

**SENTINEL HUMAN SURVEILLANCE FOR INFLUENZA
IN BHUTAN**

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Protocol Title:

Sentinel Human Surveillance for Influenza in Bhutan

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Mr. Sonam Wangchuk as co-principal investigator in Bhutan will also serve as in country team leader and coordinate activities within Bhutan for proper case selection, acquiring informed consent, specimen collection, specimen storage and transport and contribute protocol training. He will contribute to the planning, preparation and administration of the protocol. He will furnish Bhutanese language translated version of the information sheet and consent form. He will guide the protocol through Ethical Review Committee in Bhutan. He will also contribute to the publication of any reports related to this study.

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Mr. Binay Thapa will contribute to protocol related training, proper case selection, acquiring informed consent, supervise specimen collection, storage and transport. He will contribute to the planning, preparation and administration of the protocol.

Study Location:

Jigme Dorji Wangchuck National Referral Hospital, Thimphu, Punakha Hospital, Paro Hospital, Monggar Regional Referral Hospital, Phuntsholing Hospital, Gelephu Regional Referral Hospital, Trongse Hospital, Tsirang Hospital and Trashigang Hospital

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3. Walter Reed Army Institute of Research (WRAIR)
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Source of Funding:

Department of Defense - Global Emerging Infectious Disease Surveillance (DoD - GEIS), and other funding sources as they become available.

Study Duration:

10 years

Anticipated Start Date:

August 2010

Synopsis

Require Number and Type of Study Subjects:

The investigator will enroll male and female patients ages over 6 months from in and out patient department. We estimate about 3000 subjects to be enrolled per year from the health care institutions and both in-and out-patient clinics or from respiratory disease outbreaks in Bhutan

Study Objective

Primary Objectives:

- a. To collect and characterize influenza viruses circulating within the human population in Bhutan.
- b. To provide influenza surveillance data to the US CDC and WHO surveillance network towards the annual re-formulation of the influenza vaccine.
- c. To report the circulating influenza strains and other respiratory pathogens to Ministry of Health, Bhutan.

Secondary Objectives:

- a. To evaluate new technologies for specimen collection and preservation as well as different molecular platforms to identify respiratory pathogens.
- b. To maintain an influenza sample repository for evaluation of newer diagnostics as they are developed.
- c. To provide early warning of H5N1 and other novel influenza strains circulating in human population.

Number of Subjects: We estimate about 3000 subjects to be enrolled per year from the health care institutions and both in-and out-patient clinics or from respiratory disease outbreaks in Bhutan.

Study Design and Methodology: This protocol describes prospective surveillance for influenza and other viral respiratory pathogens in Bhutan. The sites of this surveillance activity are the health care institutions and both in-and out-patient clinics in Bhutan: Jigme Dorji Wangchuck National Referral Hospital, Thimphu, Punakha Hospital, Paro Hospital, Monggar Regional Referral Hospital, Phuntsholing Hospital, Gelephu Regional Referral Hospital, Trongse Hospital, Tsirang Hospital and Trashigang Hospital. In accordance with the methods used by DoD Influenza Surveillance Program, patients who meet a case definition for influenza-like illness

(ILI) will be requested to provide clinical information as well as a nasal / throat swab sample. Blinded influenza specimens without personal identifier provided by Bhutan Ministry of Health and WHO network will also be tested to provide influenza diagnostic support service to Bhutan. Specimens for viral isolation and molecular assays will be collected as part of routine patient care through a surveillance network of emergency rooms, outpatient clinics, inpatient clinics, or from respiratory disease outbreak investigations.

Study Institution Affiliation

Institution	Affiliation
Royal Centre for Disease Control, Department of Public Health in Bhutan	Subject enrollment, Demographic/Clinical data and Specimen collection, rapid diagnostic tests, storage and transportation of specimens. -A RT- PCR assay for influenza
Armed Forces Research Institute of Medical Sciences (AFRIMS)	AFRIMS will provide: - Collaborating institutions rapid diagnostic tests based on antigen detection (either QuickVue and/or other US-FDA approved rapid tests) for on-site testing and providing the results to the attending clinicians for patient management. - A RT- PCR assay for influenza - Virus isolation for respiratory viruses - Sequencing
U.S. Air Force School of Aerospace Medicine (USAFSAM)	For viral isolation, typing and subtyping, antigenic and molecular characterization.
Centers for Disease Control and Prevention (CDC)	For advanced antigenic and molecular characterization, viral pathogenesis characterizations.
Walter Reed Army Institute of Research (WRAIR)	Advanced Molecular Characterizations

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List of Abbreviations

AFRIMS	Armed Forces Research Institute of Medical Sciences
ARI	Acute Respiratory Infection
CDC	Centers for Disease Control and Prevention
Co-PI	Co- Principal Investigator
DoD- GEIS	Department of Defense - Global Emerging Infectious Disease Surveillance
FDA	Food and Drug Administration
ILI	Influenza Like Illness
IRB	Institutional Review Board
RT-PCR	Reverse Transcriptase - Polymerase Chain Reaction
VTM	Viral Transfer Media
SOP	Standard Operating Procedure
RSV	Respiratory Syncytial Virus
U.S. FDA	United States Food and Drug Administration
PI	Principal Investigator
USAFSAM	U.S. Air Force School of Aerospace Medicine
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research

1. Background

1.1. Introduction

Influenza is usually a self-limited illness characterized by an abrupt onset of high fever, cough, sore throat, body aches, chills, headache, or fatigue lasting several days. Influenza can cause pneumonia, either viral or from bacterial super infection which may be lethal, particularly in the very young, very old, and chronically-ill. Its clinical presentation is usually indistinguishable from other respiratory viruses. Of the 3 types of influenza virus (A, B and C), type A or B is most often associated with disease outbreaks during the late fall and early winter each year. In the tropics and subtropics influenza may occur throughout the year and/or with more intense activity during the rainy season. Consequently the morbidity and mortality in these regions are probably greatly underestimated. Transmission of influenza virus is by person-to-person contact, airborne droplets, or fomites. Importantly, influenza is occasionally transmitted to people by pigs or birds.

Antigenic drift or antigenic shift of influenza virus produces new strains yearly. The most devastating impact of influenza in the twentieth century occurred in 1918-1919 when one quarter of the world's population was infected resulting in 20 million deaths including 550,000 in the United States. The number of deaths associated with this pandemic was greater than those that occurred as a result of World War I and was also distinctive because of the larger number of fatalities among young adults. There have been three other pandemics, though less devastating, during the 20th century and there is worldwide concern about an imminent pandemic.

The viral surface of influenza types A and B has two strain-specific molecules, hemagglutinin (H) and neuraminidase (N). Hemagglutinin allows the virus to attach to cells and neuraminidase allows the virus to spread from cell to cell. Four subtypes of H (H1, H2, H3 and in 1997 H5) and two subtypes of N (N1 and N2) have been recognized as causing human disease. Each strain with the subtype is specifically identified by influenza type/site of viral isolation/date of viral isolation/subtype. Infection with a virus of one subtype provides little protection against viruses of other subtypes. An antigenic shift, which occurs at undefined time intervals, refers to a change from one H or N subtype to another and has the potential to cause pandemics. In pandemics, the population is not immune to the new subtype. An antigenic drift results from minor alternations in H or N antigens without a change in subtype. Epidemics due to antigenic drifts occur every one to three years. Recent vaccines have included two A-type and one B-type strains and a protective immune response develops in about two weeks but wanes over a year.

The highly pathogenic avian influenza A (H5N1) epizootic (animal outbreak) in Asia, Europe, the Near East, and Africa is not expected to diminish significantly in the short term. It is likely that H5N1 virus infections among domestic poultry have become endemic in certain areas and that sporadic human infections resulting from

direct contact with infected poultry and/or wild birds will continue to occur. So far, the spread of H5N1 virus from person-to-person has been very rare, limited and unsustained. However, this epizootic continues to pose an important public health threat. This strain poses a considerable human public health risk. Recently there was an outbreak among poultry in Thailand. Not only can these viruses infect humans, causing severe disease with high mortality, but there is also potential for them to adapt, or recombine with other influenza viruses, and give rise to a pandemic viral strain. For close global monitoring of the situation and coordination of the global response, WHO is recommending enhanced surveillance for influenza A/H5 until further notice. AFRIMS is uniquely positioned to take on the surveillance for avian influenza through seasonal influenza surveillance.

Critical Positioning of AFRIMS Virology to Conduct Influenza Surveillance in Asia

The Department of Virology, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand, has had an active regional presence for the past four decades with active field stations in the region including Thailand, Nepal and the Philippines. Its mission has at its core, infectious disease surveillance and product development (vaccine and field diagnostics). This protocol describes surveillance for influenza (and other viral respiratory pathogens) and testing of viral diagnostic devices in Asia including Bhutan.

DoD Influenza Surveillance Program

AFRIMS is part of the DoD Influenza Surveillance Program, a laboratory-based program that was begun in 1976 as Project Gargle under the Air Force (AF) Surgeon General. There are 19 AF sentinel bases and additional sites that have been established in cooperation with the Army and the Navy. The sites represent an effective inter-service cooperative effort providing surveillance from areas not otherwise covered. Respiratory specimens collected from each site are shipped to USAFSAM (previously known as Air Force Institute for Occupational Health) for viral isolation by culture for influenza A and B, parainfluenza 1, 2, or 3, adenovirus, enterovirus, and herpes simplex virus. Selected viral isolates are forwarded to CDC for further subtyping. These submissions are compared with others for vaccine component determinations. The DoD Influenza Surveillance program is a flexible system that provides valuable public health information as well as worldwide surveillance of an important and continually adapting threat.

World Health Organization (WHO) Surveillance Network

Approximately 80 countries participate in the WHO surveillance network for influenza. The DoD Influenza Surveillance Program participates in the WHO Surveillance Network. Since new influenza strains frequently emerge in Asia, comprehensive influenza surveillance is especially important in those regions. As part of the U.S. Department of Defense's emerging infectious disease effort, we aim to study influenza strains in various locations in Asia by obtaining specimens using respiratory specimens from patients who meet the case definition for Influenza-Like

Illness (ILI). Rapid testing will be completed on-site to benefit the subjects and clinicians. Specimens and clinical data will be sent to AFRIMS for initial diagnostic and confirmatory testing, and then some or all specimens may be forwarded to USAFSAM for confirmation and advanced diagnosis. Definitive test results will be shared with CDC and WHO Flu Net. Feedback on the results of virus typing/sub-typing as well as virus isolation will also be provided to health care institutions in Bhutan as they become available.

Recent Role of AFRIMS in Influenza Surveillance in Bhutan

AFRIMS has been involved in the specimen collection as part of DoD Influenza Surveillance Program since 1996. Collaboration between the Royal Centre for Disease Control, Bhutan Ministry of Health and AFRIMS has been well established since 2007 to enhance the country's capabilities to perform influenza and other emerging pathogen surveillance by significantly increasing laboratory and information technology infrastructure and through the provision of training. A unique opportunity exists to take advantage of the current infrastructure in Bhutan to perform influenza surveillance.

Convergence of Host Nation Interests and Military Relevance

Bhutan is a small landlocked country located in the heart of Himalaya with a population of 650,000. Communicable diseases are still major public health concern in Bhutan especially respiratory illnesses. Acute respiratory infection (ARI) is ranked as the most common illness in Bhutan causing a high rate of morbidity and has been considered among top ten health problems in the country for the past five years (Annual Health Bulletin 2007). ARI is also the most common illness afflicting children less than five years of age and accounts for 20% of infant mortality in combination with other communicable diseases (Annual Health Bulletin 2007). ARI with diarrhea accounts to 40-51% of morbidity amongst the population in the country per annum.

Bhutan remains an under-reported region for influenza as laboratory support for influenza surveillance is inadequate. However, it is strategically located near other countries of disease interest such as India, China and Nepal. There are also significant flow of people between Bhutan and India where Avian Influenza is endemic. Very recently Avian Influenza in poultry was reported from Nepal. These factors underscore the need to establish an efficient surveillance system for monitoring influenza illness in Bhutan to detect viruses with both seasonal changes and/or pandemic potential.

Acute respiratory diseases including viral pathogens such as influenza are of special interest to the U.S. military. Influenza affects people of all ages in all parts of the world and outbreaks have affected all three branches of the Department of Defense. In fact, the influenza pandemic of 1918 had a devastating impact on both civilian and military operations. The DoD is in the process of developing a plan to maintain force readiness in the event that a majority of active U.S. military forces are affected by

influenza worldwide. Other respiratory viruses such as adenovirus are also a serious threat to military forces. Coordinated global surveillance for viral respiratory pathogens is a key component of any program whose objective is to minimize the impact of future epidemics among civilians and military personnel.

2. Study Objective

2.1 Primary Objectives:

- a. To collect and characterize influenza viruses circulating within the human population in Bhutan.
- b. To provide influenza surveillance data to the US CDC and WHO surveillance network towards the annual re-formulation of the influenza vaccine.
- c. To report the circulating influenza strains and other respiratory pathogens to Ministry of Health, Bhutan.

2.2 Secondary Objectives:

- a. To evaluate new technologies for specimen collection and preservation as well as different molecular platforms to identify respiratory pathogens.
- b. To maintain an influenza sample repository for evaluation of newer diagnostics as they are developed.
- c. To provide early warning of H5N1 and other novel influenza strains circulating in human population.

3 Study Design (See Flu Surveillance Flow Diagram in Annex B)

3.1 Overview:

This protocol describes prospective surveillance for influenza and other viral respiratory pathogens in Bhutan. The sites of this surveillance activity are the health care institutions and both in-and out-patient clinics in Bhutan: Jigme Dorji Wangchuck National Referral Hospital, Thimphu, Punakha Hospital, Paro Hospital, Monggar Regional Referral Hospital, Phuntsholing Hospital, Gelephu Regional Referral Hospital, Trongse Hospital, Tsirang Hospital and Trashigang Hospital. In accordance with the methods used by DoD Influenza Surveillance Program, patients who meet a case definition for influenza-like illness (ILI) will be requested to provide clinical information as well as a nasal/throat swab sample. Blinded influenza specimens without personal identifier provided by Bhutan Ministry of Health and WHO network will also be tested to provide influenza diagnostic support service to Bhutan. Specimens for viral isolation will be collected as part of routine patient care

through a surveillance network of the health care institutions and both in-and out-patient clinics in Bhutan.

3.2. Sample Size:

We estimate about 3000 subjects to be enrolled per year from the health care institutions and both in and out-patient clinics or from respiratory disease outbreaks in Bhutan.

3.3. Study Duration: 10 years

3.4. Data and Specimen Collection and Analysis

3.4.1. Locations of the study:

Clinical Component

The clinical component will be patients presenting with influenza like illness (ILI) at a surveillance network of health care institutions and both in-and out-patient clinics or from a respiratory disease outbreaks in Bhutan. The surveillance network includes; Jigme Dorji Wangchuck National Referral Hospital, Thimphu, Punakha Hospital, Paro Hospital, Monggar Regional Referral Hospital, Phuntsholing Hospital, Gelephu Regional Referral Hospital, Trongse Hospital, Tsirang Hospital and Trashigang Hospital. This surveillance may be further expanded to include other hospitals as surveillance capacities are assessed, deficiencies are identified and funding permits. This surveillance activity will be coordinated by Royal Centre for Disease Control, Department of Public Health, Ministry of Health, Bhutan. A protocol amendment with local Institutional Review Board (IRB) and the WRAIR IRB approval will be obtained before additional host nation activities are initiated.

Logistical Component

The respiratory specimens from the surveillance sites or outbreak areas will be tested on-site or at Royal Centre for Disease Control with rapid diagnostics (to potentially include molecular techniques). Royal Centre for Disease Control can also perform PCR on the respiratory specimens. Additional respiratory specimens will be transported under cold-chain to the Armed Forces Research Institute of the Medical Sciences (AFRIMS) Bangkok, Thailand where viral isolation and PCR will be used identify and subtype influenza and other respiratory disease. Samples may be transferred to reference laboratory collaborators in the United States for further characterization.

Reference Laboratory Component

U.S. Air Force School of Aerospace Medicine (USAFSAM), San Antonio, Texas and the Influenza Branch, U.S. Center for Disease Control (CDC), Atlanta, Georgia may perform viral isolation, as well as more advanced and confirmatory testing on selected specimens to include sequencing and drug resistance.

Research Laboratory Component

Royal Centre for Disease Control will perform RT-PCR on respiratory samples as part of routine surveillance. AFRIMS Dept. of Virology will perform confirmatory assays RT-PCR and virus isolation and will store respiratory samples for evaluation of new influenza detection tests. AFRIMS may also conduct initial molecular testing, hemagglutination inhibition assay, and advance molecular characterization to include sequencing on select samples.

3.4.2. Data Analysis:

Descriptive statistics, including demographics, sampling method used, and the number and type of influenza isolates, will be done for samples submitted. Subtypes will be identified and reported by month, year and demographical categories. Descriptive statistics will be done separately for unique situations such as a focal epidemic. Respiratory pathogens other than influenza will be assessed on selected samples to identify other viruses causing significant diseases and to identifying co-infections.

Sequencing of HA and possibly other genes will be analyzed for antigenic drift and shift by phylogenetic analysis. Potential influenza viral evolution will be assessed by advanced phylogenetic analysis. Identification of key mutations by sequencing may be further characterized by neuraminidase inhibition assay at AFRIMS or the USCDC.

The evaluation of diagnostic tests (if there are any) will be done against defined gold standards, such as viral isolation for product evaluation. Sensitivities and specificities will be calculated. Definitive test results will be shared with WHO Flu Net through the host country and can be used in the determination of future influenza vaccines.

3.4.3. Disposition of Specimens:

Samples from patients identified by subject number only will be shared only with collaborating organizations (Royal Centre for Disease Control, USAFSAM, WRAIR and US-CDC). Respiratory specimens will be maintained at AFRIMS after study completion. Viral isolates from human specimens will be maintained at AFRIMS indefinitely. Isolation from human specimens may be sent to other

laboratories specialized in advanced techniques or to health authorities for vaccine considerations or advanced studies. Any use of the samples outside of this defined plan will be submitted as a protocol amendment or a new protocol.

3.5. Endpoints

The study will continue for 10 years, unless funding is terminated or expires.

4. Method

4.1. Recruitment of Subject/Screening Procedures

Eligible patients will be identified in the outlined study hospitals by the primary health care staff based on the eligibility criteria. Eligible subjects will be presented the study explanation form and asked to participate in the study. If they agree, written informed consent will be obtained and medical history collected as outlined in the Demographic/Clinical Form (Annex C). Study participants will receive no monetary compensation for participation in this study. Additional diagnostic tests and any treatment decisions will be solely made by the attending physician, in accordance with local standards and the available medical data. The study site will follow the guidelines of Bhutan Ministry of Health on screening and treatment of Avian Influenza. Patients with severe influenza or pneumonia who tested positive by a rapid test and have history of exposure to dead/sick poultry or reside in the area with abnormal poultry death during the past two weeks or history of contact with other pneumonia cases in the past ten days will be referred to the Bhutan health care system for consideration for antiviral therapy.

4.2. Informed Consent Process

Potential subjects will have the study explained in detail by an investigator or designee. The informed consent and assent forms will be translated in Bhutanese language. The informed consent and assent form will be verbally explained in Bhutanese language when necessary to the subject or child. The subjects will be given time to review the study explanation form before signing the informed consent. Legal consenting age is 18. Parents or guardians will provide consent for children younger than 18 years. Consent will be obtained from one parent or guardian. If a child capable of assenting refuses to give his/her assent he/she will not be enrolled in the study. A copy of the signed consent/ assent form will be provided to the subject for their record. The example of inform consent and assent are provided in Annex D.

4.3. Determination of Eligibility

4.3.1 Inclusion Criteria:

Subjects meeting all of the following criteria will be considered eligible for enrollment in the study:

- Male or female patients \geq 6 months of age
- Fever (oral temperature \geq 100.5°F or 38°C; axillary temperature \geq 99.5°F or 37.4°C; rectal temperature \geq 101°F or 38.6°C) AND cough or sore throat
- Presentation to health care within 5 days of reported fever onset
- Signed informed consent by patient and parent or legal guardian. For minor ages between 7-17 years, a signed assent is required.

4.3.2. Exclusion Criteria:

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

- Immunocompromised host such as Acquired Immune Deficiency Syndrome, Lymphoma or Leukemia
- Suspected case of TB

4.3.3. Discontinuation Criteria:

- Once a sample is taken there is no further contact with the subject unless they contact the study investigators.
- Patients may discontinue participation at any time without penalty or loss of benefits.
- The study will continue for 10 years, unless funding is terminated or expires.

4.4. Specimen (or Data) Collection and Testing

Specimen Collection:

Although nasal wash is preferred to nasal/throat swab as it is superior for direct specimen testing by RT-PCR, nasal swab and throat swab are recommended for both adults and children in this study as it is generally better tolerated than other methods. Specimen collections can be done by nasal or throat swab. Nasal specimen is recommended for rapid on-site test with QuickVue. The details of respiratory specimen collection procedures are provided in Annex E. A maximum of 3 swabs (one swab for on-site testing and two for Viral Transfer Media) will be collected. Proper and thorough collection along with careful maintenance of the cold chain in handling of specimens will significantly increase viral recovery.

Swabs:

AFRIMS will provide the appropriate swabs (for adult and pediatric patients) for collection. Swab material should be made of Dacron or other synthetic material, avoiding the use of calcium alginate swabs for virus isolation since they are toxic to many enveloped viruses. Wooden-shafted swabs will not be used as they can contain toxins and formaldehydes that inhibit virus recovery.

Viral Transfer Media (VTM):

AFRIMS will provide the VTM to Royal Centre for Disease Control. VTM may be stored at room temperature or refrigerator prior to specimen inoculation. For VTM inoculation, the study personnel will unscrew the VTM, place the culturette into the viral media, and break off the tip of the culturette so that the top can be closed securely leaving the swabs in place. The tube will be labeled with identification number and will then be placed on ice for transportation or 2-8°C if the sample can be delivered to the laboratory within 5 days and -70°C if the specimen need to be held longer than 5 days.

Storage and Transportation:

Respiratory samples from enrolled subjects will be stored temporarily on site to maintain the required temperature. Respiratory specimens will be batched and shipped under cold-chain to a selected central secure location on a regular basis where they will be temporarily stored at -70 C until ready for further shipment to AFRIMS Bangkok. The specimens will then be re-batched and shipped under cold-chain to AFRIMS Bangkok where viral isolation and PCR will be used to identify and subtype influenza and other respiratory diseases. Samples that are designated to be sent to a confirmatory laboratory will be periodically batch (no more than quarterly) and forwarded to USAFSAM. While all attempts are made to maintain the cold chain, specimens that are shipped under adverse conditions will be duly noted in the Cold Chain Maintenance Table (according to the institutional SOP) and tested. A break in cold chain will have less effect on the molecular assays than the viral isolation.

On-site Diagnostics:

AFRIMS will provide collaborating institutions rapid diagnostic tests based on antigen detection to detect influenza A and B (e.g. QuickVue Influenza A+B and/or other US-FDA approved rapid tests) for on-site testing and providing the results to the attending clinicians for patient management. Study personnel will be provided a package insert of the rapid antigen detection test and training to conduct and interpret the results.

A real time RT- PCR assay has been set up at Royal Centre for Disease Control and AFRIMS. Virus isolation will be performed at AFRIMS. The assay will be performed in accordance to departmental Standard Operating Procedure (SOP). Selected samples may be confirmed with definitive diagnostics at USAFSAM. Package inserts or standard operating procedures for rapid diagnostic tests are available on request.

Selected samples will be sequenced at AFRIMS to identify key mutation and to identify antigenic drift and shift.

Definitive Diagnostics:

Definitive diagnostics (viral isolation including influenza A and B, parainfluenza 1, 2, or 3, adenovirus, and respiratory syncytial virus (RSV) and typing, sub-typing and sequencing will be performed at AFRIMS.

USAFSAM and the Influenza Branch, U.S. Center for Disease Control (CDC), Atlanta, Georgia may perform more advanced and confirmatory testing (to include viral isolation, HA/HAI subtyping full genome sequencing, drug resistance assay, and animal pathogenesis (ordered most to least likely) on selected specimens. The advanced test results will be made available to AFRIMS once completed and the results will promptly be sent to Royal Centre for Disease Control for information purpose only. Laboratory protocols for the definitive and advanced diagnostic testing at USAFSAM are available on request.

Additional Diagnostic Testing:

Additional diagnostic tests might include MassTag PCR. The Mass Tag PCR would allow detection of multiple respiratory pathogens of both viral and bacterial origin simultaneously as well as identifying co-infection. The current Multiplex primer sets were designed to identify influenza A, B viruses, respiratory syncytial virus group A, B, human parainfluenza virus type 1, 2, 3, 4, human respiratory coronavirus, human metapneumovirus, enterovirus, adenovirus, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Streptococcus pneumoniae*, *Legionella pneumophila*, *Chlamydia pneumoniae*, and *Neisseria meningitidis* however the panel may be updated according needs. No additional sample collection is required for this test.

Non-commercial tests may be evaluated under appropriate agreement with the company involved.

A human influenza specimen repository will be maintained to evaluate newer diagnostic tests as they are developed. HIV as well as human DNA testing will not be performed on these samples.

Medical Care and Equipment Required:

No medical care beyond the routine patient care to the participating physician is required for patient volunteers. VTM, influenza rapid test kits, as well as insulated boxes and gel ice will be supplied by the Department of Virology, AFRIMS. The specimens will be batched and delivered to AFRIMS. The transportation to and testing of specimens at USAFSAM will be performed under the MOU (Memorandum of Understanding) of sending and handling of samples which is available upon request.

5. Ethical Considerations

5.1. Study Explanation Form

Study Explanation Form will be distributed in Bhutanese language to all patients. An investigator or designated staff will explain the details of study and will allow time to read the study explanation form.

5.2. Subject/Specimen Identification and Confidentiality

The subject number will be created by using the letters FLU and country code then collection site code and ended up with the running number of subject (example FLU-BTA-00000). The subject number will identify the patient by using a Subject Enrollment log sheet that will be maintained in a locked office at enrollment site to track specimen accountability and volunteer notification. Only persons collecting samples, on-site PI, and database manager are authorized to access the data. Subject names will be kept confidential within the host country and will be used only for the purposes of notifying the subject of any new confirmatory testing.

5.3. Risks and Benefits

This protocol involves collecting only clinical information and respiratory specimens and is thereby considered minimal risk to the subjects. There are no major risks involved except for some mild discomfort, sneezing or coughing when collecting specimens. Children and pregnant women will benefit in the same way as all other subjects. Specimen collection will be performed by well trained medical personnel in order to get a good clinical sample with minor discomfort to the patient. Every effort will be made to keep the subject's confidentiality.

An FDA-approved rapid test for influenza which detects both influenza A and B will be provided to all collaborating health care institutions. Subjects will benefit by knowing the diagnosis early and avoiding the inappropriate use of antibiotics. Subjects might possibly benefit from the satisfaction felt from contributing to medical surveillance that may lead to better protection of the public against viral respiratory pathogens.

Subject will receive no monetary compensation for participation in this study. Subjects will benefit by NOT having to pay for inappropriate antibiotics and risking antibiotic side effects. If the on-site test is negative subjects benefit by more consideration to alternative diagnoses.

Unanticipated problems involving risk to subjects or others related to participation in the study will be promptly reported by phone (301-319-9940) or by facsimile (301-319-9961) or by e-mail to usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil of WRAIR Institution Review Board. A complete written report should follow the initial telephone call. In addition to the methods above, the complete report can be sent to the Director, Division of Human Subjects Protection, Walter Reed Army Institute of Research, 503 Robert Grant Ave., Silver Spring, MD 20910-7500.

5.4. Medical Care for Injury or Illness

In the event that a subject injury or become ill as a direct result of study participation, the proper medical care will be provided at no cost to the subject follow Royal Centre for Disease Control standard. The subject will not receive any injury compensation, only medical care.

6. Administrative Procedures

6.1. Access to Source Data and Documents

The investigators, members of the WRAIR IRB, MRMC, DOD representatives and REBH are authorized access to the study data as part of their duties and part of their responsibility to protect human subjects in research.

6.2. Disposition of Data

Source data sheets (consent form/ clinical form) and diagnostic test results will be maintained at the study site or AFRIMS. All data forms will be kept in a locked office when not in use. Database files will be kept in a password protected computer in a locked room and with a limited access for authorized personnel only. All data without personal identifier will be compiled, computerized, carefully edited using standard procedures and analyzed at AFRIMS, Thailand and USAFSAM, USA. All specific information pertaining to patients will remain confidential.

Data used for analyses or publication will be based on study subject number and not contain individual identifying information. Both interim and final reports will be made available to all investigators and collaborating institutions listed on this protocol as well as GEIS.

6.3. Disposition of Source Documents

Laboratory source documents generated of this study in Bhutan will be maintained in Royal Centre for Disease Control and/or AFRIMS in a safe place with limited access. The source documents related to this study will be kept for a maximum of 10 years after study completed and they will be disposed by the institutional SOP.

6.4. Protocol Amendments

Any change or amendment to the protocol affecting study volunteers, study objectives, study design, study procedures, significant administrative aspects or any future or new diagnostic test will require a formal amendment to the protocol. The protocol will be revised to concur with the amendment. Such amendment will be submitted to the WRAIR IRB and National Ethical Review Committee of Bhutan for review and approval.

6.5. Training

Personnel including physicians, research nurses and designates working at surveillance sites will be trained on specimen collection, rapid diagnostic tests, storage, transportation of specimens and biosafety issues by study personnel before the study starts. Training will be documented in both Royal Centre for Disease Control and in AFRIMS.

6.6. Protocol Deviations

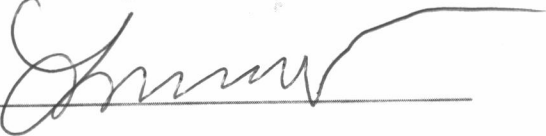
Major deviations that pose an immediate hazard to subject will be promptly reported by phone (301-319-9940) or by facsimile (301-319-9961) or e-mail (wrairdhsp@amedd.army.mil) as soon as investigator becomes aware of the deviation and then follow up in written form within 10 working days to the Director, Division of Human Subjects Protection, Walter Reed Army Institute of Research, 503 Robert Grant Ave., Silver Spring, MD 20910-7500. Minor deviations will be report in the continuing review report.

6.7. Principal Investigator Agreement

1. I agree to follow this protocol version as approved by the IRBs/ERCs.
2. I will conduct the study in accordance with applicable IRB/ERC requirements, Federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.
3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.
4. I will not modify the protocol without first obtaining an IRB/ERC approved amendment and new protocol version unless it is necessary to protect the health and welfare of study participants.
5. (For Greater than Minimal Risk studies or studies of public interest) In accordance with Command Policy 2008-35, I will ensure that the Commanding General receives a pre-brief (or Executive Summary) and approves the study prior to execution.
6. I will ensure that the data (and/or specimens) are maintained in accordance with the data (and/or specimen) disposition outlined in the protocol. Any modifications to this plan should first be reviewed and approved by the applicable IRBs/ERCs.
7. I will promptly report changes to the research or unanticipated problems to the WRAIR IRB immediately via the WRAIR of Human Subjects Protection Branch at (301) 319-9940 (during duty hours) or to the usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil and submit a written report within 10 working days of knowledge of the event.
8. I will prepare continuing review reports at an interval established by the IRB/ERC, and a study closure report when all research activities are completed.
9. I will immediately report to the WRAIR of Human Subjects Protection Branch knowledge of any pending compliance inspection by any outside governmental agency.
10. I agree to maintain adequate and accurate records in accordance with IRB policies, Federal, state and local laws and regulations.

I have read the foregoing protocol and agree to conduct the study as outlined herein.

Principal Investigator:

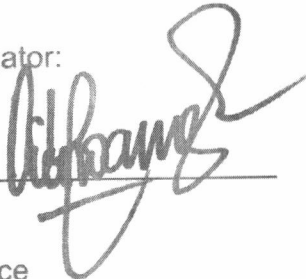


13 June 2017

Date

LOUIS R. MACAREO
COL, MC
Chief, Department of Virology
Armed Forces Research Institute of Medical Sciences

Co- Principal Investigator:



13/6/2017

Date

Sonam Wangchuk
Chief Laboratory Office
Royal Centre for Disease Control
Department of Public Health

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