include medical and psychiatric conditions, that in the opinion of the Investigator, might interfere with the safety of the subject or the study objectives.

4 ENROLLMENT OF RECIPIENTS FOR CHIVITT

From among the pool of already screened subjects under the EMIT-2 Recipient Protocol (UMB IRB HP-97730), we will contact these potential volunteers for agreement to consent to participate in a CHIVITT. These subjects will have recent (within the past year) serological assessment of anti-influenza antibody which may predict whether they may be vulnerable to an influenza strain (matching that of the Donor virus strain); this will be done from serum specimens collected under the EMIT-2 Recipient Protocol.

4.1 Recipient Eligibility Criteria

Inclusion Criteria

1. Enrolled in the Recipient Protocol (UMB IRB HP-97730)
2. Provides written informed consent, able to comply with the planned study procedures, be available for an up to ~14-day stay for the CHIVITT and have the ability to attend the scheduled follow-up visits.
3. Subjects must be able to comprehend the study requirements, as evidenced by a score of ≥70% or better on the comprehension assessment (two attempts permitted).
4. No significant change (for the worse) in general health history or in concomitant medication use, as compared from their responses collected during screening (EMIT-2 Recipient Protocol).
5. Agree not to meet with other participants (recipients or donors) outside of the programmed exposure events during the course of their participation in the CHIVITT.

Exclusion Criteria

1. Female of childbearing potential who has a positive urine pregnancy test within 24 hours of admission to the hotel quarantine unit or is breastfeeding or planning to become pregnant within 2 months after entry into a CHIVITT.
2. Presence of infection with influenza, SARS-CoV-2, or other respiratory pathogens detected via a multiplex nucleic acid amplification test (e.g., Biofire) at admission to the hotel quarantine facility.
3. Within the past 72 hours, presence of influenza-like illness, as defined as fever of ≥100.2°F AND cough or sore throat, in the absence of an alternative cause.
4. Receipt of any blood products within the past 2 months.
5. Does not agree to provide permission for secondary research use of extra samples collected and stored specimens.
6. Habitual smoker of tobacco, marijuana, or e-cigarettes per self-report. (*Habitual smokers are those who smoke or vape more than four cigarettes, other tobacco products, e-cigarettes or marijuana in a week for more than three months or use an inhaled nicotine or marijuana product more than 3 days a week on average. Edible or patch forms of tobacco or marijuana products do not constitute an exclusion.*)
7. Self-reported or known history of alcohol or drug abuse in the past two years and/or illicit drug use within the last 30 days. (*Prescribed stimulants for the treatment of ADHD and cannabinoids use do not constitute exclusionary criteria*)
8. Has an ongoing symptomatic condition[1] for which the subject has had or has ongoing medical investigations but has not yet received a diagnosis or treatment plan.
   [1]*e.g., ongoing chronic fatigue without a diagnosis for symptom.*
9. Presence of self-reported or medically documented significant medical or psychiatric condition(s)[2]
   [2]*Significant medical or psychiatric conditions include but are not limited to:*
   a. *Respiratory disease (e.g., chronic obstructive pulmonary disease [COPD], asthma, cystic fibrosis) requiring daily medications* currently or any treatment of respiratory disease exacerbations or hospitalizations for acute respiratory illnesses (e.g., asthma exacerbation) in the last 5 years.*
      ** Asthma medications: inhaled, oral, or intravenous (IV) corticosteroids, leukotriene modifiers, long and short acting beta agonists, theophylline, ipratropium, biologics.*
   b. *Significant cardiovascular disease (e.g., congestive heart failure, cardiomyopathy, ischemic heart disease) or history of myocarditis or pericarditis as an adult.*
   c. *Neurological or neurodevelopmental conditions (e.g., epilepsy, stroke, seizures, encephalopathy, focal neurologic deficits, Guillain-Barré syndrome, encephalomyelitis or transverse myelitis).*
   d. *Ongoing malignancy or recent diagnosis of malignancy, including leukemia; treated, non-melanoma skin cancers are permissible.*
   e. *An autoimmune disease.*
   f. *An immunodeficiency of any cause.*
   g. *A blood disorder (e.g., sickle cell disease)*
   h. *Endocrine disorders (e.g., diabetes)*

i. Liver, kidney, metabolic disorders

j. BMI ≥40 kg/m2

k. Any other condition or behavior that in the opinion of the PI would affect the ability to participate in the screening or future transmission studies.

10. Presence of immunosuppression or any medications that may be associated with impaired immune responsiveness[3].

    [3]*Including, but not limited to, corticosteroids exceeding 10 mg/day of prednisone equivalent, immunoglobulin, interferon, immunomodulators, cytotoxic drugs, or systemic corticosteroids or other similar or toxic drugs during the preceding 2-month period. Low dose topical and intranasal steroid preparations used for a discrete period are permitted.*

11. Known allergy or intolerance to treatments for influenza and other respiratory infections (including but not limited to oseltamivir, baloxavir, acetaminophen/paracetamol).

12. History of a previous severe allergic reaction to medicines of any kind with generalized urticaria, angioedema, or anaphylaxis.

13. Participating in any other interventional clinical trial that has a scheduled intervention 30 days prior to the start of the CHIVITT or 30 after discharge from the research quarantine unit.

5 CHIVITT OBJECTIVES

Table: Objectives and Endpoints (Outcome Measures)

Objectives	Endpoints (Outcome Measures)
Primary	
• To determine the relative contribution of the various modes of transmission	• SAR of influenza infection will be determined through virological confirmation, symptomatic infection, or serological evidence
Secondary	
• To measure influenza viral parameters in Donors and Recipients	• Incidence, duration, and quantity of virus shedding in donors and recipients
• To measure influenza illness parameters in Donors and Recipients to assess the impact of aerosol exposure to influenza on disease severity	• Disease severity parameters in donors and recipients
• To evaluate the role of serologic antibody levels on influenza transmission, susceptibility, and immunologic response to infection	• HAI, NI, and FRNT titers, HA/NA anti-stalk ELISA, and ADCC reporter assays.
	• Measurement and modeling of aerosol size, virus number per aerosol particle,

• To estimate the infective dose of influenza virus	deposition and environmental dispersion characteristics, and rate of shedding
Exploratory	
• To evaluate the role of mucosal antibody and cellular immunity in transmission and susceptibility.	• Potential assays include IgA in nasal secretions and collection and analysis of cellular immune markers.
• To characterize the quantum of airborne influenza infection and extend models of aerosol inhalation risk from well-mixed to non-well mixed conditions and the personal breathing zone.	• Exhaled breath viral loads and ambient viral concentrations, environmental parameters, video analysis of individual movement and interpersonal proximity and behaviors.

6 CHIVITT STUDY DESIGN

Each CHIVITT is planned to be conducted with discrete cohorts of Recipients and Donors. The size of each cohort and numbers of Recipients and Donors will vary from cohort to cohort, but the general size of a cohort may consist of up to 20 Recipients and up to 5 Donors per CHIVITT, which would span an approximate 2-week period. The number of Recipients will be determined by each individual's availability and whether their most recent serum antibodies have demonstrated a relative low titer against the influenza virus to be transmitted in the CHIVITT. To maintain the size of the recipient group, we may consider bringing in new recipients during the quarantine study if a significant number of original recipients are infected on the same day. The number of Donors will be determined by the number of participants which are identified with acute symptomatic influenza infection during the time of a CHIVITT cohort's stay.

The CHIVITT studies are anticipated to occur during the peak of local seasonal influenza transmission (e.g., January and February). Timing of the study will be constrained by the necessity to rent the facility for prespecified calendar dates. It is intended that the local seasonal influenza activity will be closely monitored, including the virus subtypes which are predominantly circulating. During the peak of the local seasonal influenza activity, a cohort of Recipients will be contacted to request their willingness to reside up to ~2 weeks on the quarantine research unit for a CHIVITT. The selection of Recipients from the pool of potential Recipients will be partially determined by the demonstration of low antibody titers against the circulating influenza virus, which has been measured in the past year (under the EMIT-2 Recipient Protocol, UMB IRB HP-97730). Potential Recipients will be given some time (≤ one week) to decide whether to participate and they must complete an IRB-approved consent form for the CHIVITT study.

Meanwhile, during the peak of community-wide local seasonal influenza the study team will be attempting to rapidly identify healthy adults with acute and symptomatic influenza infection for consent to participate as Donors in the CHIVITT cohort. It is intended that there could be multiple Donors identified during a ~2-week period; each of these Donor individuals could be introduced into the CHIVITT cohort in an overlapping or sequential fashion, to provide more opportunities for transmission events.

The success of each CHIVITT cohort will be influenced by the unpredictability of an influenza season, which influenza viruses may be circulating, willingness or availability of Recipients for each cohort, and the ability to identify and consent Donors for each cohort. To mitigate these factors, we will enroll sufficient potential

recipients that only a fraction are needed in any one season, multiple cohorts will be studied each year, and multiple clinical sources will participate in recruiting donors.

6.1 General Outline of Activities During Each CHIVITT Cohort

The CHIVITT is to be conducted as a randomized, open, controlled study. Recipient participants for each cohort will enter the study hotel quarantine facility and be housed in individual hotel rooms. After a two-day run-in period to ensure that Recipients are free of preexisting respiratory infections *Exposure Events* will be held in a specially prepared event room.

Recipient participants of each cohort will be randomized to an intervention group or no intervention (Control Recipients, CR). Before entering their first *Exposure* Event each Intervention Recipients (IR) will be trained on how to comply with the intervention. IRs will be required to wear a lightweight plastic face shield, comply with hand hygiene (i.e., using a hand sanitization product every 15 minutes, plus periodic hand washing with soap and water), and avoid face touching during each planned *exposure event*. The face shield may only be removed after leaving the exposure room to go to the bathroom, for other short comfort breaks, and at mealtimes. In these instances, hand hygiene will be used after removing or replacing the face shield; and Donors will not be present. Hand washing with soap and water will be required before meals. IR will be separated from Donors by more than 6 feet during meals. The exposure room will be supervised by a trained member of the study staff who will monitor to ensure that Intervention Recipients wear the face shield continuously, are separated from Donors at meals, and do not touch their faces. Small sticks (e.g., coffee stirrers) will be provided to allow IRs to scratch their faces.

Exposure events are to occur as controlled (prescribed) activities within the exposure room. Exposure events will extend for up to 15 hours per day when Donors are available and able to participate (e.g., 15 hours 8am to 11pm). Each Recipient will participate in up to 60 hours of *Exposure Events* (e.g., 4 days at 15 hours/day) which may be spread over up to 9 or 10 days. During each *Exposure Event* multiple 1-3 hour group activities will be planned and supervised by trained members of the study staff. The group activities are intended to be entertaining and may consist of participating in a card game, board game, foosball game, karaoke, or other group activity which facilitates crowding, loud talking, etc. for potential influenza transmission.

Meanwhile, the exposure room environment will be regulated to control room temperature, humidity, and air hygiene for each CHIVITT. The overarching design of the EMIT-2 program is the evaluation of the contribution of aerosol inhalation to influenza transmission. In order to evaluate the effect of aerosol inhalation, we may alternate the air quality and cleaning between high and low air hygiene conditions during each quarantine cohort with a wash-out period (for example 2 days). High air hygiene conditions will be those where ventilation, filtration, and/or germicidal UV will achieve effective air exchanges of ≥10 air changes per hour (eACH), or >10 liters/second/person, or a mean target CO2 concentration of ≤200 parts per million (ppm) above the outdoor concentration (if high air hygiene is achieved with ventilation alone). Low air hygiene conditions will be those where effective air exchanges will be ≤ 0.7 ACH or <1 liter/second/person (similar to an unventilated dorm room at the University of Maryland College Park), or a mean target CO2 concentration of ≥2,500 ppm (similar to a poorly ventilated public-school classroom), or as low as achievable while controlling temperature in a comfortable range and relative humidity <40%. It is intended that the first CHIVITT (Year 1) will be performed with the low air hygiene, ventilation, filtration, and no germicidal UV conditions so that we can determine the attack rate of influenza under

the circumstances which will most facilitate inhalation exposure and influenza transmission. In subsequent years, the air hygiene conditions will be alternated within each CHIVITT cohort.

Within the exposure room, digital cameras will be mounted for photography and video recording of the *exposure events*. The photos and video information are used to monitor and characterize the direction of exhaled breath, duration and proximity of persons from each other, and other positional information which will be needed to develop the analytical models of aerosol transmission. Although the content of conversations is not the intent of the recordings, some audio data may be analyzed for "loudness" and audible coughs and sneezes, as a proxy for periods during which higher exhaled breaths may be generated. In some CHIVITTs we may employ newer technologies, such as wearable biosensors with geo-positional data collection to allow for the investigators to collect data on minute respiratory rate, heart rate, and proximity.

When Recipients and Donors are not participating in exposure events, the participants will be separated as much as possible to avoid transmission outside of the exposure events. Anti-septic hand hygiene products will be widely available throughout the area. In-room air filtration and/or germicidal UV fixtures will also be used to decontaminate the air of individual hotel rooms and hallways.

6.2 Duration of Donor Participation

It is intended that symptomatic influenza-infected Donors be introduced into the CHIVITT as soon as possible after onset of symptoms, while still shedding influenza viruses. When the daily influenza testing (e.g., using BioFire Respiratory Panel testing or exhaled breath testing) demonstrates no detectable virus, which is anticipated to occur by approximately 3 to 5 days from the initiation of symptoms, or more than three days has elapsed since onset of symptoms, discharge plans for the participant may be entertained. Safe discharge will be contingent upon the absence of sufficient illness which may require escalation of care (section 6.8). Donors may also be allowed to withdraw from the study early, for their own personal reasons (see section 6.7).

6.3 Duration of Recipient Participation

Recipients will be requested to be available for participation in a CHIVITT cohort for approximately two contiguous weeks. However, they may voluntarily consent to participate for a longer period. Alternatively, if they join the cohort late to replace a significant number of discharged recipients, they may participate for a shorter period. It is possible that there may be multiple exposure event activities in a single day or there may be days where no exposure event activities are planned (e.g., because there are no donors available with shedding virus). Total time for participation in exposure events will be up to 60 hours over the course of a CHIVITT.

6.4 Recipient Confinement

When Recipients are not engaged in an *exposure event* activity they will be in their designated private room (hotel room which includes private bathroom and shower). While in their private room, they will be allowed to use the amenities available to them, including watching TV and using the WiFi internet. They may engage in their professional work activities (telework) but must understand that their work schedules must be highly flexible given the nature of the timing of Donor availability and the timing of some *exposure events* which will be according to the Donor's symptomatology and ability to engage in prescribed activities. Recipients must understand that they are housed in "isolation" conditions such that they will

not be allowed to ingress and egress from the isolation unit at will. Guests will not be permitted during the Recipient's stay on the isolation unit. Limited personal deliveries may be possible but must be negotiated for approval with the investigators and may not consist of illicit drugs, weapons, or other forms of contraband which could present a hazard to the welfare of the participants or staff members. An exercise room equipped with treadmill and/or stationary bicycle is intended to be provided on the quarantine research unit; use of the exercise room's equipment will be single occupancy with sign-up so that inadvertent transmission events can be minimized from this common use space. All meals are to be provided through the facility's food services and outside food deliveries will not be permitted, unless there is a specific need from a participant which has been authorized by the investigators (e.g., special dietary needs which cannot be accommodated sufficiently by the facility food service).

Recipients will be notified that hallways are monitored by video camera and that leaving their room when not authorized by study personnel will result in a reduction in compensation (i.e., the payment scale will be adjusted for failures of compliance).

Should a recipient become laboratory-confirmed influenza infected (a countable SAR) and have accompanying symptoms during the CHIVITT, then the Recipient may be allowed to continue in the CHIVITT as a Donor. In order to accept the Recipient into the Donor status, the Donor informed consent form must be reviewed and signed by the participant. Recipients who are a countable SAR and do not want to continue in the CHIVITT may be safely discharged, contingent upon the absence of sufficient illness which may require treatment of influenza (section 6.6). However, once the 2 weeks have transpired, and if they have not become influenza infected, they will be allowed to end their participation in the CHIVITT. Recipients will also be allowed to withdraw from the study early, for their own personal reasons (see section 6.7).

6.5 Staffing of the Facility

The facility will be staffed by research team members who will be present in the facility 8 am through 5 pm each day (including weekends). An on-call physician will be available for all hours, including evenings and weekends, for the management of illness or to address any questions or concerns from any study participants. The on-call physician can make decisions on the treatment of influenza, use of supportive care, and escalation of care. For overnight hours, a thermometer and over the counter antipyretics will be made available. Participants will be able to request video or phone call contact with the covering physician to discuss any concerns. The on-call physician will use their judgement whether they may need to report into the quarantine research unit to directly assess the participant.

6.6 Treatment of Influenza

For the healthy young adult, influenza infection is largely a self-limiting viral infection which only requires rest and supportive care. No later than 72 hours of a Recipient demonstrating any criteria which satisfy the primary outcome of SAR influenza infection and immediately upon onset of fever >102˚F, meeting other criteria for a grade 3 adverse event (see Appendix A), or as required by the clinical judgement of study physicians, therapeutic intervention with an approved influenza antiviral agent (i.e., oseltamivir or baloxavir marboxil) will commence. Meanwhile, the subject will be requested to provide exhaled breath specimens; the subject will not be required to participate in additional *exposure event* group activities.

Recipients that become secondarily influenza infected may provide voluntary consent to continue in the CHIVITT as a Donor; the Donor consent form must be reviewed and signed in order for the participant to

continue as a Donor. Under this circumstance, treatment with the influenza antiviral agent will be withheld and the subject (now considered an infectious subject) will be instructed to participate in *exposure event* group activities, as a Donor participant. The participant may choose to terminate their participation as a Donor participant at any time before the maximum 5 days, as originally intended for a Donor participant. Upon deciding to terminate in participation in the CHIVITT, the subject can be administered an influenza antiviral agent and may be discharged. Under all circumstances, symptoms of an influenza infection are intended to be followed to resolution and this may be done through phone call contacts upon discharge; the dates of resolution of influenza symptoms are intended to be documented.

6.7 Supportive Care

The management of illness by the study physicians will allow for the use of supportive care measures. The study physician may treat the subject with symptom-directed, over-the-counter therapies, such as non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen, decongestants, or antihistamines. Oral hydration (oral fluids) may be provided, should a subject become dehydrated due to poor appetite. Humidification of room air may also be performed to improve the comfort of the participant. Nonetheless, support care is intended to be limited for subjects that do not demonstrate any illness criteria that may satisfy conditions requiring escalation of care.

6.8 Escalation of Care

In rare instances a subject's illness may meet criteria for escalation of care, wherein more in-depth evaluation and/or treatment of illness may be necessary. Escalation of care may involve referral to the local Emergency Room or Urgent Care Center, as the investigator has deemed the severity of illness to be beyond the simple supportive care which can be provided by the EMIT-2 facility. Subjects infected with influenza will be monitored for the possible development of severe symptoms and any influenza complications (as outlined in section 2.5.1). If a subject has a severe influenza complication(s) or has shortness of breath with an SpO_2 <89% or has any 2 severe grade vital signs (see Appendix A for severity grading for vital signs), then escalation of care is to be considered.

Therapeutic intervention with an approved influenza antiviral agent (oseltamivir or baloxavir marboxil) should commence immediately upon recognition that escalation of care for influenza is necessary and if not yet already initiated. Study physicians have the authority to exercise clinical judgement to initiate referral for pulmonary function testing, chest radiography, institution of supplemental oxygen, or the initiation of bronchodilators for management. Escalation of care may also include immediate referral to the local urgent care or emergency department for rapid diagnostic testing and/or management. The intent is that there will be no delay in providing appropriate escalation of care to study participants and that the study physicians document their decision-making process. UMB and/or its affiliated institutions or healthcare groups will not provide financial compensation or reimbursement for the cost of referred care that may be provided. The institution or group providing medical treatment will charge the participant's insurance carrier.

Meanwhile, the ill subject will not be required to participate in *exposure event* group activities and will be afforded the time to rest and recover or be referred for higher-level care. Participant preference is intended to be honored. However, when the investigator judges that escalation of care is necessary for the welfare of the participant then participation in *exposure event* activities will be prohibited.

6.9 Discharge Criteria

Subject discharge is planned at approximately 14 days (for Recipient participants) or 2-5 days (for Donor participants) after entry. To be eligible for discharge home, the participant must meet the following criteria: no greater than moderate severity influenza signs or symptoms (Appendix A) and are clinically stable during their stay. In addition, the participant must attest that they feel well enough to provide their own self-care. Otherwise, Recipients will be discharged as describe in escalation of care (Section 6.8). Recipients must be at least 2 days post the end their last exposure event to be eligible for discharge. We may discharge Recipients with a home influenza test to ensure that we do not miss late onset, long latency, influenza infection. However, recipients who have contracted the flu but do not wish to be a donor may be discharged earlier. In this case, they must meet the discharge criteria described above for discharge home. All other instances will be handled as described in Section 6.8.

6.10 Subject Withdrawal

Subjects may voluntarily withdraw their consent for participation at any time and for any reason, without penalty or loss of benefits to which they are otherwise entitled. The PI or appropriate co-investigator may also withdraw a subject for any reason. Withdrawal requests may be temporary (e.g., a single CHIVITT cohort) or permanent.

A subject may withdraw or be withdrawn from this study for any of the following reasons:

- Medical disease or condition, or any new clinical finding for which continued participation, in the opinion of the PI or appropriate co-investigator, would compromise the safety of the subject, or would interfere with the subject's successful participation in CHIVITT
- Subject withdrawal of consent.
- Subject found to have SARS-CoV-2 or other respiratory virus infection.
- Termination of this protocol.
- As deemed necessary by the PI or appropriate co-investigator for noncompliance or other reasons.

6.11 Oversight of Safety and Sponsor-Required Reporting

A monthly safety review will be conducted throughout the CHIVITT periods of the study. The safety review members will consist of the principal investigator, the DMID Medical Officer (MO), Medical Monitor (MM), and Clinical Project Manager (CPM). The information to be reviewed will consist of the following

- Enrollment
- Unsolicited, Non-Serious, Severe or Potentially Life-Threatening Adverse Events or Grade 3+ Adverse Events
- Severe Solicited Influenza Symptoms
- Grade 3 + Abnormal Clinical Laboratory Results Related to Influenza
- SAEs Related to Influenza
- Unanticipated Problems related to trial participation

These listings will include summaries if needed and will be presented as unblinded data from UMD. The reviews may be conducted by email or conference call. Whereupon any safety concerns are potentially detected, a temporary halt may be declared, based on the PI's judgment in consultation with the DMID MM.

The PI should discuss safety events with DMID on an ad hoc basis if deemed by the PI to be of significant concern. Telephone ad hoc meetings will be scheduled as needed to include DMID MO, MM, CPM, and UMD PIs.

Any Unanticipated Problems related to trial participation are to be reported to DMID at the time of IRB reporting. UPs should be submitted to DMID as narratives or a copy of the IRB report for review by MO, MM, and CPM monthly by email to the CPM.

7 SAFETY AND OTHER ASSESSMENTS

7.1 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

The site PI or designated clinician licensed to make medical diagnoses will record, assess, and follow abnormal laboratory test values and abnormal clinical findings to collect adverse events (AEs) that are observed or reported during the study.

Signs, symptoms, and laboratory findings that are part of mild to moderate influenza disease, including mild or moderate fever and lymphopenia from the time of onset of illness through 7 days post-onset, will not be considered AEs. These signs, symptoms, and laboratory findings (mild and moderate) include the following: runny nose, stuffy nose, sneezing, sore throat, headache, cough, malaise (tiredness), body aches, chills, feverish, shortness of breath, earache, fever, and lymphopenia. Mild to moderate fever and mild to moderate lymphopenia is defined in Appendix A. Influenza infection-related symptoms that are deemed by the investigator to be severe, in addition to severe fever and severe lymphopenia as defined in Appendix A, will be considered as AEs.

Safety will be assessed by the frequency and severity of:

- Study intervention-related serious adverse events occurring from the time of the first exposure event until 2 months post-discharge from the hotel quarantine facility).
- Clinical safety laboratory adverse events occurring from the time of the first exposure event through discharge from the hotel quarantine facility. Parameters to be evaluated include white blood cells (WBCs), absolute lymphocyte count, hemoglobin, platelets, alanine transaminase (ALT), and creatinine (Cr).
- Adverse Events – non-serious adverse events occurring from the time of the first exposure through approximately 28 days post-last-exposure. See below for how adverse events are defined.

7.2 Adverse Events and Serious Adverse Events

Definition of Adverse Events (AE)

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical intervention regardless of its causal relationship to the study treatment. FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related (21 CFR 312.32 (a)).

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) intervention. The

occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

In this study, solicited symptoms of symptomatic influenza infection will be collected from the time of first exposure event through 7 days post-last-exposure and will **not** be considered adverse events. Objective clinical examination findings consistent with the solicited symptom, such as oropharyngeal erythema or lymphadenopathy, will also **not** be considered AEs. Any influenza signs, symptoms or lab findings determined by the clinician to have exceeded the expected severity or any moderate or severe complications, as listed in Section 2.5.1, will be considered unsolicited adverse events and captured on the appropriate data collection form and electronic case report form (eCRF). Events that are not consistent with illness due to influenza will be considered unsolicited AEs as well. Events that occur after administration of antivirals will also be considered unsolicited AEs. Any chronic or stable medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it should be recorded as an AE.

Classification of an Adverse Event

The determination of seriousness, severity, and causality will be made by an on-site investigator who is qualified (licensed) to diagnose AE information, provide a medical evaluation of AEs, and classify AEs based upon medical judgment. This includes but is not limited to physicians, physician assistants, and nurse practitioners.

Definition of Serious Adverse Events

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes (21 CFR 312.32 (a)):

- death
- a life-threatening adverse event
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, convulsions that do not result in inpatient hospitalization, etc.

SAEs will be:

- Assessed for severity and relationship to study intervention and alternate etiology (if not related to study intervention) only by the site Principal Investigator or Co-investigator with the training and authority to make a diagnosis.
- Recorded on the appropriate SAE data collection form and eCRF.
- Followed through resolution by a licensed study clinician.
- Reviewed and evaluated by the principal investigator, the DMID MO, MM, CPM, and the IRB/IEC.

Severity of Event

All AEs or SAEs will be assessed for severity according to the toxicity grading scales in Appendix A.

For adverse events (AEs) not included in the protocol-defined grading system, the following guidelines will be used to describe severity.

- Mild (Grade 1): Events that are usually transient and may require only minimal or no treatment or therapeutic intervention and generally do not interfere with the subject's usual activities of daily living.
- Moderate (Grade 2): Events that are usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.
- Severe (Grade 3): Events interrupt usual activities of daily living, or significantly affect clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.

AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity.

Relationship to Study Intervention

The licensed study clinician's assessment of an AE's relationship to study intervention is part of the documentation process, but it is not a factor in determining what is or is not reported in this study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. The relationship to study intervention must be assessed for AEs using the terms: related or not related.

- Related – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

Time Period and Frequency for Event Assessment and Follow-Up

In this study, solicited symptoms of influenza infection will be collected from the time of first exposure event through 7 days post-last-exposure and will **not** be considered adverse events. Unsolicited AEs will be captured starting at the time of exposure through 28 days post-exposure. AEs will be followed through resolution. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Adverse Event Reporting

Investigators Reporting of AEs

Information on all AEs should be recorded on the eCRF. Information to be collected for AEs includes event description, date of onset, assessment of severity, relationship to study intervention and alternate

etiology (assessed only the site Principal Investigator or Co-investigator with the training and authority to make a diagnosis), date of resolution, seriousness, and outcome. AEs occurring during the study will be documented appropriately regardless of relationship.

All clearly related signs, symptoms, and results of diagnostic procedures performed because of an AE should be grouped together and recorded as a single diagnosis. If the AE is a laboratory abnormality that is part of a clinical condition or syndrome, it should be recorded as the syndrome or diagnosis rather than the individual laboratory abnormality. Each AE will also be described in terms of duration (start and stop date), severity, association with the study intervention, action(s) taken, and outcome.

Serious Adverse Event Reporting

Investigators Reporting of SAEs

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the PI (Wilbur Chen), at the following address: wilbur.chen@som.umaryland.edu. Any SAEs that satisfy a reportable new information, according to the UMB IRB, will be reported to the UMB IRB.

In addition to the SAE form, select SAE data fields must also be entered into the DCC system. Please see the protocol-specific MOP for details regarding this procedure.

The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the site principal investigator or appropriate co-investigator becomes aware of an SAE that is suspected to be related to study intervention, the site principal investigator or appropriate co-investigator will report the event to the UMB IRB.

Unanticipated Problems

Definition of Unanticipated Problems (UP)

The Department of Health and Human Services Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to DMID, the reviewing Institutional Review Board (IRB) and to the Statistical and Data Management Coordinating Center (SDMCC)/study Sponsor and the lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;

- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and as per reportable new information definition. for Serious Adverse Event Reporting within 24 hours of the investigator becoming aware of the event.
- Any other UP which satisfies one of the categories of a Reportable New Information (RNI) event will be reported to the IRB within five days of the investigator becoming aware of the problem.

8 CHIVITT STUDY PROCEDURES/EVALUATIONS

8.1 Study Procedures

8.1.1 Admission/entry to a CHIVITT cohort

- Obtaining of informed consent or confirmation of signed informed consent
- Vital signs (including SpO_2, HR, RR, BP, and oral temperature) and height and weight will be obtained
- A targeted physical examination may be performed, as directed by the medical history, medications, and vital signs assessment. An absence of significant past medical history, concomitant prescription medications, and abnormalities in vital signs will not require a physical examination.
- Review and record medical history* and concomitant medications†.

  *All significant past and current diagnoses will be documented. All signs and symptoms (regardless of severity) that the subject has experienced within 30 days prior to screening will be documented. Medication allergies will also be documented on this form.

  †All prescription and non-prescription medications, alternative therapies, and vaccines taken within 90 days prior to screening or since the last study visit should be documented.

- For Donors and Recipients, performance of a multiplex influenza, SARS-CoV-2, respiratory virus panel test (e.g., BioFire Respiratory Virus panel)
- For Donors, collection of a respiratory sample for viral quantitation
- Venous whole blood (~10 mL) collection for HAI and FRNT assays and the storage of remaining volumes for secondary research
- For Recipients only, Nasal wash specimen (~5-10 mL) will be collected.
- Additional nasal swab and a saliva sample for research tests (Participants will be asked to collect a saliva sample using a standard protocol and by spitting into a screw cap specimen cup or 50 mL polypropylene screw cap tube.
- For Donors, Exhaled breath sampling within 24 hours of entry.
- Point of care urine pregnancy test for donor and recipient females of childbearing potential within 24 hours of admission to the hotel quarantine unit (to be completed prior to any exposure events)

8.1.2 Exposure Events

- Exposure events will be held in a large hotel room each lasting up to several hours. Each cohort will experience multiple exposure events over the course of their stay. Each exposure event will be either a high or low air hygiene exposure.
- Low air hygiene conditions will be limited by the quarantine hotel's standard mechanical ventilation (~0.7 air changes per hour) and may be further limited by sealing windows and or blocking bathroom exhaust fans and hallway supply as necessary to control carbon dioxide levels to simulate a poorly ventilated public space while remaining within the OSHA permissible exposure limit.
- High air hygiene conditions will supplement the building's mechanical ventilation with portable HEPA filter units and / or germicidal ultraviolet air disinfection (either upper-room 254 nm or direct 222 nm UV-C or similar technologies and wavelengths with monitoring to confirm compliance with current ACGIH Threshold Limit Values).
- The room in which the exposure events occur will have the ventilation rate, CO_2 concentration, temperature, and relative humidity continuously monitored and supplemental heating, cooling, and dehumidification will be supplied to maintain comfort (~20˚C ± 3) and relative humidity <40%.
- Recipients will be randomized to spray-borne and touch transmission intervention (Intervention Recipients, IR) and control (Control Recipients, CR) within each exposure event, except that the initial exposure event may include only CR to assess the SAR and power of the study design for the remaining events.
- Prescribed group activities will be planned by the study team to involve close-distance interactions between study participants (donors and recipients). Due to the unpredictability of when donors may become available, it is intended to be flexible for the scheduling of these events for the time of day, duration, and frequency of number of days. For example, donors and recipients will engage in supervised playing of board games, watching films, eating meals together while ensuring that randomized designated IR individuals comply with spray-borne and touch transmission prevention interventions (face shield use, hand sanitization, and no-touch-face rules). Outside of the exposure events, donors and recipients are to be housed in separate rooms to prevent inadvertent "contamination" or transmissions outside of the prescribed group activities.
- The conditions of each exposure event, time, duration, and which participants engaged in the activity are to be documented in study logs.

8.1.3 Daily Assessments

- Vital signs (including SpO_2, HR, RR, BP, and oral temperature) will be obtained at least twice daily (intended to be at least once in morning and once in afternoon). After the first two days, vital signs will be collected once a day until after exposure to a Donor or at PI discretion.
- A targeted physical examination may be performed, as directed by the medical history, medications, and vital signs assessment. An absence of significant past medical history, concomitant prescription medications, and abnormalities in vital signs will not require a physical examination.
- Once daily collection of a respiratory sample for viral quantitation and performance of a combined influenza and SARS-CoV-2 test (e.g., BioFire Respiratory Virus panel).After the

first two days of negative tests these daily assessments may be paused until after exposure to the first Donor.
- Twice daily completion of a Modified Jackson Score, Investigator Assessment Tool (Appendix C).
- Once daily completion of a self-reported Symptom Diary, FLU-PRO Survey Instrument and Validation Diary (Appendix B).
- Additional nasal swab and a saliva sample for research tests. However, these two samples will not be collected between the period after the incubation and before the first donor arrives.
- For Donors, Exhaled breath sampling

8.1.4 Intermittent Assessments

- Exhaled breath sampling, daily post-onset of infection in Recipients and in Donors
- Venous whole blood (~10 mL) collection
- Nasal swab for viral detection and nasal swabs and saliva for research tests at PI discretion.

8.1.5 Assessment within 48 hrs from Influenza Infection (Recipients)

Approximately 48 hrs (window 36-60 hrs) from virologic-confirmation of influenza infection (e.g., BioFire positive test), a blood volume of ~10 mL will be collected for the following safety labs: white blood cells (WBC), absolute lymphocyte count (ALC), hemoglobin (Hgb), platelets (PLT), alanine transaminase (ALT), and creatinine (Cr). The ALC is a variable under the Modified Jackson score. (see Appendix C for Score).

8.1.6 Ad hoc Assessments

At the request of the study physician, ad hoc assessments may be performed to facilitate the close monitoring and management of influenza illness. These assessments must be documented with signed orders and accompanied by a doctor's note which provides clinical assessment and judgement.

- 12-lead electrocardiogram may be performed for complaints of chest pain, pressure or tightness, feelings of heart fluttering, or sustained tachycardia.
- Vital signs measurements may be changed in frequency (e.g., increased frequency) for the management of influenza illness
- Clinical laboratory assessments which might include serum chemistry, hematology, coagulation, cardiac enzymes, and urinalysis (listing not exhaustive of possible lab assessments which could be performed)

8.1.7 Discharge/exit from a CHIVITT cohort

- Vital signs (including SpO2, HR, RR, BP, and oral temperature) will be obtained, if not completed within the past 12 hours
- A targeted physical examination may be performed, as directed by the signs and symptoms, medications, and vital signs assessment. An absence of concurrent signs or symptoms and abnormalities in vital signs will not require a physical examination.
- Performance of a combined influenza and SARS-CoV-2 test (e.g., BioFire Respiratory Virus panel), if not done within the past 12 hours

- Collection of a respiratory sample for viral quantitation and saliva sample, if not done within the past 12 hours
- Venous whole blood (~10 mL) collection, if not done within past 48 hours
- Review follow-up procedures, discharge medications (if any), and indications for seeking additional medical care.

8.1.8 Outpatient Clinic Follow-up Visit (~1 month from CHIVITT)

Each subject who participated in a CHIVITT cohort will be contacted approximately 4 weeks (allowable window is 3-5 weeks) from their discharge from a CHIVITT cohort. The participant will be asked whether they have fully recovered from their influenza illness (if any) and whether there were any interim unscheduled medical visits. Venous whole blood (~10 mL) will be collected. Nasal wash specimen (~5-10 mL) will be collected. The participant will be afforded the ability to ask questions or raise concerns and to be provided the answers.

8.1.9 Phone call follow-up (~2 months from CHIVITT)

Each subject who participated in a CHIVITT cohort will be contact approximately 2 months (allowable window is 7-10 weeks) from their discharge from a CHIVITT cohort. The participant will be asked whether they have fully recovered from their influenza illness and whether there were any interim unscheduled medical visits. The participant will be afforded the ability to ask questions or raise concerns and to be provided the answers.

8.2 Research Laboratory Evaluations

HAI and FRNT Assays

The ability of antibodies to inhibit the interaction of hemagglutinin (HA) protein on the surface of influenza virus with sialic acids on the surface of red blood cells (RBCs) will be examined by hemagglutinin inhibition (HAI) assay performed on serum samples from study participants at the specified collection timepoints. The readout will be the ability of antibodies to inhibit hemagglutination, HAI titers. For H3N2 viruses that have no HA activity, serum antibody will be titred using a focus reduction neutralization test (FRNT) in which the ability of the serum to inhibit the formation of infectious foci in cell culture will be measured. The HAI and FRNT assays are to be performed with deidentified specimens at a collaborating lab (Dr. Florian Krammer, Icahn School of Medicine at Mount Sinai, New York, NY). The virus strain-specific HAI or FRNT titer will be considered the primary method for determining a participant's immunity.

Potential Antibody Assays if funding is available

A briefing listing of some of the potential additional antibody assays which may be performed on serum and/or nasal wash specimen includes the following:

- Microneutralization (MN) assay, measures the ability of antibodies to prevent cytopathologic effects in cell cultures infected with influenza viruses.
- Neuraminidase inhibition (NI) assay, measure antibodies that block the enzymatic activity of the neuraminidase protein (NA), a surface glycoprotein which cleaves sialic acid residues.
- Enzyme-linked immunosorbent assay (ELISA), measures binding antibodies against full-length HA, HA-stalk, NA, and other influenza antigens.

- Influenza virus protein microarray, an approach which can measure the "landscape" of serum antibodies by measuring the binding against hundreds of HA and NA proteins simultaneously and spanning multiple virus subtypes and strains.
- Antibody-dependent cellular cytotoxicity (ADCC) assay, functional antibody assay.

8.3 Secondary research use of stored specimens

Subjects will be asked to consent to the storage and secondary research use of leftover residual specimens. If they choose to not provide permission for leftover specimens for secondary research use, they will not be eligible for the study. However, a subject has the right to withdraw from secondary research at any time and will be instructed that they must submit this decision in writing to the principal investigator.

The secondary research use of the banked samples and data will require a separate study protocol with IRB approval before the use of the specimens. These residual samples will be stored with a barcode for an indefinite period of time at the CVD or the University of Maryland (College Park). The linking information (to PHI) will be maintained separately in a secured and locked location to which only authorized study staff members have access. Subjects will be notified that their residual samples (e.g., serum and nasal specimens) may be used for possible use in secondary research. Subjects will be informed that they cannot take part in this study if they do not agree to allow for the identifiable (coded) samples and data to be used for secondary research. Possible use in other research studies may include but is not limited to non-traditional immune assay development, assessing innate immune factors, cytokines, and other virologic evaluations. These residual samples will be stored indefinitely at a central clinical storage facility and may be shared with investigators at the participating site and with other investigators at other institutions. No genetic tests will be performed on samples. These specimens are not intended for any genetic studies or the creation of cell lines or commercialized products. These specimens would be used in laboratory-based assays to try to answer questions related to influenza or other respiratory viruses or vaccine-induced immune responses. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject confidentiality.

8.4 Exhaled Breath Sampling

All Donors and secondarily influenza-infected Recipients will be asked to provide exhaled breath samples using the Gesundheit-II cone collector (and/or next generation iEBA sampler, when available). The participant will be instructed to sit inside a tented apparatus containing the cone collector. The participant's face will be placed near the wide part of a metal funnel (the cone-shaped sampling instrument) or newer transparent cone with conductive coating. Breath samples are collected through the instruments gentle and controlled suction of air into the narrow part of the funnel and into the instrument's collection system. Samples will be collected for a 30-minute period with the participant breathing normally. Participants will also be instructed to perform several prescribed maneuvers. For example, at 5-minute intervals over 25 minutes the participant will be asked to recite standard text (e.g., the alphabet), talk loudly, shout, or sing. The iEBA sampling instrument is able to fractionate different sized particles and these fractionated samples can be assayed by quantitative viral culture, serial passaged for detection of low concentrations of virus, and RTqPCR. Within the EMIT-2 program, a newer iEBA collection instrument (e.g., Gesundheit-III) will be developed and is intended to be a supplement and replace the Gesundheit-II over the course of the 5-year grant. It is anticipated that newer instruments may replace the tent with a hood resembling a loose-fitting powered air-purifying respirator. It is also anticipated that some shorter duration (5-15 minute) samples may be collected using a spirometry

mouthpiece and nose clip while the participant breathes slowly and deeply or coughs, and that a few deep breaths while vocalizing through a spirometry mouthpiece may also be collected.

8.5 Environmental and Personal Sampling

Sampling will also be conducted throughout the exposure room and may also be performed in other areas where CHIVITT participants reside, including the common rooms, bathroom, showers, and bedrooms. Surface swabs of common contact surfaces (fomites) will be collected and evaluated for the presence of influenza virus by PCR and culture.

Within the EMIT-2 program's Advanced Bioaerosol Technology Core (ABTC), a novel ambient sampling system will be developed. The following description of the device provides the aspirational goal of the device. The device will use a 3-stage water-based condensational growth technology to enlarge airborne nanoparticles as small as 10 nm to micrometer size under moderate temperatures without using steam injection or heat. The water condensation technique encapsulates each airborne particle within a water shell that enables direct impaction of these particles onto solid or liquid surfaces for delivery to in vitro assays. Gentle impaction will be achieved by delivering the particle-containing flow through multiple nozzles. Because condensation is achieved at moderate temperature, and impaction velocities are optimized to minimize membrane damage, this technology retains high infectivity of airborne pathogens. Significantly, the technology will allow for each system to be tuned for collection into buffer for bulk analysis of total viral RNA or into culture media cell inoculation by live virus; onto hydrogel membranes for efficient collection and culture of viable virus; or into an oil phase to form water emulsions enabling single aerosol particle analysis.

Environmental and personal samples will also be collected using NIOSH BC251 cascade samplers – the current standard method for bioaerosol collection. The NIOSH BC251 is a two-stage dry cyclone employing two 1.5 mL microcentrifuge Eppendorf tubes screwed into a small metal base with a backup filter as a final third stage. Coupled with a small 3.5 liter/minute personal air sampling pump it has been extensively used for area as well as personal sampling to monitor viral aerosol exposures of healthcare workers. Personal air samples may be collected from Recipients by providing them with a waist belt mounted personal air sampling pump (weight, approximately 22 oz) and attached shoulder strap for mounting the BC251 (<6 oz) in their breathing zone.

8.6 Data for Secondary Research

Data from this study may be used for secondary research. Only individual subject data that has been coded will be made available for secondary research. The Statistical Analysis Plan (SAP) and Analytic Code will also be made available. Data will be available immediately following publication, with no end date. De-identified data may be used for secondary research without further consent of the subject. Any coded or identifiable data used for secondary research may occur if the subject agreed to use by signing this study consent form and has not withdrawn their consent.

9 OUTCOMES AND DATA ANALYSIS

9.1 Primary Outcome Measure – Attack Rate of Influenza

The SAR of influenza infection will consist of virological confirmation, symptomatic infection, or serological evidence of an influenza infection.

9.1.1 Viral Confirmation of Influenza Infection

BioFire Respiratory Panel. This CLIA-approved, commercially available molecular diagnostic test will be the primary means for determining influenza infection. The test result will be used for the day-to-day management and decision-making process for the care of study participants.

Cepheid 4-Plex. This CLIA-waived, commercially available molecular diagnostic test (which runs the Xpert® Xpress SARS-CoV-2/Flu/RSV assay) can be a satisfactory alternative means for determining influenza infection from the BioFire Respiratory Panel.

Qualitative RT-PCR. This research diagnostic test will be used as a secondary means for determining influenza infection. The test result may not be available for day-to-day decisions and the test may be performed in batched analysis.

Quantitative RT-PCR. This research diagnostic test will be used as another secondary means for the characterization of influenza infection. Peak viral shedding will be calculated for each subject as the maximum shedding during their CHIVITT participation period. Total viral shedding will be calculated with an area under the curve (AUC) with study day on the x-axis and shedding on the y-axis. The test result may not be available for day-to-day decisions and the test may be performed in batched analysis.

9.1.2 Symptomatic Influenza Infection

Modified Jackson Instrument score. (Appendix C) Symptomatic influenza infection for determining AR will be assessed using a Modified Jackson score collected by study staff twice a day (approximately 8 AM and after 3 PM) from the time of admission until the time of discharge. The Modified Jackson score will be calculated after evaluation of vital signs, laboratory results, and the Modified Jackson score across multiple days.

FluPro® participant reported tool. (Appendix B) A previously validated, patient-reported outcome (PRO) measure to standardize the assessment of influenza symptoms in clinical research (i.e., FLU-PRO Survey Instrument and Validation Diary) will be completed by subjects once a day (after 3:00 PM) from the time of admission through discharge. The investigators will review the FLU-PRO and Validation Diary responses each day.

Fever. A febrile illness, with or without accompanying symptoms, will be defined as an occurrence of an oral temperature of ≥100.2°F (≥37.9°C). *Note: a qualifying temperature of 100.2°F-100.5°F does not constitute a gradable toxicity (see Appendix A).* Elevated temperatures must be confirmed with a repeat measurement within 5-60 minutes.

Clinical Findings of Respiratory Tract Infection. At least once daily, the study physician will perform at least a limited physical examination of the head, neck, and lungs; a directed or more detailed physical examination may be performed at the discretion of the study physician for any reason. Physical examination findings which would be consistent with an influenza infection will include:

- Nasal discharge
- Otitis
- Sinus tenderness
- Pharyngitis
- New wheeze, rales, rhonchi, or other lower tract signs

9.1.3 Serological Confirmation of Influenza Infection

Seroconversion. A positive seroconversion will be defined as achieving ≥4-fold increase in serum HAI titer from baseline. The serological assessment will be performed in batched analyses and will not be available for day-to-day decisions.

Seropositive. A seropositive will be defined as a serum HAI titer of ≥40 at 1-month post-CHIVITT. The serological assessment will be performed in batched analyses and will not be available for day-to-day decisions.

9.2 Sample Size Considerations

We estimate effect sizes of interest based on an expected SAR of 25% and two alternate hypotheses that: a) long-range aerosol transmission accounts for 75% of transmission and b) contact, fomite, and spray-borne transmission account for 60% transmission. These hypotheses are essentially attributable fractions, not the expected effect size of the interventions. The interventions, unfortunately, cannot cleanly block one mode without impacting transmission via other modes. Therefore, we derived the estimated effect sizes for the interventions by analysis of planned interventions and their impact on each mode of transmission. It is assumed that hand hygiene and face shield is 100% effective for contact and spray-borne transmission and the effectiveness for short-range aerosols is based on the work of Lindsley[5] and Bischoff[6]. The air hygiene (ventilation, filtration, and germicidal UV light) intervention will be designed to be 100% effective against long-range aerosol transmission using a validated computational model of these systems, to be developed by Research Project 2 of the EMIT-2 U19 Project, to achieve the equivalent of > 60 ACH. However, air hygiene interventions are relatively less effective against short-range aerosol transmission.

Because of incomplete blockage of aerosol transmission by air sanitation and partial blockage of short-range transmission by the face shields, we assume marginal effect size of 79% reduction in the observed SAR. To compute power, we use one-sided alternatives because we do not expect that either intervention is capable of increasing transmission. Given a sample size of 54 in an intervention group, we will have 84% power to detect the intervention effect with an alpha level of 5%.

For power to detect an effect of contact, fomite, and spray-borne transmission, we assume a marginal effect size of 76% reduction in observed SAR. Given a sample size of 63 in an intervention group, we will have 84% power to reject the null hypothesis of no effect with the hand hygiene and face shield.

9.3 Analysis Plan

We will plan to analyze the binary outcome (SAR of influenza infection) using a generalized estimating equation (GEE) with a logistic link function. Individuals sharing a CHIVITT cohort will be considered as a cluster and the dependence will be adjusted by the working correlation in the GEE model. The effect of the interventions will be treated as independent, and the analysis will be controlled for sex, race-ethnicity, and age ≥ or < 30 years. We will examine sex as a biological variable for a main effect on infection risk but will not have sufficient power to perform subgroup analysis. R markdown and reproducible statistical analysis will be applied to ensure maximal reproducibility of the data analysis.

Continuous data will be transformed (e.g., Box-Cox transformation) to meet the normality assumption in the analysis. To compare proportions among unpaired samples, we will use logistic regression and adjust covariates as needed. For paired samples, we will use the conditional logistic regression. To examine the group effect on the continuous effect, we will use the general linear model. To test for a trend across

groups, we will use the chi-squared test for trend to compare proportions or Cuzick's test for trend to compare continuous data. Spearman correlation tests will be used to assess correlations between non-binary ordinal variables. The association of immunological measures and other covariates on the risk of infection will be examined using generalized estimating equations (GEE) with a Poisson distribution and robust standard errors to overcome the potential overdispersion. Principal components analysis (PCA) will be used to assess the extent to which variables can be summarized using a smaller number of components. The potential effects of age and sex will be examined in all multivariable models.

10 OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

This study will be conducted in accordance with the ethical principles set out in the World Medical Association Declaration of Helsinki, The Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines for Biomedical Research Involving Human Subjects, and in conformity with ICH GCP regulations.

This study will be conducted under the auspices of the University of Maryland, Baltimore Institutional Review Board (UMB IRB), Baltimore, Maryland, U.S.A. (DHHS OHRP Federal Wide Assurance number 00007145).

When additional sites are added for the recruitment of Donors, the respective IRB must also provide review and approval of the study prior to the recruitment of participants. In instances where a Reliance Agreement can be negotiated, the UMB IRB will maintain oversight of the study.

The principal investigator will obtain IRB approval for this protocol and send supporting documentation to the DMID before initiating recruitment of subjects. The IRB must be registered with OHRP as applicable to the research. DMID must receive the documentation that verifies IRB-approval for this protocol, associated informed consent documents, and upon request any recruitment material and handouts or surveys intended for the subjects, prior to the recruitment and enrollment of subjects. Any amendments to the protocol or consent materials will be approved by the IRB before they are implemented. IRB review and approval will occur at least annually throughout the enrollment and follow-up of subjects and may cease if annual review is no longer required by applicable regulations and the IRB. The investigator will notify the IRB of deviations from the protocol and reportable new information, as applicable to the IRB policy.

10.2 Informed Consent Process

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout the subject's participation in the study. Before any study-related activities and in agreement with ICH-GCP and the applicable local ethical and regulatory requirements, the site investigator must ensure that the subject is fully informed about the objectives, procedures, potential risks, and potential benefits of study participation. Subjects will receive a concise and focused presentation of key information about the study, verbally and with a written or electronic consent form. The explanation will be organized and presented in lay terminology and language that facilitates understanding why one might or might not want to participate. Additional screening activities may occur by phone using an IRB approved process that ensures confidentiality. The key information about the purpose of the study, the procedures and experimental aspects of the study, risks and discomforts, any

expected benefits to the subject, and alternative treatment will be presented first to the subject. The subject will be asked to consent for future use of specimens for secondary research; the subject will be instructed to submit a written request for stored specimens to be destroyed. Information will also include the foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated. The subjects will be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled. The rights and welfare of the subject(s) 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.

Subjects will be informed that records identifying the subject will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the subject's identity will remain confidential. Subjects will be informed whether private information collected from this research and/or specimens will be used for additional research, even if identifiers are removed.

Subjects will be allowed sufficient time to consider participation in this research trial and have the opportunity to discuss this trial with their family, friends or legally authorized representative, or think about it prior to agreeing to participate.

10.3 Subject Confidentiality

Subjects will be assigned coded subject identification numbers and will not be identified by name. The subject's names and code numbers (code key) will be kept at the site so that subjects can be re-contacted to participate in studies. The study site will maintain the subject's information and code key in locked files or on password protected secured computer servers. Subject confidentiality is strictly held in trust by the site principal investigator and study personnel. This confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to participating subjects.

No information concerning study data will be released to any unauthorized third party without prior written approval of the Principal Investigator. Research teams who maintain the code key will contact subjects who qualify for future CHIVITT studies. As we are using research immunology assays and not CLIA-certified assays, we will not disclose the results of immunology testing to the participants.

All data and information generated by the clinical site as part of this study (other than a subject's medical records) will be kept confidential by the site principal investigator and other study personnel to the extent permitted by law. This information and data will not be used by the site principal investigator or other study personnel for any purpose other than conducting the study and for potential recruitment into future CHIVITT studies. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the site principal investigator or other study personnel; (2) information which is necessary to disclose in confidence to an IRB solely for the evaluation of the study (3) information which is necessary to disclose to provide appropriate medical care to a study subject; or (4) study results which may be published. Authorized representatives of the Sponsor, university ethics board (IRB), or other auditing agencies may inspect all documents and records required to be maintained by the site principal investigator. This includes, records related to the samples used for this study. The study site will permit access to such records.

To protect privacy, the NIH has provided a Certificate of Confidentiality, CoC. With this CoC, the participating sites cannot be forced to release information that may identify the participant, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The participating sites will use the CoC to resist any demands for information that would identify the participant, except as explained below.

The CoC cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects, like this study or local laws, such as for reporting of communicable diseases.

A CoC does not prevent the participant from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive study information, then the participating sites may not use the CoC to withhold that information.

The CoC does not prevent the participating sites from reporting without the participant's consent, information that would identify the subject as a participant in the study regarding matters that must be legally reported including child and elder abuse, sexual abuse, or wanting to harm themselves or others.

Hotel staff will be providing services as per their standard duties and are instructed to maintain confidentiality as per the standards of the hotel industry. There will be no further control or oversight from the study team over the hotel staff with regards to assurances of confidentiality. Nonetheless, any breaches of confidentiality will be immediately brought to the attention of hotel executives and supervisors to minimize further occurrences.

Electronic data, photo, and video recordings are intended to be collected and stored using secure and password protected means, as described in Section 2.5.1. Participants and the IRB will be informed should there be breaches in confidentiality with these forms of data.

10.4 Data Handling and Record Keeping

The PI is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. Data will be entered into REDCap to record and maintain data for each subject enrolled in this study. For paper source documents, entries should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, cross out the original entry with a single line and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

All data must be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete. All data collection forms and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the site principal investigator or designee. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site principal investigator. During the study, the investigator must maintain complete and accurate documentation for the study.

Study related records, including the regulatory file, study product accountability records, consent forms, subject source documents and electronic records will be maintained indefinitely. No records will be destroyed without the written consent of DMID. Consent forms with specimen retention linked to identifiable specimens will be maintained for as long as the specimens remain in identifiable format.

11 CLINICAL MONITORING

Clinical Monitoring will be conducted to ensure that the rights and well-being of trial subjects are protected, that the reported trial data are accurate, complete, and verifiable. Clinical Monitoring also ensures conduct of the trial is in compliance with the currently approved protocol, ICH, GCP, and applicable regulatory requirements. Clinical monitoring will also verify that any critical study procedures are completed following specific instructions in the protocol-specific Manual of Procedures (MOP).

Monitoring for this study will be performed by a designated independent monitor(s). Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports. Monitoring visits will include, but are not limited to, review of regulatory files, source data, eCRFs, ICFs, medical and laboratory reports, study record storage, training records, and protocol and GCP compliance. Site monitors will have access to study staff and all study documentation. Study monitors will meet with the PI to discuss any problems and outstanding issues and will document site visit findings and discussions.

Quality Assurance and Quality Control

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

- Routine internal quality control (QC) and quality assurance (QA) activities
  - for the purposes of measuring, documenting and reporting study conduct, protocol adherence, human subjects' protections, and reliability of the protocol-driven data collected,
  - independent of sponsor site monitoring.
- A process for identifying data quality issues (i.e., data collection, 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.

The site principal investigator will provide direct access to all study-related sites, source data/data collection forms, and reports for the purpose of monitoring, auditing by the sponsor, and inspection by local and regulatory authorities. The site principal investigator will ensure all study personnel are appropriately trained and applicable documentation are maintained on site.

The data system will implement quality control procedures and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site for clarification and resolution.

12 PUBLICATION POLICY

All investigators funded by the NIH must submit, or have submitted for them, an electronic version of their final, peer-reviewed manuscripts, upon acceptance for publication, to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/). The NIH Public Access Policy ensures the public has access to the published results of NIH-funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central

upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after the official date of publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of Extramural Research (OER) Grants and Funding, http://grants.nih.gov/grants/oer.htm

Following completion of this clinical trial, the PI will publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry, such as ClinicalTrials.gov (http://clinicaltrials.gov/), which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies.

13 LITERATURE REFERENCES

1. Milton DK, Fabian MP, Cowling BJ, Grantham ML, McDevitt JJ. Influenza virus aerosols in human exhaled breath: particle size, culturability, and effect of surgical masks. PLoS Pathog 2013;9(3):e1003205. DOI: 10.1371/journal.ppat.1003205.

2. Paules C, Subbarao K. Influenza. Lancet 2017;390(10095):697-708. DOI: 10.1016/S0140-6736(17)30129-0.

3. Erbelding EJ, Post DJ, Stemmy EJ, et al. A Universal Influenza Vaccine: The Strategic Plan for the National Institute of Allergy and Infectious Diseases. J Infect Dis 2018;218(3):347-354. DOI: 10.1093/infdis/jiy103.

4. Nguyen-Van-Tam JS, Killingley B, Enstone J, et al. Minimal transmission in an influenza A (H3N2) human challenge-transmission model within a controlled exposure environment. PLoS Pathog 2020;16(7):e1008704. DOI: 10.1371/journal.ppat.1008704.

5. Killingley B, Enstone JE, Greatorex J, et al. Use of a human influenza challenge model to assess person-to-person transmission: proof-of-concept study. J Infect Dis 2012;205(1):35-43. DOI: 10.1093/infdis/jir701.

14 APPENDIX A: GRADING OF SEVERITY FOR ADVERSE EVENTS

Abnormal vital signs will be recorded as adverse events according to the severity scores tabulated below. The table is not intended to be all inclusive and non-listed abnormal vital signs will require investigator judgement. In general, abnormal vital signs alone which are otherwise asymptomatic will not qualify as grade 4, potentially life-threatening events.

Vital Sign Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-Threatening (Grade 4)
Oral Temperature*	38.1°C – 38.4°C 100.6°F – 101.1°F	38.5°C – 38.9°C 101.2°F – 102.0°F	>38.9°C >102.0°F	*abnormal vital signs without other symptoms (e.g., asymptomatic grade 3 vital sign alone) cannot qualify as a grade 4 severity*
SpO_2 (%)	92-94	89-91	<89	
Tachycardia – beats per minute	100 – 130	131 – 155	>155	
Hypotension (systolic) mmHg	80 – 84	75 – 79	<75	
Hypotension (diastolic) mmHg	50 – 54	45 – 49	<45	
Respiratory Rate – breaths per minute	21-24	25-29	>30	

** Fever must be confirmed by a repeat oral temperature measurement at least 5 minutes later; no eating or drinking anything hot or cold within 5 minutes prior to taking oral temperature is necessary for a valid oral temperature measurement*

The Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.1 consists of parameters, or AEs, with severity grading guidance that are to be used in DAIDS clinical trials for safety data reporting to maintain accuracy and consistency in the evaluation of AEs. The EMIT-2 study intends to report AEs in general alignment with the DAIDS grading guidance. The following table contains the grading of some common or expected reactions and is not intended to be an exhaustive listing. In addition, all deaths related to an AE are to be classified as grade 5. The complete DAIDS Grading Table is at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables.

Adverse Event Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-Threatening (Grade 4)
Arrhythmia	No symptoms AND no intervention indicated	No symptoms AND non-urgent intervention indicated	Non-life-threatening symptoms AND non-urgent intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Hemorrhage	NA	Symptoms AND no transfusion indicated	Symptoms AND Transfusion of ≤ 10 cc/kg of packed RBCs indicated	Life-threatening hypotension OR Transfusion of > 10 cc/kg of packed RBCs indicated
Bruising	Localized to one area	Localized to more than one area	Generalized	NA
Rash	Localized rash	Diffuse rash OR Target lesions	Diffuse rash AND Vesicles or limited number of bullae or superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Stevens-Johnson syndrome OR toxic epidermal necrolysis
Bloating or Distension (of abdomen)	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Constipation	NA	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated	Life-threatening consequences (e.g., obstruction)
Dysphagia or Odynophagia	Symptoms but able to eat usual diet	Symptoms causing altered dietary intake with no intervention indicated	Symptoms causing severely altered dietary intake with intervention indicated	Life-threatening reduction in oral intake

Diarrhea	Liquid stools (more unformed than usual) by usual number of stools	Liquid stools with increased number of stools OR mild dehydration	Liquid stools with moderate dehydration	Life-threatening consequences (e.g., liquid stools resulting in severe dehydration, hypotensive shock)
Nausea	Transient (<24 h) or intermittent AND no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24-48 h	Persistent nausea resulting in minimal oral intake for >48 h OR Rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Vomiting	Transient or intermittent AND no or minimal interference with oral intake	Frequent episodes with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Arthralgia	Joint paint causing no or minimal interference with usual social & functional activities	Joint pain causing greater than minimal interference with usual social & functional activities	Joint pain causing inability to perform usual social & functional activities	Disabling joint pain causing inability to perform basic self-care functions
Arthritis	Stiffness or joint swelling causing no or minimal interference with usual social & functional activities	Stiffness or joint swelling causing greater than minimal interference with usual social & functional activities	Stiffness or joint swelling causing inability to perform usual social & functional activities	Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Myalgia (generalized)	Muscle pain causing no or minimal interference with usual social & functional activities	Muscle pain causing greater than minimal interference with usual social & functional activities	Muscle pain causing inability to perform usual social & functional activities	Disabling muscle pain causing inability to perform basic self-care functions
Altered Mental Status	Changes causing no or minimal interference with usual social & functional activities	Mild lethargy or somnolence causing greater than minimal interference with usual social & functional activities	Confusion, memory impairment, lethargy, or somnolence causing inability to perform usual social & functional activities	Delirium OR Obtundation OR Coma

Ataxia	Symptoms causing no or minimal interference with usual social & functional activities OR No symptoms with ataxia detected on examination	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Disabling symptoms causing inability to perform basic self-care functions
Seizures	Seizure lasting < 5minutes with < 24 hours postictal state	Seizure last 5 to < 20 minutes with < 24 hours postictal state	Seizure lasting ≥ 20 minutes OR > 24 hours postictal state	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
Syncope	Near syncope without loss of consciousness (e.g., pre-syncope)	Loss of consciousness with no intervention indicated	Loss of consciousness AND Hospitalization or intervention required	NA
Acute Bronchospasm	Forced expiratory volume in 1 sec or peak flow reduced to ≥70 to <80% OR Mild symptoms with intervention not indicated	Forced expiratory volume in 1 sec or peak flow 50 to <70% OR Symptoms with intervention indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Forced expiratory volume in 1 sec or peak flow 25 to <50% OR Symptoms causing inability to perform usual social & functional activities	Forced expiratory volume in 1 sec or peak flow <25% OR Life-threatening respiratory or hemodynamic compromise OR Intubation
Dyspnea or Respiratory Distress	Dyspnea on exertion with no or minimal interference with usual social & functional activities OR Wheezing OR Minimal increase in respiratory rate for age	Dyspnea on exertion causing greater than minimal interference with usual social & functional activities OR Nasal flaring OR Intercostal retractions OR Pulse oximetry 90 to <95%	Dyspnea at rest causing inability to perform usual social & functional activities OR Pulse oximetry <90%	Respiratory failure with ventilator support indicated (e.g., CPAP, BPAP, intubation)

Acute Allergic Reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with intervention indicated OR Mild angioedema with no intervention indicated	General urticaria OR Angioedema with intervention indicated OR Symptoms of mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR Laryngeal edema
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Fatigue or Malaise	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating symptoms of fatigue or malaise causing inability to perform basic self-care functions
Clinical adverse event **NOT** identified elsewhere in the complete grading table	Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

15 APPENDIX B: FLU-PRO – PARTICIPANT REPORTED TOOL

Flu-Pro survey

Please complete the survey below.

Thank you!

Time of Flu-Pro survey ___

Date and time of Flu-Pro survey ____

Please rate the extent to which you had each symptom during the past 24-hours.

	Not at all	A little bit	Somewhat	Quite a bit	Very much
Runny or dripping nose	☐	☐	☐	☐	☐
Congested or stuffy nose	☐	☐	☐	☐	☐
Sinus pressure	☐	☐	☐	☐	☐
Scratchy or itchy throat	☐	☐	☐	☐	☐
Sore or painful throat	☐	☐	☐	☐	☐
Difficulty swallowing	☐	☐	☐	☐	☐
Teary or watery eyes	☐	☐	☐	☐	☐
Sore or painful eyes	☐	☐	☐	☐	☐
Eyes sensitive to light	☐	☐	☐	☐	☐
Trouble breathing	☐	☐	☐	☐	☐
Chest congestion	☐	☐	☐	☐	☐
Chest tightness	☐	☐	☐	☐	☐
Dry or hacking cough	☐	☐	☐	☐	☐
Wet or loose cough	☐	☐	☐	☐	☐
Felt nauseous	☐	☐	☐	☐	☐
Stomach ache	☐	☐	☐	☐	☐
Felt dizzy	☐	☐	☐	☐	☐
Head congestion	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐

Please rate the extent to which you had each symptom during the past 24 hours.

	Not at all	A little bit	Somewhat	Quite a bit	Very much
Lack of appetite	☐	☐	☐	☐	☐
Sleeping more than usual	☐	☐	☐	☐	☐
Body aches or pains	☐	☐	☐	☐	☐
Weak or tired	☐	☐	☐	☐	☐
Chills or shivering	☐	☐	☐	☐	☐
Felt cold	☐	☐	☐	☐	☐
Felt hot	☐	☐	☐	☐	☐
Sweating	☐	☐	☐	☐	☐

In the past 24 hours, how often have you had any of the following symptoms?

	Never	Rarely	Sometimes	Often	Always
Sneezing	☐	☐	☐	☐	☐
Coughing	☐	☐	☐	☐	☐
Coughed up mucus or phlegm	☐	☐	☐	☐	☐

	0 times	1 time	2 times	3 times	4 or more times
How many times did you vomit?	☐	☐	☐	☐	☐
How many times did you have diarrhea?	☐	☐	☐	☐	☐

Daily Diary

1. Did you take any medication for your flu symptoms?

- Yes
- No

2. Do you have asthma, COPD (chronic obstructive pulmonary disease) or both?

- Yes
- No

3. Did you use any rescue medication today for your asthma or COPD?

- Yes
- No

(Question 3 skipped)

4. Overall, how severe were your flu symptoms today?

- No flu symptoms today
- Mild
- Moderate
- Severe
- Very severe

5. Overall, how were your flu symptoms today compared to yesterday?

- Much better
- Somewhat better
- A little better
- About the same
- A little worse
- Somewhat worse
- Much worse

6. How much did your flu symptoms interfere with your usual activities today?
   - Not at all
   - A little bit
   - Somewhat
   - Quite a bit
   - Very much
7. Have you returned to your usual activities today?
   - Yes
   - No
8. In general, how would you rate your physical health today?
   - Excellent
   - Very Good
   - Good

- Fair
- Poor

9. Have you returned to your usual health today?
   - Yes
   - No

16 APPENDIX C: MODIFIED JACKSON SCORE – PARTICIPANT REPORTED TOOL

Subject ID Today's Date Current Time

Please complete the survey below.
Thank you!

1) Time of Modified Jackson Score _____
2) Date and time of Modified Jackson Score ____

Symptoms as reported by subject

	Subject has NO symptoms	Just noticeable	Bothersome from time to time, but doesn't prevent me from doing activities	Bothersome most or all the time, and prevents me from doing activities
3) Runny Nose	0 ☐	1 ☐	2 ☐	3 ☐
4) Stuffy Nose	0 ☐	1 ☐	2 ☐	3 ☐
5) Sneezing	0 ☐	1 ☐	2 ☐	3 ☐
6) Sore Throat	0 ☐	1 ☐	2 ☐	3 ☐
7) Earache	0 ☐	1 ☐	2 ☐	3 ☐
8) Malaise (tiredness, fatigue)	0 ☐	1 ☐	2 ☐	3 ☐
9) Headache	0 ☐	1 ☐	2 ☐	3 ☐
10) Muscle and/or joint ache	0 ☐	1 ☐	2 ☐	3 ☐
10) Chills	0 ☐	1 ☐	2 ☐	3 ☐
11) Feverish	0 ☐	1 ☐	2 ☐	3 ☐
12) Chest tightness	0 ☐	1 ☐	2 ☐	3 ☐
13) Shortness of Breath	0 ☐	1 ☐	2 ☐	3 ☐
14) Cough	0 ☐	1 ☐	2 ☐	3 ☐

17 APPENDIX D: SCHEDULE OF EVENTS

Recipients Participating in a CHIVITT Cohort

		CHIVITT – approx. 14 days on Quarantine Research Unit								
	Pre-Adm	Admission	Daily*	Intermittent or Additional	(w/in 48h) upon influenza infection	(w/in 72h)	(as requested) Ad Hoc	Discharge, ~Day 14	Outpt Clinic Follow-Up ~1 month	Phone Call Follow-Up ~2 months
Inform of upcoming CHIVITT, ~1 wk prior	X									
Sign Recipient CHIVITT Consent Form		X								
Vital signs (SpO_2, HR, RR BP, temp)		X	X[1]				X	X[3]		
Targeted physical exam		X	X					X		
Nasal Swab, resp virus panel		X	X[2]	X				X[3]		
Nasal Swab, viral quantification		X	X[2]	X				X[3]		
Saliva Sample, viral quantification		X	X[2]	X				X[3]		
Nasal Wash, ~5-10 mL		X							X	
Point of care urine pregnancy for FOCBP		X								
Research Blood, ~10 mL		X		X				X[4]	X	
Clinical Safety Lab Blood, ~10 mL					X[5]		X[6]			
Modified Jackson Score, twice daily			X							
FLU-PRO survey[7], once daily			X					X9		
Exposure Events				X						
Exhaled Breath Sampling				X						
12-lead electrocardiogram							X			
Influenza Therapy (anti-viral)						X8				
Adverse Event Reporting		x	x	x	x	x	x	x	x	x

* – Daily including Day 1 unless completed as part of admission procedures.
1 – routine daily vital signs are to be performed twice daily (morning and afternoon), except once a day if the Recipient has been observed on the unit for more than two days and has not been exposed to a Donor.
2 – routine daily swabs and saliva may not be necessary prior to first exposure to a Donor and after the first two days of negative tests.
3 – to be performed/collected if not done within the past 12 hours
4 – to be collected if not done within the past 48 hours
5 – these clinical safety labs include: WBC, ALC, Hgb, PLT, ALT, and Cr
6 – these clinical safety labs are to be determined by the requesting clinician; may include: chemistry, hematology, coagulation, cardiac enzymes, and urinalysis
7 – the FLU-PRO survey is completed by the participant
8- Unless consenting to continue in the CHIVITT as a Donor
9 – the FLU-PRO survey won't be administered at discharge if the discharge occurs before 3pm, as the survey becomes available at 3pm.

Donors Participating in a CHIVITT Cohort

		CHIVITT – up to 5 days on Quarantine Research Unit						
	Pre-Adm	Admission	Daily*	Intermittent or Additional	(as requested) Ad Hoc	Discharge, ~Day 5	Outpt Clinic Follow-Up ~1 month	Phone Call Follow-Up ~2 months
Identification of potential influenza case	X							
Lab-confirmation influenza infection, w/in 48h	X							
F≥100.2°F AND cough or sore throat, w/in 48h	X							
Sign Donor CHIVITT Informed Consent Form	X	X						
Vital signs (SpO_2, HR, RR BP, temp)		X	X[1]		X	X[2]		
Targeted physical exam		X	X			X		
Nasal Swab, resp virus panel		X	X	X		X[2]		
Nasal Swab, viral quantification		X	X	X		X[2]		
Saliva Sample		X	X	X		X[2]		
Nasal Wash, ~5-10 mL							X	
Point of care urine pregnancy for FOCBP		X						
Research Blood, ~10 mL		X		X		X[3]	X	
Clinical Safety Lab Blood, ~10 mL					X[5]			
Modified Jackson Score, twice daily			X					
FLU-PRO survey[6], once daily			X			X7		
Exposure Events			X					
Exhaled Breath Sampling		X	X					
12-lead electrocardiogram					X			
Influenza Therapy (anti-viral)					X			
Adverse Event Reporting		x	x	x	x	x	x	x

* – Daily including Day 1 unless completed as part of admission procedures.
1 – routine daily vital signs are to be performed twice daily (morning and afternoon)
2 – to be performed/collected if not done within the past 12 hours
3 – to be collected if not done within the past 48 hours
5 – these clinical safety labs are to be determined by the requesting clinician; may include: chemistry, hematology, coagulation, cardiac enzymes, and urinalysis
6 – the FLU-PRO survey is completed by the participant
7 – the FLU-PRO survey won't be administered at discharge if the discharge occurs before 3pm, as the survey becomes available at 3pm.