

TITLE

Human Influenza Surveillance of Health Care Centers in the United States and Taiwan

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Statement of Compliance

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

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 45 CFR 164.508-514)
- International Conference on Harmonisation (ICH) E6; 62 Federal Register 25691 (1997)
- National Institutes of Health (NIH) Clinical Terms of Award
- Federal Wide Assurance (FWA) for the Protection of Human Subjects (as approved by the US Department of Health and Human Services (DHHS), Office for Human Research Protections (OHRP))

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

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.

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List of Abbreviations

BAL	Bronchoaveolar Lavage
cc	Cubic Centimeter
CEIRS	Center of Excellence for Influenza Research
CFR	Code of Federal Regulations
CGMH	Chang Gung Memorial Hospitals
CLIA	Clinical Laboratory Improvement Amendments
DCF	Data Collection Form
DFA	Direct Immunofluorescence Assays
DHHS	Department of Health and Human Services
ED	Emergency Department
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ID	Identification
ILI	Influenza-Like Illness
IRB	Institutional Review Board
JH	Johns Hopkins
JH CEIRS	Johns Hopkins Center of Excellence for Influenza Research and Surveillance
JHU	Johns Hopkins University
kg	Kilogram
mL	Milliliters
MOP	Manual of Operating Procedures
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
NP	Nasopharyngeal
OHRP	Office for Human Research Protections
PCR	Polymerase Chain Reaction
PI	Principal Investigator
QC	Quality Control
QA	Quality Assurance
REDCap	Research Electronic Data Capture
TFDA	Taiwan Food and Drug Administration
US	United States
WHO	World Health Organization

Protocol Summary

Title:

Human Influenza Surveillance of Health Care Centers in the United States and Taiwan

Population:

Active Surveillance: Adult subjects (ages 18-100 years) presenting to Johns Hopkins or Chang Gung Emergency Departments (ED) with (symptomatic, n=400) and without (asymptomatic, n=100) influenza like illness each year.

We will recruit an additional 100 adult subjects (ages 18-100 years) presenting to Johns Hopkins or Chang Gung EDs with laboratory confirmed influenza (for purposes of ensuring adequate sample size for serological studies) each year.

Passive Surveillance: Adult and pediatric subjects (ages 0-100 years) who tested positive for Influenza A in either the inpatient service, ED or outpatient clinics of the Johns Hopkins (n=125) or Chang Gung Healthcare Systems (n=125) each year.

Sites:

Johns Hopkins Healthcare System – United States National Capital Region
Chang Gung Memorial Hospitals – Taiwan National Capital Region

Study Duration:

7 years

Subject Duration:

Active Surveillance: 3-4 weeks

Initial Visit –Occurs in person at time of subject enrollment

Follow-up visit –Occurs 3-4 weeks after initial visit

Passive Surveillance: No duration

Objectives:

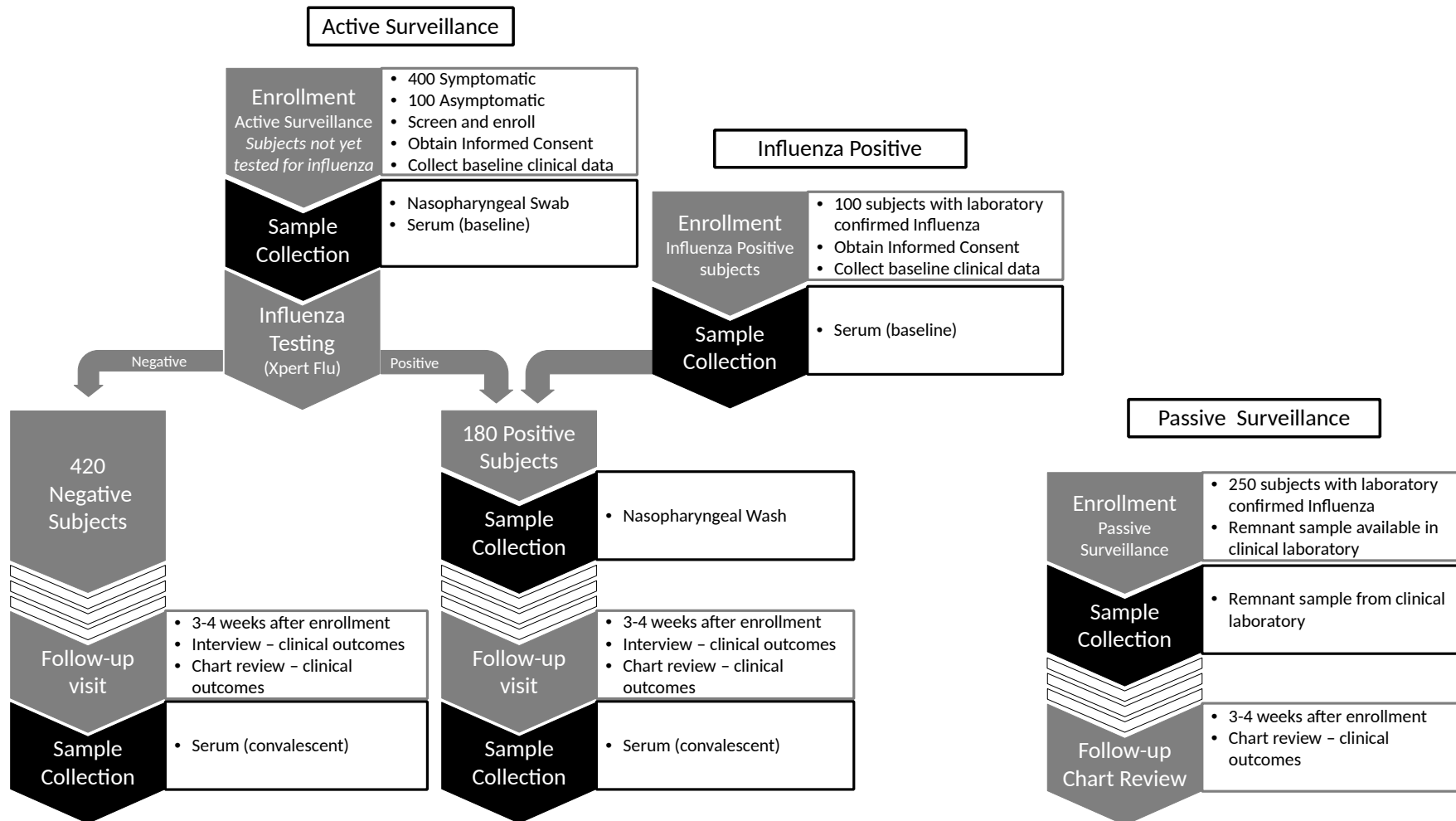
Primary:

- To develop a robust domestic and international influenza surveillance network which will allow rapid identification of circulating influenza viruses, including those with pandemic potential in human populations.

Secondary:

- To create a sample bank of nasopharyngeal and serum samples from patients with suspected and confirmed influenza.
- To identify the added benefit of serology when combined with PCR for identification of influenza infections.
- To characterize the immunologic basis of severe illness due to influenza.

Schematic of Study Design:



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KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Influenza is a rapidly evolving respiratory pathogen which causes significant morbidity and mortality, as evidenced by annual epidemics which result in three to five million severe influenza cases and between 250,000 and 500,000 estimated deaths each year.¹ Emergence of the recent 2009 H1N1 pandemic emphasized the impact that variant evolving strains of influenza have on both clinical outcomes and public health planning and preparedness. Those observations reinforce the need for new approaches to rapid high-throughput prospective molecular surveillance. Optimal surveillance includes the capacity for timely identification and characterization of current viruses with a specific focus on viruses with pandemic potential. Advancement of such methods could help fill an important gap, not only in informing vaccine development, but also guiding other key clinical and public health practice decisions.^{2,3}

We propose an innovative multi-disciplinary approach to address existing limitations associated with current surveillance methods, through development of a robust domestic and international human surveillance network, which will be closely coupled with novel, rapid, high-throughput laboratory techniques. Broad systematic influenza surveillance of human populations, including patients who seek medical care in EDs, outpatient clinics and inpatient settings, will provide a rich unbiased source of human respiratory specimens for purposes of rapid characterization of circulating and emerging virus strains with pandemic potential. Specimens will be evaluated via the Johns Hopkins Center of Excellence for Influenza Research and Surveillance (JH CEIRS) virologic analysis pipeline which permits characterization of both viral and host factors responsible for virulence, transmission and virus evolution. Collecting and linking these specimens with detailed epidemiologic and clinical data will allow timely assessment of the genotypic relationship between circulating virus, and the clinical features of disease in the community relevant for public health planning and response.

In order to facilitate rapid diagnosis and treatment of influenza, clinical laboratories use rapid but low sensitivity assays such as antigen detection or direct immunofluorescence assays (DFA), or the time-intensive but highly sensitive polymerase chain reaction (PCR) assays.^{4,5} For this study we will use Cepheid's Xpert Flu, a new molecular-based rapid influenza test which yields a result in 80 minutes and has recently obtained Food and Drug Administration (FDA) approval for

influenza testing. Previous validation studies performed in comparison to Luminex rt-PCR report a sensitivity of 91.2% and specificity of 99.4% giving an overall positive predictive value of 99.2% and negative predictive value of 93.1%.⁶

In addition to evaluating the circulating strains of influenza, we will also perform serology testing. Although the selected assay, Xpert Flu, is highly sensitive for detecting influenza in the sample, the nasopharyngeal (NP) swab used may not accurately reflect the subject's influenza status. In particular, asymptomatic patients, and/or those with lower respiratory disease, may not have sufficient viral shedding in the nasopharynx to be detected by PCR on an NP sample.⁷ Alternatively, influenza virus may be present in the NP sample, but the PCR primers may not detect the pathogen if it has a new or unusual structure that is not intrinsic to the range of PCR targets used in this platform. Influenza serologic testing permits detection of exposure and immune response to an influenza virus, but is generally not considered useful in the acute setting due to the prolonged time to test result. However, serologic testing can have value as secondary testing in order to identify patients who may have a falsely negative NP swab result. The combination of PCR on a NP sample with the addition of serology, will give a more accurate and complete perspective for purposes of population based surveillance.

Additionally, we will also perform an evaluation of individual's immune response to influenza. Although the Centers for Disease Control and Prevention (CDC) has identified several broad categories of underlying medical conditions associated with increased risk of death, hospitalization, and other influenza-related complications, many individuals with severe disease due to influenza do not have these underlying conditions.⁸ However, the relative contribution of virus-driven morbidity and mortality versus illness resulting from a dysregulated immune response due to some other cause is not known.

Elegant mechanistic studies in animal models have defined the core features of the immune network activated in response to influenza infection and the consequences when individual nodes are compromised, for example, disruption of cytokines, cytokine receptors, or entire cell lineages. Virus clearance is often reduced and delayed with severe consequences for the host. Alternatively, over-exuberant (e.g. cytokine "storm") inflammatory responses have the potential to control the virus at the expense of the physiological health of the host, with equally severe outcomes. The available data indicate that severe pathology in humans is a complex phenotype that is unlikely to reflect catastrophic gene deletions analogous to those in knockout animals. However, published data in human samples are very limited and the molecular correlates of severe disease are as yet of fairly low resolution.

Previous attempts to evaluate the inflammatory response to influenza in humans have been hindered by small sample sizes, and insufficient clinical information of the affected individual. In previous studies “severe disease” is often defined as inpatient hospitalization, which includes contributing factors beyond influenza related severity of disease. Lack of sufficient attention to potential confounders, at both the data collection and analytic stages has led to clouding of the outcome measurement of “hospitalization”, and limited investigators ability to effectively evaluate the association between immunologic response and clinical outcome.

In order to more accurately understand the interaction between immune response and severity of disease in patients with influenza, we propose to evaluate the immune response of individuals seeing medical care at the emergency department with influenza, over a range of disease severities.

2.2 Scientific Rationale

Our surveillance network includes human populations at high risk for influenza exposure or severe influenza, and leverages existing partnerships between Johns Hopkins University (JHU) in the US and Chang Gung Memorial Hospitals (CGMH) in Taiwan. Nasopharyngeal and blood (serum) samples will be collected via both prospective active and passive surveillance (permitting broad sampling and representation of the full range of illness), and include corresponding key descriptive demographic and epidemiologic parameters. These samples will undergo rapid evaluation for influenza, creating a repository of influenza viruses which can then undergo further characterization and/or distribution for studies of viral pathogenesis.

The surveillance population will intentionally include those with varying levels of illness severity from influenza, including those managed in the community outpatient setting, to those requiring intensive care. This will be accomplished via a mix of passive surveillance (selecting nasopharyngeal samples from local cluster outbreaks and/or intensive care unit populations from each of the regional hospital networks), and active surveillance (selecting from the thousands of patients with acute respiratory symptoms evaluated these EDs every year). EDs are widely recognized as the clinical venue where patients most frequently seek care during the surge associated with severe pandemics. They serve as the primary site for initial evaluation and treatment for over 200,000 US patients who are ultimately hospitalized with influenza each year and are responsible for the care of close to 10 million patients with influenza related illnesses who are treated and discharged.⁹⁻¹¹

2.3 Potential Risks and Benefits

This study will represent minimal risk to subjects. Subject involvement in this protocol is limited to obtaining nasopharyngeal swabs, nasopharyngeal washes, and blood (serum) as well as collection of clinical data.

2.3.1 Potential Risks

This is a minimal risk study. Subjects will be asked to complete a questionnaire that asks relevant questions related to their illness. Blood (serum) and nasopharyngeal specimens will be collected. Subject confidentiality will be protected.

Nasopharyngeal swabbing is momentarily uncomfortable but is not otherwise associated with risk. It may cause brief pain, itchy nose, eye watering or sneezing. Nasopharyngeal wash may cause discomfort, coughing or gagging.

The blood drawing procedure could cause infection, some discomfort and/or leave a temporary bruise and/or infection. Repeated blood drawing may be associated with the development of iron deficiency anemia. To avoid this risk, no more than 100 cubic centimeters (cc) of blood will be obtained over 8 weeks in adults and children >12 years of age, and the lesser of 50 cc or 3 cc per kilogram (kg) in children less than 12 years of age.

To minimize these risks and discomforts, a fully trained physician, nurse, study coordinator, or designee will collect the specimens. Care will be taken to obtain these specimens in a safe and hygienic manner. Infection control practices will be an integral part of the Manual of Operating Procedures (MOP) of this study.

Questions that subjects may be asked to complete during the study may contain questions that could be embarrassing and/or make them feel uncomfortable. Subjects may get tired or bored when we are asking questions or they are completing questionnaires. Subjects do not have to answer any question they do not want to answer.

All subjects will be assigned a randomly generated study ID number to protect private information. Privacy will be protected by de-identifying all private information which is entered into the electronic database system.

There are no alternative procedures for this study.

2.3.2 Known Potential Benefits

Subjects will derive no direct benefit from involvement. Subjects testing positive for influenza, and their treating provider, will be immediately informed and thus will benefit by the additional knowledge of their influenza infection.

It is possible that the study investigators will learn something new during the study that may affect subjects health or the decision as to whether they want to stay in the study or not. If this happens, we will tell the subject about it. Then they can decide if they want to continue to be in this study or not.

Data from this study may yield a better surveillance of influenza infections in humans over time. Our findings from monitoring active influenza infections among humans can act as an early warning system alerting the healthcare system. Such results will be especially beneficial to country officials involved in avian and human influenza preparedness activities.

3 OBJECTIVES AND OUTCOME MEASURES

3.1 Study Objectives

The overall objective of this study is to improve the ability of the medical and public health infrastructure to respond to influenza pandemics. The specific objectives of the protocol are as follows:

- Develop a robust domestic and international influenza surveillance network which will allow rapid identification of circulating influenza viruses, including those with pandemic potential in human populations.
- Create a sample bank of nasopharyngeal and serum samples from patients with suspected and confirmed influenza.
- To identify the added benefit of serology when combined with PCR for identification of influenza infections
- To characterize the immunologic basis of severe illness due to influenza

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

Develop a robust domestic and international influenza surveillance network which will allow rapid identification of circulating influenza viruses, including those with pandemic potential in human populations.

- We will collect a nasopharyngeal specimen and clinical, demographic and epidemiologic data on 850 subjects. We will enroll 500 subjects through active surveillance (250 US, 250 Taiwan), add an additional 100 influenza positive subjects, and retrospectively collect nasopharyngeal samples and clinical data on 250 subjects (125 US, 125 Taiwan) through passive surveillance.
- We will test each active surveillance subject for influenza and notify subjects and the subject's provider of positive influenza test results.
- We will send samples (nasopharyngeal swabs, nasopharyngeal washes, and serum) to the JH CEIRS research surveillance laboratory for further characterization, analysis, and viral recovery.

Subjects enrolled through active surveillance will be tested for influenza, which will be estimated as a proportion with 95% confidence interval.

Nasopharyngeal swab specimens obtained from subjects who have an Influenza A positive rapid test will be further tested at the JH CEIRS research surveillance laboratory in order to characterize the influenza virus causing the infection using molecular techniques. Additionally, 5% of the samples negative for Influenza A will also be sent for further testing.

3.2.2 Secondary Outcome Measures

Create a sample bank of nasopharyngeal and serum samples from patients with suspected and confirmed influenza.

- We will send samples (nasopharyngeal swabs, nasopharyngeal washes, and serum samples) to the JH CEIRS research surveillance laboratory for storage to support future research endeavors.
- We will establish a shareable database for other CEIRS projects and collaborators which will be linked to remnant sample.

To identify the added benefit of serology when combined with PCR for identification of influenza infections.

- Initial and convalescent serum from 200 selected subjects will be evaluated for evidence of influenza infection.
- Serology results will be compared to the results of the PCR assay of the NP swab performed at enrollment.

Following collection of initial and convalescent serum, samples will be evaluated for evidence of immune response to influenza. These results will then be compared to the results of the PCR assay to determine the sensitivity and specificity of routine NP swabbing techniques to detect influenza infection.

To characterize the immunologic basis of severe illness due to influenza.

- Nasopharyngeal wash and serum samples from 100 influenza positive individuals will be sent to the laboratory of Paul Thomas at St. Jude CEIRS center for detailed characterization of immunologic response.

We will ship 100 influenza positive samples (and make available the corresponding clinical information) to Dr. Paul Thomas, to enrich the serologic analysis and ensure adequate sample size for that analysis.

4 STUDY DESIGN

This is a multicenter prospective observational cohort study with two surveillance components recruiting throughout the year: primarily during high rates of influenza season, but surveillance may occur during lower rates off-season as well:

For the purposes of this study, “influenza season” will begin when the site has 2 or more positive influenza samples within 7 days, and continue until there have been 3 weeks with no confirmed influenza. Surveillance may continue outside of flu season if the research may benefit from longer period of surveillance.

Active Surveillance: Active surveillance will occur in the ED where individuals present for acute care. For this component, we will recruit both adults with and without symptoms of influenza like illness. Following informed written consent, eligible, consented subjects whom enrolled will complete a questionnaire detailing the demographic information, current symptoms, and past medical history. Then, we will collect a nasopharyngeal swab and serum sample from the subjects. The nasopharyngeal sample will be tested for influenza using a rapid PCR-based influenza test, and the result will be given to the subject’s provider and the subject. If the subject is positive for influenza, we will also collect a nasopharyngeal wash sample. We expect to enroll 500 subjects (400 symptomatic; 100 asymptomatic) each year for the duration of the study with 250 subjects from JHU and 250 subjects from CGMH.

Note: For Taiwan sites only, nasal wash will be collected from all 250 active surveillance subjects. Only NP wash samples with influenza positive confirmed cases will be sent to the JH CEIRS Central Laboratory. Negative NP wash samples will be stored at the Linkou study site laboratory in Taoyuan County.

Additional Influenza Positives: We anticipate that approximately 20% of the symptomatic population will test positive for influenza yielding a total of 80 influenza positive subjects from the active surveillance arm. In order to ensure we have sufficient numbers of influenza positive subjects, we will also recruit adults presenting to the ED who are newly diagnosed with influenza through routine clinical laboratory testing. These subjects will undergo written informed consent, and complete the enrollment questionnaire detailing demographics, symptoms and past medical history. Then, we will collect nasopharyngeal wash and serum samples. We will recruit 100 subjects (50 at JHU and 50 at CGMH) with laboratory confirmed influenza each year.

Three to four weeks after enrollment, all subjects will return for a follow up visit where they will give an interview to assess their clinical course and a serum sample. We will also review their medical record to further define their clinical course. All clinical information will be linked with stored nasopharyngeal and serum samples via an

anonymous study ID to create a database linking samples and detailed clinical, demographic and epidemiologic information.

The samples collected from these surveillance efforts will move forward to support our key objectives:

In order to further viral characterization, aliquots of the initial NP swab from the surveillance population will be used by the JH CEIRS research surveillance laboratory for additional viral characterization. This will include all subjects who tested positive for influenza and 5% of those who tested negative for influenza. Additional NP samples will be provided as needed.

Active Surveillance: Serology will be performed on 200 subjects using the baseline serum and the 3 week convalescent serum. For this aim we will select subjects who have:

- 1) No clinical symptoms (50 JHU, 50 CGMH)
- 2) Evidence of lower airway disease defined as a diagnosis of pneumonia (20 JHU, 20 CGMH)
- 3) Severe disease resulting in intensive care unit (ICU) admission mechanical ventilation or death (5 JHU, 5 CGMH)
- 4) Randomly selected samples from the remaining subjects (25 JHU, 25 CGMH)

Additional Influenza Positives: Samples collected from 100 laboratory confirmed influenza positive subjects (50 JHU, 50 CGMH) will be used to evaluate the immunologic basis of severe disease. For this aim, baseline serum, baseline nasopharyngeal wash, and 3 week convalescent serum will be sent to St. Jude CEIRS for further analysis.

Passive Surveillance: Passive surveillance will occur through the clinical laboratory, which processes samples for the outpatient, emergency department, and inpatient populations from Johns Hopkins Healthcare System Hospitals. We will not collect samples which are collected from the active surveillance population in order to avoid overlap. Waste material from nasopharyngeal samples testing positive for Influenza A will be collected and corresponding clinical information will be collected. This NP waste sample may come from NP swab, NP wash, or bronchoalveolar lavage (BAL) specimens. The clinical information will be linked with the nasopharyngeal samples through an anonymous study ID and permanently deidentified after completion of all data abstracted.

We expect to collect enroll 250 subjects each year for the duration of the study with 125 subjects from JHU and 125 subjects from CGMH. Using the corresponding epidemiologic information, samples will be selected based on their potential for novel or severe infection to move forward for further characterization.

5 Study Population

Active Surveillance: The study population will consist of adult subjects (ages 18-100 years) presenting to the ED at either JHU or CGMH with (symptomatic, n=400) and without (asymptomatic, n=100) influenza like illness each year.

Additional Influenza Positives: The study population will consist of 100 adult subjects (ages 18-100 years) presenting to the ED at either JHU or CGMH with a clinically obtained laboratory test confirming influenza infection (for purposes of ensuring adequate sample size for serological studies) each year.

Passive Surveillance: The study population will consist of 250 outpatient, ED, and hospitalized adult and pediatric patients (ages 0-100 years) who had a clinically-obtained influenza test which is influenza A positive each year.

5.1 Selection of the Study Population

Active Surveillance: This study will enroll adult subjects presenting to the ED. Children, prisoners, and those unable to provide informed consent will be excluded. Subjects who are pregnant will be included. There are no exclusion criteria based on gender or race/ethnicity. Each site (JHU and CGMH) will enroll 250 subjects per year. The anticipated gender and ethnic breakdown per year is as follows:

	Johns Hopkins University	Chang Gung University	Total
Total	250	250	500
Male	125	125	250
Female	125	125	250
African American	160	0	160
Caucasian	75	0	75
Hispanic	10	0	10
Asian	5	250	255

Additional Influenza Positives: The study population will consist of adult subjects presenting to the ED. Children, prisoners, and those unable to provide informed consent will be excluded. There are no exclusion criteria based on gender or race/ethnicity. Each site (JHU and CGMH) will enroll 50 subjects per year. The anticipated gender and ethnic breakdown per year is as follows:

	Johns Hopkins University	Chang Gung University	Total
Total	50	50	100
Male	25	25	50
Female	25	25	50
African American	32	0	32
Caucasian	15	0	15
Hispanic	2	0	2
Asian	1	50	51

Passive Surveillance: This study will retrospectively enroll subjects who have a positive clinically-obtained influenza test. This population includes outpatients, emergency department patients, and hospitalized patients. As a retrospective, minimal risk study, subjects will be enrolled under an Institutional Review Board (IRB)-approved waiver of consent. Each site (JHU and CGMH) will enroll 125 subjects per year. The anticipated gender and ethnic breakdown per year is as follows:

	Johns Hopkins University	Chang Gung University	Total
Total	125	125	250
Male	62	63	125
Female	63	62	125
African American	80	0	80
Caucasian	38	0	38
Hispanic	5	0	5
Asian	2	125	127

5.2 Inclusion/Exclusion Criteria

Passive surveillance nasopharyngeal samples will be obtained under a waiver of consent. Subjects (ages 0-100 years) will be enrolled if they 1) have a clinically obtained influenza test which is positive for Influenza A and 2) have sufficient waste nasopharyngeal sample, at least 1 mL, from NP swab, NP wash, or BAL specimens for further analysis.

Below are detailed inclusion and exclusion criteria for the active surveillance and additional influenza positive samples.

5.2.1 Inclusion Criteria

Inclusion Criteria for Active Surveillance Subjects

Active Surveillance: Subjects must meet all of the following inclusion criteria to participate in this study. Subjects will:

- Present to an eligible emergency department
- Age 18 years of age or older
- Meet the additional criteria for either symptomatic or asymptomatic subjects as listed below:

Symptomatic subjects:

Must have new onset within the past 7 days of:
Documented fever ($\geq 38^{\circ}\text{C}$) or report of fever
Either cough, headache, or sore throat

Asymptomatic subjects:

Must have none of the following symptoms within the past 7 days:

Documented fever ($\geq 38^{\circ}\text{C}$)
Report of fever
Cough
Headache
Sore throat
Myalgia
Rhinorrhea/nasal congestion
Shortness of breath

Inclusion Criteria for Additional Influenza Positive Subjects

Additional Influenza Positives: Subjects must meet all of the following inclusion criteria to participate in this study. Subjects will:

- Present to an eligible emergency department
- Age 18 years of age or older
- Have a positive laboratory test for influenza from the current ED visit

5.2.2 Exclusion Criteria

Exclusion Criteria for Active Surveillance Subjects

Active Surveillance: Subjects who meet any of the following exclusion criteria will be excluded from study involvement:

- Do not speak and understand:
English (JHU) or
English or Chinese (CGMH)
 - Unable to provide informed consent
 - Unable to provide follow-up telephone number
 - Incarcerated
 - Previously enrolled in the study
-

Exclusion Criteria for Additional Influenza Positive Subjects

Additional Influenza Positives: Subjects who meet any of the following exclusion criteria will be excluded from study involvement:

- Do not speak and understand:
 English (JHU) or
 English or Chinese (CGMH)
- Unable to provide informed consent
- Unable to provide follow-up telephone number
- Incarcerated
- Previously enrolled in the study

5.3 Criteria for Discontinuation

- Study subjects may withdraw their consent for study participation at any time during the study without penalty.
- The principal investigators determine that it is in the best interest of the subject to discontinue participation.
- The study is terminated.

6 STUDY SCHEDULE

6.1

Screening

Active Surveillance:

Eligible subjects will be recruited by dedicated trained research coordinators at the participating EDs. Screening and enrollment occur at the same visit. The clinical study staff will be responsible for collecting specimens and completing all study forms. When a potential subject is identified, he/she will be asked to consent for screening to determine eligibility. If the subject consents and is eligible and willing to participate, then study staff shall proceed with the written informed consent process.

Written informed consent will be obtained in a private area. Subjects will be provided with a description of the study (purpose and study procedures) and asked to read and sign the informed consent form. The informed consent form will be signed prior to performing any study procedures and prior to enrollment. Subjects will be given an opportunity to ask questions to assess understanding of participation.

Additional Influenza Positives:

Eligible subjects will be recruited by dedicated trained research coordinators at the participating EDs. Screening and enrollment occur at the same visit. The clinical study staff will be responsible for collecting specimens and completing all study forms. When a potential subject is identified, he/she will be asked to consent for screening to determine eligibility. If the subject consents and is eligible and willing to participate, then study staff shall proceed with the written informed consent process.

Written informed consent will be obtained in a private area. Subjects will be provided with a description of the study (purpose and study procedures) and asked to read and sign the informed consent form. The informed consent form will be signed prior to performing any study procedures and prior to enrollment. Subjects will be given an opportunity to ask questions to assess understanding of participation.

Passive Surveillance:

Dedicated research coordinators will review at minimum once a week the clinical laboratory logs to identify patients who tested positive for influenza. Patients who tested positive for influenza and have sufficient waste nasopharyngeal sample, at least 1 mL, will be enrolled under a waiver of consent in the passive surveillance arm. This NP waste sample may come from NP swab, NP wash, or BAL specimens.

6.2

Enrollment

Active Surveillance:

Patients will be considered enrolled in the study when they sign the informed consent form and are assigned a study identification number. Therefore, if for any reason a subject, after they have been assigned a study identification number, decides not to continue their participation, he/she needs to be discontinued from the study. For each enrollee, research coordinators will complete a set of brief structured clinical data forms to include basic demographic data, vaccination history, co-morbid illness, initial vital signs and history of present illness. To complete this set of forms, study coordinators will verbally ask each subject to respond to the listed questions in a step-by-step manner. After completion of the clinical data forms, research coordinators will immediately review the subject's past medical history with what is reported in the medical record. Any discrepancy between subjects report and the medical record will be brought to the subject's attention and corrected on the enrollment form as appropriate based upon the subject's report.

For each JH active surveillance subject (indeterminate influenza) a nasopharyngeal swab sample will be collected using a flocked swab and universal viral transport media according to the MOP. Part of this sample will be used for rapid testing using Xpert Flu according to manufacturer's instructions in a CLIA certified laboratory. The influenza test result will then be given to the subject and the subject's provider. The remaining sample will be aliquoted, stored, frozen and shipped to the JH CEIRS central laboratory as detailed in the MOP.

For each CGMH active surveillance subject (indeterminate influenza) a nasopharyngeal swab will be collected using a flocced swab and universal viral transport media according to the MOP. Part of this sample will be used for rapid testing using Sofia FIA according to manufacturer's instructions in a certified laboratory. The influenza test will then be given to the subject and the subject's provider. The remaining sample will be used for rapid testing using Xpert Flu; these results will not be given to the subject nor the subject's provider. Any remaining sample will be aliquoted, stored, frozen, and shipped to the JH CEIRS central laboratory as detailed in the MOP. All CGMH subjects will provide NP wash samples, and only subjects with confirmed influenza will be shipped to JH CEIRS central laboratory. Negative NP wash samples will be stored at the Linkou study site laboratory in Taoyuan County .

All subjects who have a laboratory confirmed positive influenza test will then be asked to provide a nasopharyngeal wash sample. The nasopharyngeal wash sample will be collected, processed, stored, frozen and shipped to the JH CEIRS central laboratory according to the MOP.

Additionally, 10 mL of blood will be collected from each subject, processed, stored, frozen and shipped to the JH CEIRS central laboratory according to the MOP.

Additional Influenza Positives:

Patients will be considered enrolled in the study when they sign the informed consent form and are assigned a study identification number. Therefore, if for any reason a subject, after they have been assigned a study identification number, decide not to continue their participation, he/she needs to be discontinued from the study. For each enrollee, research coordinators will complete a set of brief structured clinical data forms to include basic demographic data, vaccination history, co-morbid illness, initial vital signs and history of present illness. To complete this set of forms, study coordinators will verbally ask each subject to respond to the listed questions in a step-by-step manner. After completion of the clinical data forms, research coordinators will immediately review the subject's past medical history with what is reported in the medical record. Any discrepancy between subjects report and the medical record will be brought to the subject's attention and corrected on the enrollment form as appropriate based upon the subject's report.

All subjects who have a laboratory confirmed positive influenza test will be asked to provide a nasopharyngeal wash sample. The nasopharyngeal wash sample will be collected, processed, stored, frozen and shipped to the JH CEIRS central laboratory according to the MOP.

Additionally, 10 mL of blood will be collected from each subject, processed, stored, frozen and shipped to the JH CEIRS central laboratory according to the MOP.

Passive Surveillance:

For each enrollee, research coordinators will complete a set of brief structured clinical data forms to include basic demographic data, vaccination history, co-morbid illness, initial vital signs and history of present illness based on the medical record of the visit in which the patient received a positive influenza test. The waste nasopharyngeal sample, at least 1 mL, from NP swab, NP wash, or BAL specimens will be collected from the clinical laboratory, processed, stored, frozen and shipped to the JH CEIRS central laboratory according to the MOP.

6.3 Follow-up Visit

Active Surveillance and Additional Influenza Positives:

Follow-Up Visit

Each subject will have a follow-up visit planned for 21 – 35 days following their enrollment. At the visit, research coordinators will complete the follow-up questionnaire inquiring about additional hospitalizations or care the patient required. Additionally, 10 mL of blood will be collected, processed and stored according to the MOP. If a subject is unavailable to return for the follow up visit, they may be contacted via telephone to obtain the follow up information.

Chart Review

At least twenty-eight days after the date of subject enrollment, the subject's electronic medical record will be reviewed for records from the subject's ED visit, as well as any subsequent ED visits, outpatient visits or hospitalizations and other follow up doctor visits noted in the medical center's medical record. Chart review will capture the subject's presentations, treatments, and course of medical visits made within 21 days of subject enrollment.

Passive Surveillance:

At least twenty-eight days after the day the influenza A positive NP (waste) sample was originally collected, the subject's electronic medical record will be reviewed for records from the subject's ED visit as well as any subsequent ED visits, outpatient visits or hospitalizations, and other follow up doctor visits noted in the medical center's electronic medical record. Chart review will capture the subject's

presentations, treatments and course of medical visits made from 1 day prior the day the influenza A positive NP (waste) sample was collected until 21 days after the influenza A positive NP (waste) sample was collected.

7 STUDY PROCEDURES AND EVALUATIONS

7.1 Clinical Evaluations

Active Surveillance:

- Enrollment
 - o Screening to determine eligibility
 - o Written consent
 - o Enrollment questionnaire including basic demographic data, vaccination history, co-morbid illness, initial vital signs and history of present illness.
 - o Collection of serum sample
 - o Collection of nasopharyngeal swab sample
 - o Collection of nasopharyngeal wash sample (Taiwan only)
 - o Test for influenza
 - o If influenza positive, then collect nasopharyngeal wash sample
- Follow up visit
 - o Follow up questionnaire to assess clinical course
 - o Collection of serum sample
 - o Medical record review to assess clinical course

Additional influenza positive subjects:

- Enrollment
 - o Screening to determine eligibility
-

- o Written consent

- o Enrollment questionnaire including basic demographic data, vaccination history, co-morbid illness, initial vital signs and history of present illness.

- o Collection of serum sample

- o Collection of nasopharyngeal wash sample

- Follow up visit
 - o Follow up questionnaire to assess clinical course
 - o Collection of serum sample
 - o Medical record review to assess clinical course

Passive Surveillance:

- Screening to determine eligibility

- Chart review including basic demographic data, vaccination history, co-morbid illness, initial vital signs and history of present illness.

- Collection of nasopharyngeal waste sample from NP swab, NP wash, or BAL specimens

- Medical record review to assess clinical course

7.2 Laboratory Evaluations

7.2.1 Laboratory Evaluations/Assays

In the active surveillance arm, we will test for influenza using Xpert Flu. Xpert Flu is a US FDA-cleared PCR based rapid influenza diagnostic test, which utilizes the GeneXpert platform to provide accurate influenza results in approximately an hour. Overall, Xpert Flu has a 93% sensitivity and 99% specificity for detection of Influenza A and B when compared with laboratory developed rt-PCR assays.^{12,13} Xpert Flu is a Clinical Laboratory Improvement Amendments (CLIA) moderate, laboratory test and will be performed in the clinical laboratory at JHU by trained personnel. CGMH will use a Sofia fluorescent immunoassay (FIA) for influenza

testing to give results to providers and subjects. Overall, Sofia FIA has a 78% sensitivity and 86% specificity for detecting Influenza A and B when compared with the rt-PCR assays.¹⁴ Simultaneously, CGMH will test subjects for influenza using Xpert Flu which is not yet Taiwan Food and Drug Administration (TFDA)-cleared and therefore, results will not be given to providers or patients. These research samples will be completely de-identified in order to release any ethical obligations to report a positive result.

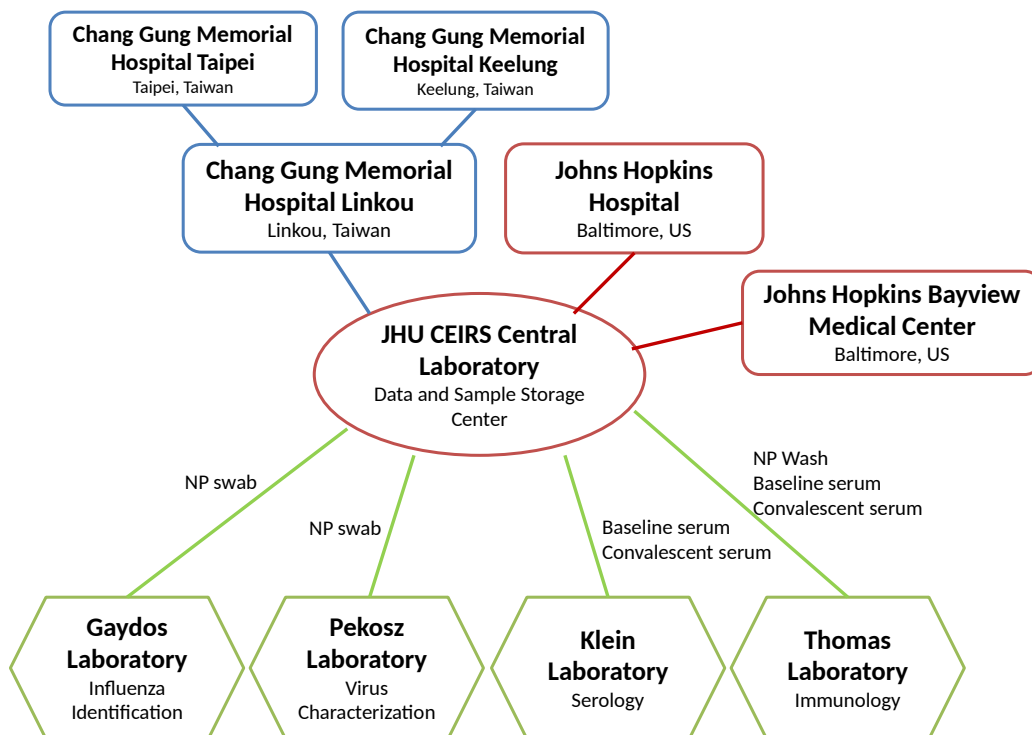
Samples from this study will fuel multiple downstream laboratory analyses as displayed in section 7.2.2. Protocols for the specific laboratory analysis using these samples will be further detailed in the laboratory specific protocols for:

- Dr. Gaydos – Influenza Identification
- Dr. Pekosz – Viral Characterization
- Dr. Klein - Serology
- Dr. Thomas – Human Immunology

Please refer to these protocols for additional information on laboratory analysis

7.2.2 Specimen Collection, Preparation, Handling and Shipping

All samples will be collected, prepared, aliquoted and frozen as detailed in the MOP. Frozen samples will be transported to the JH CEIRS central laboratory as diagramed below. Samples from the CGMH hospitals in Taiwan will first be shipped to Linkou, which will function as a Taiwan hub for sample and data collection and storage. Samples will then be shipped in bulk from Linkou to the JH CEIRS central laboratory. In the US, samples will be shipped directly to the JH CEIRS central laboratory. The JH CEIRS central laboratory will in turn distribute the samples to the individual laboratories as required.



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

If we are able to determine that a subject is acutely infected with an influenza virus, the following actions will be taken:

- If the subject is in the ED, we will notify the subject and the subject's provider of the positive influenza test (JH reports Xpert Flu result and CGMH reports Sofia IFA).
- If the subject has left the ED, we will contact the subject immediately (via the contact telephone number provided) and provide him/her with the information and urge him/her to seek attention at their local healthcare provider. The research coordinator will also attempt to inform the subject's provider of the test result. If they are unable to contact the subject's provider, they will inform the site PI.

8 ASSESSMENT OF SAFETY

8.1 Specification of Safety Parameters

Adverse events (AEs) include those that are the expected risks of obtaining nasopharyngeal and blood specimens (see Section 2.3.1).

8.2 Methods for Assessing and Analyzing Safety Parameters

8.2.1 Definitions: Adverse Events & Serious Adverse Events

Adverse Event (AE):

ICH E6 GCP guidelines defines an Adverse Event as any untoward medical occurrence in a patient or clinical investigation subject undergoing a study related procedure or receiving a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the study related procedure or use of a medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during/after the study procedures and interviews.

FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Serious Adverse Event (SAE):

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:

- 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
 - a congenital anomaly/birth defect

 - Important medical events that may not result in death, be life-threatening, or require hospitalizations 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, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

* Life-threatening adverse event. An adverse event is considered life-threatening if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.

Severity of Event: All AEs must be graded for severity. All AEs will be assessed by the study clinician using a protocol defined grading system. For events not included in the protocol defined grading system, the following guidelines will be used to quantify severity.

- Mild (Grade 1): events require minimal or no treatment and do not interfere with the patient's or subject's daily activities.
- Moderate (Grade 2): events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe (Grade 3): events interrupt a patient's or subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Relationship to Study Product or Study Procedure: All AEs must be assessed for relationship to study product or study procedure. The study clinician's assessment of an AE's relationship to test article (vaccine or study drug) or study procedure is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study product assessed using the terms: related or not related. In a clinical study, the study product or study procedure must always be suspect. To help assess, the following guidelines are used.

Related – There is a reasonable possibility that the study product or study procedure caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product or study procedure and the adverse event.

Not Related – There is not a reasonable possibility that the administration of the study product or study procedure caused the event.

8.3 Type and Duration of Follow-up of Subjects after Adverse Events

The occurrence of any adverse events occurring as a result of obtaining nasopharyngeal and blood specimens will require clinical review, monitoring and treatment as needed until resolution or stabilization, and assessment for severity and relatedness. Subjects will be asked about final resolution of symptoms upon completion of each visit.

8.4 Reporting Procedures and Safety Oversight

This is a minimal risk study. Due to its nature very few, if any, serious adverse events are likely to be chronologically (temporally) or causally related to study procedures, and any adverse events that do occur are likely to be mild, transient, and self-limiting. Therefore, adverse events, including serious adverse events, will not be collected or reported to the IRB or DMID, an Independent Safety Monitor will not be required, and a formal Safety Monitoring Committee will not be constituted.

9 STATISTICAL CONSIDERATIONS

9.1 Study Hypotheses

The key objectives of this study are to perform influenza surveillance and identify viruses with pandemic potential. Specifically, the objectives of this study are:

- Develop a robust domestic and international influenza surveillance network which will allow rapid identification of circulating influenza viruses, including those with pandemic potential in human populations.
- To create a sample bank of nasopharyngeal and serum samples from patients with suspected and confirmed influenza.
- To identify the added benefit of serology when combined with PCR for identification of influenza infections.
- To characterize the immunologic basis of severe illness due to influenza.

9.2 Sample Size Considerations

All sample size calculations below are listed by year with the assumption that that sample size would be used each year for the duration of the study.

Assuming a 20% percent positivity rate of symptomatic individuals, enrolling 400 symptomatic active surveillance subjects will result in samples and data from 80 subjects with influenza. We will recruit an additional 100 influenza positive subjects to ensure sufficient number of subjects with confirmed influenza to meet all objectives.

For the main objective of identifying circulating influenza viruses, we will also collect passive surveillance samples, by definition, have influenza, thus passive surveillance will contribute an additional 250 influenza samples, resulting in a total of 430 influenza positive samples each year.

Samples from all recruited subjects will contribute samples and clinical information to the sample bank. Thus, we expect to recruit 500 subjects via active surveillance, 250 subjects via passive surveillance, and 100 additional influenza positive subjects.

For serologic evaluation 200 selected subjects will undergo serology in order to evaluate those who are asymptomatic, have lower respiratory tract disease, have severe disease, and a sample of routine influenza subjects.

To characterize the immunologic basis of disease we will evaluate 100 influenza positive subjects each year with an estimated breakdown of 5 cases of severe disease, 40 cases of moderate disease and 45 cases of mild disease.

9.3 Subject Enrollment and Follow-Up

Given the short duration of this study (3-4 weeks) we anticipate 70% of subjects will complete follow up. For subjects not completing follow-up, we can still use the initial nasopharyngeal and serum samples and information to ascertain the subject's influenza status and initial clinical information. The medical record review will add additional information regarding clinical course.

9.4 Final Analysis Plan

Analysis of most data obtained in this study will be descriptive. Rates of discrete events, as well as mean values for numerical data will be calculated.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring is conducted to ensure that the human subject protections, study and laboratory procedures, and data collection processes are of high quality and meet sponsor, ICH/GCP guidelines and applicable regulations, and that the study is conducted in accordance with the protocol, protocol-specific MOP, applicable sponsor standard operating procedures and applicable in country regulatory guidelines.

DMID or its designee may conduct site-monitoring visits as applicable according to a site monitoring plan. The frequency of site visits will be determined by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, data collection forms (DCFs), informed consent forms, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to the study site, study personnel, and all study documentation according to the site monitoring plan. Study monitors will meet with site principal investigators to discuss any problems and actions to be taken and document visit findings and discussions. Clinical monitoring reports will be submitted to DMID.

11 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted protocol specific clinical quality management plan, the sites are responsible for conducting routine quality control (QC) and quality assurance (QA) activities to internally monitor study progress and protocol compliance. The Principal Investigators will provide direct access to all study-related field sites, DCFs, source documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The Principal Investigators will ensure all study staff are appropriately trained and applicable documentations are maintained on site.

Each site will implement QC procedures beginning with the data entry system and generate data QC checks that will be run on the database. Any missing data or data anomalies will be clarified and resolved by going back to the DCFs and/or source documents and checking with the field team that collected the data.

Each site will implement QC and QA procedures to ensure that laboratory analysis will be performed per instructions in the MOP. Problems with laboratory results will be resolved by repeating the analysis and re-training laboratory personnel.

12 ETHICS/PROTECTION OF HUMAN SUBJECTS

12.1 Ethical Standard

The investigators will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46, 21 CFR 50, 21 CFR 56 and/or the ICH E6; 62 Federal Regulations 25691 (1997), if applicable. The investigator's Institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

12.2 Institutional Review Board

Per regulations and prior to enrollment of subjects into this study, the Johns Hopkins Medical and Chang Gung Medical Foundation Institutional Review Boards will provide initial and continuing reviews of this research study. The study protocol, the associated informed consent documents, and any written information given to study subjects, will be reviewed and approved by the appropriate IRBs listed on their respective FWAs. Any amendments to the protocol, consent documents, or written information for subjects will be reviewed and approved by the Johns Hopkins Medical and Chang Gung Medical Foundation Institutional Review Boards before they are placed into use. The responsible official for each IRB will sign an IRB letter of approval of the protocol prior to the start of this study and a copy will be provided to DMID.

12.3 Informed Consent Process

The investigators will choose subjects in accordance with the eligibility criteria detailed in Section 5. Before any study procedures are performed, subjects must sign an informed consent form that complies with the requirements of 21 CFR Part 50 and 45 CFR 46 and the local IRB.

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. At the time the study worker will seek informed consent, the study worker will ask the eligible candidate if he/she is literate. Subjects who are not literate are not eligible for this study.

Extensive discussion of risks and possible benefits of participation in this study, including a detailed explanation of the proposed use and disclosure of their protected health

information, including specifically their specimens, will be provided to the subjects and their families before any study procedures are performed, including pre-screening of subjects for eligibility. Consent forms describing in detail the study procedures and risks are given to the subject and written documentation of informed consent is required prior to enrolling in the study and prior to performing any study procedures. Consent forms will be IRB approved and the subject will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to being enrolled in the study and prior to having any study procedures performed. The subjects should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. By signing the informed consent form, subjects agree to complete all procedures required by this study, unless the subject withdraws voluntarily, or is withdrawn or terminated from this study for any reason. The subjects may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the subjects for their records and if they refuse to take it, the study staff should document it in the subject's records. The rights and welfare of the subjects 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.

Study personnel may employ IRB-approved recruitment efforts prior to obtaining the subjects consent; however, before any study procedures are performed to determine protocol eligibility an informed consent form must be signed.

12.4 Exclusion of Women, Minorities, and Children (Special Populations)

This study will be inclusive of all individuals who meet the Subject Inclusion/Exclusion Criteria, regardless of religion, sex, or ethnic background.

12.5 Subject Confidentiality

In accordance with HIPAA regulations, no subject personal identifiers will be entered into the study database. Personal identifiers such as name, contact phone number and medical record number will be collected and recorded on a separate study identification sheet. This identifying information will be linked to the remainder of the subject information only through the study ID number, and will ultimately be stripped after all necessary data is collected. The study database, which will be accessible to the research coordinators and PI as well as key study personnel at the central coordinating center, will only contain study identification number and will not contain any subject identifiers. Personal identifiers will be destroyed upon final completion and verification of the study database.

Subject confidentiality will be held strictly in trust by the investigators and staff. This confidentiality will be extended to cover testing of biological specimens and genetic tests in addition to the clinical information relating to subjects. No information concerning the study or the data will be released to any third party without prior written approval of DMID, the sponsor of the study. The clinical study site will permit access to all documents and records that may require inspection by the sponsor or its authorized representatives, IRB, or Regulatory Agency representatives, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the subjects in this study.

Data obtained in this study will be maintained in a 21CFR11 compliant, secure encrypted database, Research Electronic Data Capture (REDCap) system. Access to RedCap is limited to approved individuals; the Principal Investigator will set the level of access for individuals to the database. Information that can be used to identify specific subjects will only be available to the Principal Investigator and other clinical staff directly involved in the study and that interact with the subjects.

12.6 Study Discontinuation

In the event that the study is discontinued, enrolled subjects will continue through the final follow up period. Given the short duration of enrollment no additional steps for study discontinuation are needed.

12.7 Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for taking part in this study. Subjects may be compensated for their participation in this study. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

If it is determined that an injury occurred to a subject as a direct result of the study procedures that are done for this study, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this study. Immediate medical treatment may be provided by the clinical study site. No financial compensation will be provided to the subject by the clinical study site for any injury suffered due to participation in this study.

12.8 Future Use of Stored Specimens

Subjects who agree to take part in this study will have specimens collected for influenza testing. Subjects will be asked for permission to keep any remaining specimens for possible use in future research studies such as future antibody determination and viral typing. Some specimens will be stored indefinitely at the JH CEIRS central laboratory and the Linkou laboratory (negative nasal wash). The specimens will not be sold or used directly for production of any commercial product. No human genetic tests will be

performed on specimens. Each specimen will be labeled only with a unique tracking number to protect subject's confidentiality. Each subject will be asked, as part of the informed consent process, to agree to have his/her specimens used for future research as noted in the protocol and consent form or have their specimens destroyed at the end of this study. This decision can be changed at any time by the subject without penalty by notifying the study staff in writing. However, if the subject originally consents to future use and subsequently changes his/her decision, any data from a previously collected specimen may still be used for this research. Stored unlinked/unidentified specimens may be shared with other researchers if authorized by DMID. There are no benefits to subjects in the collection, storage and subsequent future research use of specimens. Reports about future research done with subject's samples will NOT be kept in their health records.

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Management Responsibilities

The investigators are responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All DCFs and/or source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Blue or black ink is required to ensure clarity of reproduced copies. When making changes or corrections, 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.**

Data reported in the data collection form (DCF) derived from source documents should be consistent with the source documents or the discrepancies should be explained.

DMID and/or its designee will provide guidance to investigators on making corrections to the source documents and DCF.

Study personnel are given in-service training by investigators about forms and study procedures before the start of the study and given in-service training periodically throughout the study. Data collection is the responsibility of the research staff at the site under the supervision of the site PI. During the study, the investigators must maintain complete and accurate documentation for the study. Data analysis will be the responsibility of the PIs, under the direction of a biostatistician.

Each subject will be assigned a unique identifying number for use on DCFs and in the database. The forms that contain the subjects' name date of birth, and contact information will be kept on-site and filed in a secured cabinet. A key linking each subject to their unique identifying number will be created and kept secured by the on-site investigators.

All DCFs must be reviewed by the research staff for accuracy, clarity and completion, and by the data entry staff for completion. Study related laboratory reports will be reviewed and signed off by a study investigator. Adverse events must be assessed for severity and relationship, and reviewed by the site PI or designee. Data reported on the DCF that are derived from source documents or chart review should be consistent with the source documents or the discrepancies should be explained. The subject will not be contacted for DCF data validation.

13.2 Data Capture Methods

Data will be captured using paper DCFs and transcribed into a 21CFR11 compliant, secure, password-protected electronic database with double data entry by trained study personnel. Data capture will be ongoing throughout the period of the study. It is expected that subject DCFs would be reviewed and entered before the end of the night shift. Additional laboratory updates will be entered within 30 days of the date of final laboratory result reporting. A protocol deviation must be completed if these deadlines are exceeded by 2 weeks.

13.2.1 Source Documents and Access to Source Data/Documents

The investigators will maintain appropriate medical records and documentation related to the conduct of the research, in compliance with institutional requirements for confidentiality of subject information. These documents will be held at the study site in lockable fire-proof cabinets under the control of the study staff and entered as coded data into a 21CFR11 compliant, secure, password-protected electronic database REDCap system.

The CRF will be the primary record of the subject's participation in the study. Additional source documents may include laboratory reports, hospital records, and clinical memoranda. Informed consent will be obtained for access to subject's health information. Where additional source documents are not utilized, entries will be made directly in the DCF, which will be regarded as the source document for the purposes of the study. Source data are all information, original records of clinical findings, observations, or other activities in a clinical study necessary for the reconstruction and evaluation of the study.

The DMID monitor will have access to all source documents held at the study site during monitoring visits. DMID and/or DMID's authorized representatives, and applicable regulatory agencies may have access to clinical records for the

purpose of monitoring, auditing, quality assurance reviews and overall evaluation of the study safety and progress.

13.3 Types of Data

Data for this study will include demographic data, clinical data (including current and past medical history as well as safety data) and laboratory outcome measures (including results of study-related tests/procedures).

13.4 Timing/Reports

A final report will be prepared following the availability of the analyses of all data.

13.5 Study Records Retention

Study records, reports, and any other documentation related to the study, including, but not limited to, DCFs, source documents, informed consent forms (except for future use informed consent forms), and laboratory test results, will be retained by study personnel at each clinical study site and archived in a secure document storage facility. The site must contact DMID for authorization prior to the destruction of any study records. No records will be destroyed without the written consent of DMID. Informed consent forms for future use will be maintained as long as the specimens exist.

13.6 Protocol Deviations

A protocol deviation is any noncompliance with the study protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the site principal investigator, or other study personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2

All protocol deviations, as defined above, must be addressed in study subject source documents under 'subject notes.' A completed copy of the Protocol Deviation Form must be maintained in the Regulatory File as well as in the subject's chart.

It is the responsibility of the site principal investigator and other study personnel to use continuous vigilance to identify and report protocol deviations. The site principal investigators and other study personnel are responsible for knowing and adhering to

their IRB requirements. Only protocol deviations that are related to subject safety and/or eligibility will be reported to the local IRB.IEC per its guidelines. Line listings of protocol deviations that are reported to the IRB will be submitted to DMID on a quarterly basis.

14 PUBLICATION POLICY

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (<http://www.ncbi.nlm.nih.gov/pmc/>) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. 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 publication.

Refer to:

- NIH Public Access Policy, <http://publicaccess.nih.gov/>
- NIH OER Grants and Funding, <http://grants.nih.gov/grants/oer.htm>

Following completion of the study, the lead principal investigator is expected to 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.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase I trials), would be exempt from this policy.

It is the responsibility of the investigator to register this trial in an acceptable registry. Any clinical trial starting enrollment after 01 July 2005 must be registered on or before subject enrollment. For trials that began enrollment prior to this date, the ICMJE member journals will require registration by 13 September 2005, before considering the results of the trial for publication.

For trials in which DMID is not the IND/IDE sponsor, or there is no IND/IDE, and DMID does not provide data management services, it is the responsibility of the investigator to register the trial and post results in compliance with Public Law 110-85, the Food and Drug Administration Amendments Act of 2007 (FDAAA).

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases

*Journal Citation: De Angelis C, Drazen JM, Frizelle FA, Haug C, Hoey J, Horton R, et al. Clinical trial registration: a statement from the International Committee of Medical Journal Editors. N Engl J Med. 2004; 351:1250-1.

15 LITERATURE REFERENCES

1. World Health Organization. Influenza (Seasonal). 2009; <http://www.who.int/mediacentre/factsheets/fs211/en/>. Accessed April 5th, 2012.
2. Jouvett P, Hutchinson J, Pinto R. Critical Illness in Children with Influenza A/pH1N1 2009 Infection in Canada. *Pediatr Crit Care Med*. 2010;11(5):603-609.
3. Tuite AR, Fisman DN, Kwong JC, Greer AL. Optimal Pandemic Influenza Vaccine Allocation Strategies for the Canadian Population. *PLoS One*. 2010;5(5):e10520.
4. Gaydos CA. What is the role of newer molecular tests in the management of CAP? *Infect Dis Clin North Am*. 2013;27(1):49069.
5. Centers for Disease Control and Prevention (CDC). Evaluation of rapid influenza diagnostic tests for detection of novel influenza A (H1N1) Virus - United States, 2009. *MMWR Morb Mortal Wkly Rep*. 2009;58(30):826-829.
6. Sambol AR, Iwena PC, Pieretti M, et al. Validation of the Cepheid Xpert Flu A Real Time RT-PCR detection panel for Emergency Use Authorization. *Journal of Clinical Virology*. 2010;48:234-238.
7. Jeong JH, Kim KH, Jeong SH, Park JW, Lee SM, Seo YH. Comparison of sputum and nasopharyngeal swabs for detection of respiratory viruses. *J Med Virol*. 2014;[Epub ahead of print].
8. Fiore AE, Fry A, Shay D, Gubareva L, Bresee JS, Uyeki T. Centers for Disease Control and Prevention (CDC). Antiviral agents for the treatment and chemoprophylaxis of influenza --- recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Surveill Summ*. 2011;60(1):1-24.
9. Glaser CA, Gilliam S, Thompson WW, et al. Medical care capacity for influenza outbreaks, Los Angeles. *Emerg Infect Dis*. 2002;8:569-574.
10. Institute of Medicine. The future of emergency care in the United States health system. *Ann Emerg Med* 2006;48:115-120.
11. Schull MJ, Mamdani MM, Fang J. Community influenza outbreaks and emergency department ambulance diversion. *Ann Emerg Med*. 2004;44(1):61-67.
12. Novak-Weekley SM, Marlowe EM, Poulter M, et al. Evaluation of the Cepheid Xpert Flu Assay for rapid identification and differentiation of influenza A, influenza A 2009 H1N1, and influenza B viruses. *J Clin Microbiol*. 2012;50(1704-1710).
13. Popowitch EB, Rogers E, Miller MB. Retrospective and prospective verification of the Cepheid Xpert influenza virus assay. *J Clin Microbiol*. 2011;49:3368=3369.
14. Lewandrowski K, Tamerius J, Menegus M, et al. Detection of Influenza A and B Viruses With the Sofia Analyzer. *American Journal of Clinical Pathology*. 2013; 139:684-689

12/23/2014

<https://e-irb.jhmi.edu/eirb2/Doc/0/LV0LM2AMJ3GKJ7T5PJ30K7VE20/fromString.html>

**Office of Human Subjects Research
Institutional Review Boards**

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Date: December 22, 2014

APPLICATION APPROVAL

Review Type: Convened
PI Name: Andrea Dugas
Study #: IRB 00052743
Study Name: CEIRS: Human Surveillance of Health Care Centers in the United States and Taiwan
Committee Chair: Howard Lederman
Committee: IRB -1

Date of approval: December 2, 2014

Date of expiration: December 1, 2015

The JHMIRB approved the above-referenced Application.

Please note that the IRB made additional changes to your consent form(s) prior to approval. You may view the revised consent form(s) uploaded in the Written Consent section of the eIRB application. Click on View to the left of the document name, then click History and open the document with the name starting with 'irb_'. If you do not agree with these changes, submit a change in research application with a revised consent form(s). If you submit a change in research application, use the IRB's clean copy of the consent form(s) to make additional revisions. To maintain an accurate history in eIRB, do not delete the consent form(s). If you are making additional changes, use the Update button to upload your revised copy(ies).

IRB review included the following:

The enrollment of the non-English speaking participants. If you are using a short form consent, follow the JHMIRB Guidance on Obtaining and Documenting Informed Consent of Subjects Who Do Not Speak English.

Your application included Howard County General Hospital as a research site. You may not conduct this research at that site until you receive notification of full site approval by the Howard County General Hospital RRC. If you have already received site approval, you may begin conducting research immediately.

Your application included Suburban Hospital as a research site. You may not conduct this research at that site until you receive notification of full site approval by the Suburban Hospital RRC. If you have already received site approval, you may begin conducting research immediately.

Your application included Sibley Memorial as a research site. You may not conduct this research at that site until you receive notification of full site approval by the Sibley Memorial RRC. If you have already received site approval, you may begin conducting research immediately.

45CFR 46.404 and/or 21 CFR 50.51: This study has been approved for the inclusion of children as 'research not involving greater than minimal risk'. The permission of one parent is required.

45CFR 46.204 This study has been approved for the involvement of pregnant women and fetuses prior to delivery.

Collection of blood samples by finger stick, heel stick, ear stick, or venipuncture from healthy, nonpregnant adults who weigh at least 110 pounds. For these subjects, the amounts drawn may not exceed 550 ml in an 8 week period and collection may not occur more frequently than 2 times per week; or from other adults and children, considering the age, weight, and health of the subjects, the collection procedure, the amount of blood to be collected, and the frequency with which it will be collected. For these subjects, the amount drawn may not exceed the lesser of 50ml or 3 ml per kg in an 8 week period and collection may not occur more frequently than 2 times per week.

Date of Approval and Expiration Date: The approval and expiration date for this research are listed above. If the approval lapses, the research must stop and you must submit a request to the IRB to determine whether it is in the best interests of individual participants to continue with protocol-related procedures.

Changes in Research: All proposed changes to the research must be submitted using a Change in Research application. The changes must be approved by the JHM IRB prior to implementation, with the following exception: changes made to eliminate apparent immediate hazards to participants may be made immediately, and promptly reported to the JHMIRB.

Continuing Review: Continuing Review Applications should be submitted at least 6 weeks prior to the study expiration date. Failure to allow sufficient time for review may result in a lapse of approval. If the Continuing Review Application is not submitted prior to the expiration date, your study will be

<https://e-irb.jhmi.edu/eirb2/Doc/0/LV0LM2AMJ3GKJ7T5PJ30K7VE20/fromString.html>

1/2

