The Department of Defense
Global, Laboratory-Based
Influenza Surveillance
Program: Technical Report on
Program Methods for the
2012-2013 Influenza Season

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14. ABSTRACT
This report describes the Department of Defense (DoD) Global, Laboratory-Based Influenza Surveillance Program as of the end of the 2012-2013 influenza season, focusing on the program’s methods for surveillance, laboratory analysis and reporting, and on scientific contributions to influenza vaccine development and influenza virology. The two main state-side-based lab components of the influenza program, working in collaboration, are the Air Force (AF) sentinel influenza surveillance program at the U.S. Air Force School of Aerospace Medicine (USAFSAM) and the Navy population-based respiratory disease surveillance program at the Naval Health Research Center. USAFSAM data support the DoD, the Centers for Disease Control and Prevention (CDC), and the World Health Organization; they are used each year during strain selection for the next year’s vaccine strain components. The CDC regularly requests viral isolates from USAFSAM. The AF influenza program has expanded DoD-wide participation; improved case selection and laboratory methods; improved storage, handling, shipment, and retrieval of specimens; expanded geographic presence; and increased the influenza surveillance program in conjunction with the DoD Global Emerging Infections Surveillance and Response System and many collaborators. The AF program continues to improve the ability to rapidly identify and respond to increased respiratory disease activity by enhancing the core surveillance infrastructure. It strengthens force health protection for the military by ensuring the best vaccine is made available for U.S. forces each year, and when new influenza strains emerge, they are carefully evaluated. If emerging strains are not well covered by immunity or conferred through seasonal vaccine or prior disease, then leaders are informed and other appropriate public health measures begun. The benefits of this program are not only for the U.S. military; it protects the health of U.S. citizens as well as those who work with the World Health Organization and the CDC to prepare and defend against influenza threats worldwide.

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1.0 EXECUTIVE SUMMARY

This special report describes the Department of Defense (DoD) Global, Laboratory-Based Influenza Surveillance Program as of the end of the 2012-2013 influenza season, focusing on the program’s methods for surveillance, laboratory analysis and reporting, and on scientific contributions to influenza vaccine development and influenza virology.

U.S. military forces are vaccinated annually with the influenza vaccine. The composition of the vaccine is determined annually to keep up with circulating influenza strains and their antigenic drift. Global surveillance is required; the World Health Organization (WHO) organized a system that has now operated for over 50 years. The U.S. Air Force (AF) began its laboratory-based influenza surveillance with “Project Gargle” in 1976 at key installations and then expanded worldwide. The AF system grew to include most AF overseas bases and some contiguous U.S. (CONUS) bases, based on missions, locations, and other factors. In 1997, the year the DoD Global Emerging Infections Surveillance and Response System (DoD-GEIS) formed, the AF was designated the DoD executive agent for laboratory-based influenza surveillance by the Assistant Secretary of Defense for Health Affairs.

The two main CONUS-based lab components of the influenza program, working in collaboration, are the AF sentinel influenza surveillance program, managed at the U.S. Air Force School of Aerospace Medicine (USAFSAM), Wright-Patterson Air Force Base, OH, and the Navy population-based respiratory disease surveillance program, managed at the Naval Health Research Center, San Diego, CA. This system has been augmented considerably over the past decade and closely coordinated with DoD-GEIS and overseas lab partners. Within the last few years, DoD-GEIS has reorganized and is now within the Armed Forces Health Surveillance Center as the Division of GEIS Operations (GEIS).

USAFSAM data, acquired through an extensive military, GEIS, U.S. Government, and host nation collaborative network, support the DoD, the Centers for Disease Control and Prevention (CDC), and the World Health Organization; they are used each year during strain selection for the next year’s vaccine strain components. The CDC regularly requests viral isolates from USAFSAM.

The AF influenza program has expanded DoD-wide participation; improved case selection and laboratory methods; improved storage, handling, shipment, and retrieval of specimens; expanded geographic presence; and increased the influenza surveillance program in conjunction with GEIS and many collaborators. The AF program continues to improve the ability to rapidly identify and respond to increased respiratory disease activity by enhancing the core surveillance infrastructure. It strengthens force health protection for the military by ensuring the best vaccine is made available for U.S. forces each year, and when new influenza strains emerge, they are carefully evaluated. If emerging strains are not well covered by immunity or conferred through seasonal vaccine or prior disease, then leaders are informed and other appropriate public health measures begun. The benefits of this program are not only for the U.S. military; it protects the health of U.S. citizens as well as those who work with the World Health Organization and the CDC to prepare and defend against influenza threats worldwide.
2.0 INTRODUCTION

2.1 Influenza Vaccination in the U.S. Military

The origin of universal vaccination in U.S. military personnel against infectious diseases can be traced to George Washington, who, in the winter of 1777 at Valley Forge, Pennsylvania, ordered variolation for all new recruits in the Continental Army. As medical science progressed, increasing numbers of safe and effective vaccines were developed. U.S. military leadership incorporated vaccination into the initial phase of recruit training (and for other military personnel as well). The U.S. Army developed the first influenza vaccine in the 1940s and conducted successful clinical trials showing that the vaccine was safe and effective. While many were vaccinated in succeeding years, some influenza outbreaks occurred in vaccinees. Observing that vaccine-induced immunity waned and that the influenza virus gradually adapted to escape vaccine-induced immunity, Army scientists determined that the vaccine needed to be periodically reformulated and troops needed periodic revaccination. Annual influenza vaccination is currently a requirement for active duty U.S. military for all services [1].

Influenza prevention is recognized as a high priority in the military. During the 1918 influenza pandemic, there were over 50 million deaths worldwide and 500 million cases in the U.S. [2]. Troop movement, from training bases in the U.S. to the World War I theater in Europe, probably contributed significantly to global spread. Early in the 1918 pandemic, the attack rate was very high in military populations. For example, in 3 months, September-November 1918, the U.S. Army and Navy had between 20-40% ill with influenza [3]. There were more deaths in U.S. military personnel from the 1918 influenza A(H1N1) than in any war. Reflecting this high priority, influenza vaccination is an important medical readiness issue for commanders; vaccination status is monitored continually through electronic vaccination registries, and real-time data are available by individual, unit, organization, etc. so that leadership at all levels can easily determine the current status through electronic vaccination registry data linked with medical readiness systems.

In the U.S., influenza vaccination is voluntary except for the U.S. military and certain healthcare workers. Civilian vaccination guidance is based on recommendations of the Advisory Committee on Immunization Practices. Military beneficiaries are offered influenza vaccination through their military treatment facilities (MTFs) and through a special arrangement where TRICARE, the health care program serving uniformed service members, retirees, and their families, pays for influenza vaccination at certain pharmacies. For the Air Force (AF), the Air Force Complete Immunization Tracking Application captures data from vaccinations given at the MTF and elsewhere, to include non-military pharmacies where TRICARE has paid for the vaccination.

2.2 Global Influenza Surveillance

A global surveillance system was needed to monitor influenza strains so that the vaccine could be reformulated correctly. The World Health Organization (WHO) established a system of collaborating laboratories to do this work; this began with London in 1948 and Australia in 1951. The Centers for Disease Control and Prevention (CDC) became a WHO Influenza Center in 1956 and is now one of the six WHO Centers; U.S. collaborating laboratories share isolates or specimens with the WHO network through the U.S. CDC. Each year, the WHO reviews
currently circulating influenza strains to make a recommendation for the composition of the influenza vaccine for the next season for the Northern and Southern Hemisphere, usually in February and September, respectively. For Food and Drug Administration (FDA) licensed vaccine for the U.S., the selection is made a week later, according to the recommendation of the Vaccines and Related Biological Products Advisory Committee (VRBPAC) of the FDA. Usually VRBPAC decisions are in line with WHO recommendations.

The Air Force laboratory at Brooks Air Force Base (AFB), San Antonio, TX, began to build a global network for influenza surveillance in 1976, starting with a few participating AF bases and then expanding to include many bases in Asia and the Pacific. Brook’s laboratory became a WHO Collaborating Laboratory through the CDC. In 1999, the Air Force was designated the executive agent for Department of Defense (DoD) lab-based influenza surveillance by the Assistant Secretary of Defense for Health Affairs [ASD(HA)] (see Appendix A). Please see Table 1 for a brief description of how this came about.

**Table 1. Development of an Enduring AF/DoD Influenza Surveillance System**

<table>
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<tr>
<th>Year</th>
<th>Milestone</th>
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<tr>
<td>1976</td>
<td>AF establishes active, sentinel, laboratory-based influenza and related respiratory disease surveillance at the Brooks AFB U.S. AF School of Aerospace Medicine (USAFSAM) laboratory, initially with specimens from Lackland AFB</td>
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<td>1977-1995</td>
<td>Global expansion: Japan, Korea, the Pacific, Alaska, Europe, and southwest Asia, including deployed (especially from 1990 forward) locations - logistics (pre-positioning, resupply of collection kits; specimen transport); training; support; reporting to CDC, WHO, and FDA regarding vaccine strains</td>
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<td>1996</td>
<td>National Science and Technology Council Presidential Decision Directive (PDD-7)</td>
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<td>• Stated that the U.S. was not prepared for the threat posed by emerging and reemerging infectious diseases (EIDs)</td>
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<td>• Directed departments/agencies, including the DoD, to take action</td>
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<td></td>
<td>• Assigned roles and responsibilities</td>
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<td></td>
<td>• CDC was given the lead</td>
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<td>1997</td>
<td>DoD created DoD-GEIS in response to PDD-7 and directed DoD-GEIS to coordinate this effort</td>
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<td>Influenza was recognized as the first priority among all EID threats (DoD experts and stakeholders met to prioritize EID threats and advise DoD)</td>
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<td>1998</td>
<td>ASD(HA) Dr. Sue Bailey issued a policy memo establishing the DoD influenza surveillance system and assigning the lead to the AF (HA 99-008) (Appendix A)</td>
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<td>2003</td>
<td>DoD overseas labs (U.S. Army Medical Research Unit-Kenya, Armed Forces Research Institute of Medical Sciences, Naval Medical Research Unit-2,3 [NAMRU-2,3], and U.S. Naval Medical Research Center Detachment-Lima (now NAMRU-6)) expanded regional surveillance under GEIS coordination</td>
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<td>The two special CONUS labs, USAFSAM and NHRC, modified and expanded their programs, shifting from service-specific to DoD surveillance systems</td>
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<td>2006</td>
<td>AF/DoD severe acute respiratory syndrome surveillance developed from existing influenza surveillance</td>
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<td>Congress, in a 2006 Supplemental Appropriation, dramatically increased resources for DoD influenza surveillance</td>
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<td>ASD(HA) directed DoD-GEIS to plan and execute this expanded mission</td>
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<td>DoD overseas labs expanded regional influenza surveillance; AF providing influenza laboratory reference and standardization</td>
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Table 1. Development of an Enduring AF/DoD Influenza Surveillance System (concluded)

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<th>Year</th>
<th>Milestone</th>
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<td>2009</td>
<td>Influenza pandemic; initial U.S. cases detected by NHRC and USAFSAM</td>
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<td>2010</td>
<td>DoD-GEIS reorganized as part of the Armed Forces Health Surveillance Center (AFHSC) Division of GEIS Operations (AFHSC/GEIS)</td>
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<tr>
<td>2011</td>
<td>USAFSAM completes move to new laboratory/office space and reestablishes the program at Wright-Patterson AFB, Ohio</td>
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First DoD Combined Sequence Analysis Report (contribution for VRBPAC) with AFHSC, NHRC, DoD overseas laboratories, and other GEIS partner laboratories

AFHSC/GEIS retains its central DoD EID threat coordination role and provides significant funding and guidance for major influenza surveillance programs
- USAFSAM
  - Sentinel global, laboratory-based influenza surveillance and response
  - Influenza reference laboratory services
  - DoD/AF/CDC lab coordination
  - WHO Collaborating Laboratory
  - EID diagnostic assay development and validation
  - DoD combined influenza sequence analysis
  - Influenza vaccine effectiveness evaluation
- NHRC
  - Population-based acute respiratory disease surveillance
    - Recruit training sites (all AF, Navy, Marine Corps, Coast Guard, most Army)
    - Shipboard
    - Some civilian populations along the U.S.-Mexico border
  - Coordination with USAFSAM
    - Overseas regional surveillance through U.S. Army Medical Research Unit-Kenya, Armed Forces Research Institute of Medical Sciences, NAMRU-2,3,6
    - Other GEIS-supported projects and programs according to competitively funded annual proposals

2.3 Air Force Role in Influenza Surveillance Today

The AF influenza program was originally called “Project Gargle” until 1998 (and is still known to many by that name). Located at Brooks AFB (later renamed Brooks City-Base), it was initially associated with USAFSAM. A series of reorganizations and name changes occurred: Armstrong Laboratory; the U.S. Air Force Institute for Environment, Safety, and Occupational Health Risk Analysis; and the Air Force Institute for Operational Health. More recently, in Fiscal Year 2008 and because of the planned closure of Brooks City-Base under the Base Closure and Realignment Commission (BRAC), the Air Force Institute for Operational Health’s component sections merged with USAFSAM. The influenza program was specifically organized within the Department of Public Health and Preventive Medicine. USAFSAM was brought into the newly created 711th Human Performance Wing headquartered at Wright-Patterson AFB (WPAFB), completing its move to a new laboratory and office space at WPAFB in 2011.

The Air Force has expanded the geographic reach of the program and increased DoD-wide participation in influenza surveillance. To understand key features of the program, a 1999 event is worth recalling. CDC noted that an important influenza A(H3N2) strain had emerged in Panama. Antigenic testing indicated that it was not well covered by available vaccine strains. To CDC experts, it was likely that this strain would spread and circulate widely during the next season, but isolates from specimens CDC had already acquired and tested did not grow well in eggs, a requirement for vaccine production. The CDC contacted the AF for assistance. At the time, Howard AFB in Panama was being closed under BRAC; however, the medical elements there had the supplies and expertise needed to collect additional specimens and get them to Brooks quickly. One strain they collected became the A/Panama/2007/1999 (H3N2) vaccine...
strain used successfully for four subsequent years. A number of vaccine, reference, and other important viruses have come from the Air Force program in subsequent years (see Appendix B). Current work supporting expanded global reach includes:

- Over 80 sentinel sites, located in 18 countries, 35 U.S. states, Guam, and Puerto Rico
- Molecular testing and genetic sequencing
  - Data transmitted to CDC immediately after testing improve CDC’s situational awareness and allows them to quickly select important viruses/specimens for further evaluation for suitability as potential vaccine seed strains
  - Influenza reference laboratory capability for all DoD CONUS and OCONUS labs
- DoD standardization/sharing of sequence data (hemagglutinin) from other DoD labs (approved by many DoD labs in 2009)
  - Other labs contribute hemagglutinin data in a standard format (FASTA file) to USAFSAM’s influenza molecular lab
  - DoD combined sequence analysis
    - Periodic sequence analyses performed, shared with CDC, DoD, and the contributing labs
    - Prior to WHO consultations on the Northern and Southern Hemisphere influenza vaccine strain selections, USAFSAM scientists prepare special reports for CDC’s Influenza Division to contribute to WHO’s deliberations
- Sharing of surveillance information through weekly surveillance reports, freely available and distributed via email and websites
- Publications in peer-reviewed scientific literature
- Presentations at international professional or scientific meetings
- GENBANK publication of sequence data

USAFSAM constantly seeks to improve its ability to rapidly identify and respond to infectious respiratory disease activity by enhancing the core surveillance infrastructure. The aim is to enable the rapid discovery of novel mutations early in a pandemic (shift) and those that seasonally allow the virus to escape immunity (drift). This also applies to novel influenza and non-influenza respiratory viruses; USAFSAM will develop new molecular diagnostic assays for novel pathogens or work with CDC-developed assays, then make available clinical diagnostic testing for DoD after conducting in-house assay validation. For example, during April-May 2009, USAFSAM and NHRC were the first DoD labs to confirm the 2009 pandemic influenza A(H1N1) cases, using newly developed CDC assays. At the same time, USAFSAM set up and offered in-house training for all DoD labs on these assays, in conjunction with CDC.

For seasonal influenza, the program works with the AFHSC Division of GEIS Operations (formerly DoD-GEIS), the CDC, the FDA, and its military and civilian partners to collect, identify, and rapidly characterize circulating viruses. An important feature of the program is that aliquots of all original specimens are retained. Vaccine strains must come from original specimens, not isolates. Thus, WHO and CDC have the ability to request and receive any specimen collected through this surveillance system to use as a vaccine seed strain or other purposes. This is crucial for the national strategy for both seasonal and pandemic influenza preparedness. While DoD’s priority is to maintain readiness and protect the health of DoD
forces and beneficiaries, the contributions from this surveillance program also directly benefit
the greater global health community.

The program currently is guided by DoD and AF policy. The aims and objectives of the
influenza program at USAFSAM regarding pandemic planning are consistent with the
Department of Defense Implementation Plan for Pandemic Influenza (August 2006) [4] and the
Federal National Strategy for Pandemic Influenza: Implementation Plan [5]. Annual
surveillance activities at USAFSAM are guided primarily by Air Force Instruction 48-105 [6],
with additional guidance from Health Affairs Policy Memo 99-008; each year the program’s
sentinel sites are identified in a DoD(HA) policy memorandum. Funding and guidance comes
from the AF and DoD-GEIS. Until 2010, the program’s work was reviewed annually in
conjunction with other DoD collaborators and the CDC; the Joint Influenza Surveillance
Working Group (JISWG) meeting, required in the 1999 DoD policy, was an opportunity for
regular communication and coordination with DoD-GEIS, NHRC, the DoD overseas research
laboratories, DoD-HA, and many DoD-GEIS partners. AFHSC/GEIS has developed a
Respiratory Pathogens Surveillance Program Steering Committee that they hope will take the
place of the JISWG.

3.0 METHODS

3.1 Surveillance Methods

3.1.1 DoD Sentinel Sites. Active surveillance is performed primarily at sentinel sites consisting
of MTFs caring for U.S. military members and their families. Sites are selected according to
their location, mission, and the possibility of filling an important gap in international
surveillance. MTFs at these sites are asked to do the following:

1. Submit from 6 to 10 specimens per week
2. Collect specimen from patients meeting the criteria, or case definition, for influenza-like
   illness (ILI)\(^1\)
3. Complete a standard influenza surveillance questionnaire (correct version for the season)
4. Ship specimens to USAFSAM (see more details under Laboratory Methods section
   below)
5. Communicate with USAFSAM on outbreaks, unusual severity of illness, etc.
6. Monitor laboratory results via the Composite Health Care System (CHCS) or the Armed
   Forces Health Longitudinal Technology Application (AHLTA)
7. Review their site data on USAFSAM flu surveillance website/dashboard at least weekly
8. Report results to MTF professional staff
9. Identify site points of contact (POCs): public health/preventive medicine, laboratory,
   clinical operations
10. Identify site lead, usually public health/preventive medicine

Factors considered in selection of a sentinel site are potential for emergence of new strains,
potential for importation of new strains, impact of an outbreak or novel influenza virus on
military operations, and location, especially areas with high troop concentrations and movement

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\(^1\) ILI case definition: fever ≥100.5°F and cough or sore throat with onset in the past 72 hours
of highly mobile or rapid response units. For a list of the 2012-2013 sentinel sites, see Appendix C. For a map of the sentinel site locations, see Appendix D.

Bases where recruit training occurs have special problems with viral respiratory diseases such as influenza and adenovirus [7,8]. NHRC, San Diego, CA, conducts population-based respiratory disease surveillance at basic military training (BMT) sites including Lackland, Great Lakes, Cape May, Marine Recruit Depots at Parris Island and San Diego (covering all BMTs for the AF, Navy, Marine Corps, and Coast Guard), and five Army basic training bases. BMT bases often have other important missions and significant numbers of active duty personnel, so recruit surveillance may be augmented with sentinel surveillance, but NHRC and USAFSAM, along with AFHSC, try to work in a coordinated fashion and avoid unnecessary duplication of effort. In addition, some sites are located in countries where there are ongoing regional surveillance partnerships with the DoD overseas research laboratories. Descriptions of this system have been published [9,10].

3.1.2 Surveillance Operations. Sentinel sites institute an active, year-round influenza surveillance program at their location, coordinating with USAFSAM. USAFSAM provides the following support:

1. Develops training materials, revising them annually for the beginning of each season
2. Conducts training remotely and in person via sentinel site visits, telephone consultations, Defense Connect Online, and training slides available on website
3. Handles program logistics
   a. Works with Tryco, subcontractor, on the assembly of specimen collection kits with viral transport medium (VTM), sterile saline/syringe, collection cup, bib, instructions, influenza questionnaire, biohazard bag
   b. Covers cost of shipping specimens to USAFSAM via FEDEX or other carrier
   c. Monitors supply of collection kit materials and specimen shipments
4. Performs diagnostic virology, molecular detection, sequencing, sequence analysis (see Laboratory Methods section for details)
5. Enters test results in CHCS; once certified, results can be viewed by submitting provider and submitting location public health/preventive medicine staff
6. Performs epidemiologic analyses and prepares periodic surveillance reports
7. Maintains a website/dashboard for surveillance results (not individually identifiable) by base and aggregated by service
8. Influenza surveillance staff members (preventive medicine/public health, epidemiology, laboratory, logistics) have regular communication with the sentinel sites regarding results from surveillance and any unusual disease activity they may be experiencing.
9. If unusual ILI activity or disease severity is noted at any military installation or deployed location, USAFSAM responds by providing:
   a. Collection kits, training, and support for specimen collection and transport
   b. International Air Transport Association (IATA) training, if needed, in deployed or overseas locations
   c. Outbreak investigation support, if needed
   d. Consultation regarding control measures and any other assistance necessary
10. Communicates with sites, major commands, headquarters, DoD(HA), AFHSC/GEIS, and CDC
11. Shares data with CDC Influenza Division
12. Serves as a DoD POC for influenza with CDC’s Influenza Division concerning laboratory or surveillance methods
13. Develops, evaluates, and advocates DoD standardization in influenza surveillance methods
14. Submits program documents to Institutional Review Board; facilitates Institutional Review Board oversight of surveillance operations

USAFSAM prepares for the season by identifying the proposed sentinel sites for DoD, updating training materials, distributing training materials to the sites, revising the questionnaire (Appendix E) if needed, establishing communications with key site POCs, and shipping collection kits. For a detailed review of information disseminated to preventive medicine/public health, laboratory, and provider personnel, see Appendices F, G, and H, respectively. Prior to each season, a number of sentinel sites are visited in-person by a three- to four-person team, allowing for improved relationships and coordination with that site. During the typical influenza season, the sentinel sites ensure that specimens are collected and shipped to USAFSAM, where diagnostic testing is performed and results are entered in the electronic record (CHCS). The weekly surveillance report is distributed widely via email and is available on the program’s website and through CDC’s Epidemiology Exchange, Epi-X. Epidemiology staff analyzes and reports surveillance data weekly during the influenza season. Surveillance continues year-round but, during periods of low influenza activity, reporting is completed monthly. Prior to the WHO’s consultation for the Northern Hemisphere’s influenza vaccine strain selection and FDA’s VRBPAC meeting, both held in February, the program analyzes all data, providing information to the CDC, including the DoD Global Influenza Surveillance Summary and DoD Combined Sequence Report. These reports are also presented at the VRBPAC meeting along with the (USAFSAM) DoD mid-season vaccine effectiveness estimate. Results are summarized at the end of the season.

3.1.3 ILI Electronic Syndromic Surveillance. In addition to laboratory data, ILI is continually monitored using an electronic syndromic surveillance, Electronic Surveillance System for the Early Notification of Community-Based Epidemics (ESSENCE), which covers all sentinel and non-sentinel military installations. This was originally developed after successful pilot testing using International Classification of Diseases-Ninth Revision (ICD-9) data from MTFs in the National Capital Area [11]. Electronic data from MTF patient encounters are used. Based on ICD-9 coded diagnoses, a group of respiratory codes has been selected as indicators for ILI [12].

While ESSENCE is useful, there are some important limitations:

- Access to ESSENCE is carefully controlled (there is a high level of privacy information involved).
- Algorithms, alternate code sets, trending, etc. are not user-modifiable.
- Data are aggregated for surveillance purposes according to ESSENCE administration and are not readily reorganized in a timely manner to keep current with influenza surveillance.
- Data transmission to ESSENCE doesn’t occur until the physician has closed out the record in AHLTA. This typically results in data lags of up to 2 days; however, lags can be as long as 5-7 days.
In addition to routinely monitoring ESSENCE, the influenza program developed a web-based ILI surveillance system that is viewable by anyone with access to the USAFSAM website. It contains no privacy information and is flexibly configurable, permitting multi-year weekly averaging as well as frequent changes to keep pace with changing influenza surveillance needs. Further, ICD-9 code sets can be reassessed easily and changes made as needed (see Table 2 for the current ICD-9 code list). The proportion of ILI ICD-9 codes identified in outpatient visits is evaluated by week for the previous 6-year period for each location in a web-based, automated electronic system. The intent is to establish communication of and response to ILI activity that is above normally observed limits. If an increase of one or two standard deviations above the mean is experienced at a site or clinic, USAFSAM public health staff contacts the base public health (Air Force) or preventive medicine (Army, Navy, Marines) office and encourages the site to submit specimens (if this isn’t occurring already). For the Coast Guard, the preventive medicine officer at the Coast Guard Commandant’s office in Washington, DC, is contacted.

**Table 2. ICD-9 Code Set USAFSAM Uses to Obtain ILI Rates among DoD Beneficiaries in Outpatient MTFs**

<table>
<thead>
<tr>
<th>Code</th>
<th>Short Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>79.99</td>
<td>Unspecified viral infection</td>
</tr>
<tr>
<td>382.9</td>
<td>Unspecified otitis media</td>
</tr>
<tr>
<td>460</td>
<td>Acute nasopharyngitis (common cold)</td>
</tr>
<tr>
<td>461.9</td>
<td>Acute sinusitis unspecified</td>
</tr>
<tr>
<td>465.8</td>
<td>Acute upper respiratory infections of other multiple sites</td>
</tr>
<tr>
<td>465.9</td>
<td>Acute upper respiratory infections of unspecified sites</td>
</tr>
<tr>
<td>466.0</td>
<td>Acute bronchitis</td>
</tr>
<tr>
<td>486</td>
<td>Pneumonia organism unspecified</td>
</tr>
<tr>
<td>487.0</td>
<td>Influenza with pneumonia</td>
</tr>
<tr>
<td>487.1</td>
<td>Influenza with other respiratory manifestations</td>
</tr>
<tr>
<td>487.8</td>
<td>Influenza with other manifestations</td>
</tr>
<tr>
<td>490</td>
<td>Bronchitis not specified as acute or chronic</td>
</tr>
<tr>
<td>780.6</td>
<td>Fever and other physiologic disturbances of temperature regulation</td>
</tr>
<tr>
<td>780.60</td>
<td>Fever, unspecified</td>
</tr>
<tr>
<td>780.61</td>
<td>Fever presenting with conditions classified elsewhere</td>
</tr>
<tr>
<td>786.2</td>
<td>Cough</td>
</tr>
</tbody>
</table>

Aggregate ILI charts are shown in Figures 1-4 for DoD-wide and service-specific activity over the year. Similar charts are available on the USAFSAM website for sentinel and other installations to view their own ILI activity in near real time. Color-coded points represent the degree to which the week’s ILI diverges from prior years: blue for no difference, green for one standard deviation below the mean, yellow for one standard deviation above the mean, and red for two standard deviations above the mean. The purpose of this ILI surveillance application is to establish linkage, as described above, between active laboratory surveillance and syndromic surveillance to enhance the effectiveness of the lab-based surveillance program. Another benefit, considering the military is a highly vaccinated group, is that specimens collected from military members are actively sought in a timely manner wherever ILI patterns point toward possible
breakthrough in vaccine coverage, ensuring that emerging flu strains are collected in a timely manner and reported to CDC.

**Figure 1.** Percentage of Clinic Visits for ILI for All Services and Beneficiaries: 2007-2013

**Figure 2.** Percentage of Clinic Visits for ILI for All Services and Beneficiaries: 2012-2013
Figure 3. Percentage of Clinic Visits for ILI at WPAFB: 2012-2013

Figure 4. Percentage of Clinic Visits for ILI for U.S. Air Force, Coast Guard, Army, and Navy: 2012-2013
3.1.4 Influenza Surveillance Policy Development. The influenza surveillance program prepares a recommended list of DoD sentinel sites to be incorporated in an annual DoD influenza surveillance policy. For the 2012-2013 season policy memo, see Appendix C. Prior to 2010, the JISWG meeting was an opportunity to review the past year’s performance of the surveillance system, identify important gaps, compare results with the CDC, and make decisions regarding needed policy changes and sentinel sites. There has been close coordination among ASD(HA), AFHSC, USAFSAM, NHRC, and the Air Force Surgeon General’s office on this policy.

The DoD influenza surveillance policy also appears in other policy documents. For example, DoD(HA) issues annual influenza vaccination policy, usually just prior to shipments of vaccine are made available to supply MTFs globally. In 2011, the vaccination policy also included some surveillance policy material, but in 2012 the annual vaccination policy and the surveillance policies were fairly separate. This also happens in other commands, for example, in U.S. European Command (EUCOM). The influenza operational order (OPORD) for EUCOM combined vaccination and surveillance policy in 2011; USAFSAM, U.S. Army Public Health Command-Europe (PHCR-E), and Landstuhl Regional Medical Center (LRMC) are all named in the OPORD. Since 2007, according to a EUCOM policy, European installations have been directed to send specimens to LRMC. The 2012-2013 season OPORD directs PHCR-E to report on European regional surveillance and for LRMC to send specimens or isolates to USAFSAM. For all such policies, USAFSAM tries to coordinate and review DoD, service, and regional influenza surveillance policy in sufficient time to identify problems and improve the products, consistent with the program’s mission.

USAFSAM will also prepare policy in response to emergencies and novel situations. With the recent pandemic in 2009, the program prepared AF- and DoD-level policies for case-finding, reporting, and diagnostic testing (with special attention to the lack of reliability of rapid antigen tests); this was done in coordination with the AF and other Surgeon Generals’ offices, their public health organizations, and AFHSC. In the first weeks of the pandemic, after consultation with the flu program, the Air Force Surgeon General directed all AF MTFs to send all diagnostic lab specimens for influenza to USAFSAM. Also, the flu program modified the influenza surveillance questionnaire completion process so that all AF sites could complete questionnaires on all ILI cases, whether or not a lab specimen was collected, as they were seen in clinics; all AF MTFs were directed to follow this procedure. As a result, USAFSAM automatically generated line listings each morning for public health offices; all possible cases seen at that MTF the day before (and cumulatively) were identified on the line list. It greatly facilitated the program’s ability to detect new H1N1 outbreaks at these MTFs.

3.1.5 Surveillance Gaps. Problems with surveillance such as gaps, duplication, standardization, and other inconsistencies are addressed through coordination with the services, the CDC, and through policy development. For example, before 2007, the DoD sentinel sites in Europe sent specimens directly to USAFSAM, but from 2007 on, a new EUCOM policy directed all MTFs (including DoD sentinel sites) in Europe to send specimens to LRMC. USAFSAM has not been receiving sufficient numbers of specimens or isolates from the DoD sentinel sites in Europe for inclusion in VRBPAC reports or Northern Hemisphere WHO submissions since this occurred. USAFSAM identified a similar gap in Hawaii sentinel sites. Regional surveillance exists in these areas, and we are currently building relationships with them. To close this surveillance gap, we need to restore a regular flow of 6 to 10 specimens per week that meet the ILI case definition, along with linked questionnaire data. This past season, we have worked successfully with
EUCOM to gain access to their CHCS node. With this capability, we are able to access their respiratory surveillance data directly. Since, mid-season, we have been downloading and analyzing the EUCOM data, but because their data vary so drastically from USAFSAM’s data, we chose not to combine the data. In lieu, we created a EUCOM supplemental report. We continue to work with PHCR-E to close this surveillance gap.

NHRC conducts shipboard respiratory surveillance and recruit respiratory surveillance at DoD training centers. Since this can create duplicative efforts, we collaborate with them to minimize redundancy. We consider this while selecting our sentinel sites each season. Please refer to Appendix I for more ongoing collaborations.

3.2 Laboratory Methods

3.2.1 Accreditation. The Epidemiology Surveillance Laboratory is accredited by the Department of Defense Clinical Laboratory Improvement Program and by the College of American Pathologists.

3.2.2 Specimen Collection and Transport. Nasal wash or nasopharyngeal swabs are collected from individuals meeting the case definition or on clinical judgment. Nasal washes are the preferred specimen type because a larger volume is available for initial evaluation, additional characterization studies (i.e., sequencing), and/or sharing with the CDC. USAFSAM provides collection kits containing a sterile plastic syringe, a collection cup and lid, VTM, label, bib, and biohazard bag. The respiratory viral collection kits are prepared by Tryco Corporation and drop-shipped to locations globally; the process is coordinated by one or more dedicated USAFSAM logistics technicians. The test is ordered in CHCS, which is an electronic medical record system used widely primarily for lab data in the DoD. For those without access to CHCS, the specimen is sent with a manifest containing the necessary information, and the USAFSAM lab enters the order in CHCS.

Specimens are either transported on dry ice/gel packs to USAFSAM, within 48 hours of collection, or frozen at -70°C for later transportation on dry ice. USAFSAM provides shipping with pre-paid commercial shipping contracts. International shipments are subject to IATA rules; USAFSAM provides IATA training for deployed locations by purchasing and supplying the computer-based training modules to laboratorians needing certification while in-theater. Normally, laboratory staff maintain their training and certifications through their MTF or hospital. USAFSAM has sometimes assisted other partner organizations and staff in obtaining IATA certification, which is essential for sentinel site operations. For a more detailed overview of specimen collection, shipping, and storage guidelines, see Appendix J.

3.2.3 Virology and Molecular Lab. Once received at USAFSAM, original nasal wash or nasopharyngeal specimens are examined for the presence of viruses using traditional viral culture and molecular techniques such as real-time reverse-transcriptase polymerase chain reaction (RT-PCR) using the ABI 7500 instrument and sequencing. Leftover specimen aliquots are archived [see Appendices K and L]. All specimens are handled at a minimum of Biosafety Level-2 conditions. Culture is performed using appropriate tissue culture cell lines including Primary Rhesus Monkey Kidney and A549. Respiratory pathogens isolated include influenza virus A and B; adenovirus; parainfluenza 1, 2, and 3; enterovirus; and respiratory syncytial virus (see Appendices L and M). Influenza isolates are typed and all influenza A isolates are subtyped, and
many are further characterized molecularly. Any non-subtypeable influenza A specimens are referred to the CDC for further analysis. Virology results are typically available for influenza and the additional respiratory viruses noted above within 48 hours, but the culture tubes are held for 10 days before being considered negative.

3.2.4 Clinical Results. Results from both virology and molecular testing are reported using the CHCS. Laboratory personnel enter the results. Once certified in CHCS, the results are immediately available to USAFSAM epidemiologists, the clinical provider who ordered the test, and the laboratory and public health staff at the originating MTF. Results from USAFSAM can be used for clinical treatment decisions and are entered into the laboratory section of patients’ records in AHLTA.

3.2.5 Archive. Aliquots of all original specimens are archived and available by request if needed by the CDC for additional characterization. Since vaccine seed strains must come from original specimens rather than isolates, the program archives both original specimens and isolates.

3.2.6 Genetic Sequencing. Genetic sequencing of the hemagglutinin surface proteins is performed on influenza A and B specimens, primarily to detect variations from the vaccine component strains. Typically, the first 1200 base pairs of the hemagglutinin HA1 region are sequenced because changes in HA1 are most closely correlated with changes in influenza virus antigenicity. Results are shared with the CDC and included in the WHO phylogenic representation of circulating strains. Sequence analysis reports are prepared by the molecular lab staff. Individual viruses are shown in relation to each other and to reference vaccine strains. The degree of homology (to vaccine component strains) is one predictor of whether the virus has drifted sufficiently away from the vaccine to cause problems for vaccinated military forces. Each report includes the homology range observed for the viruses analyzed. Once the phylogenetic relationships are graphically organized, USAFSAM identifies the vaccination status and vaccine type used (if known) for each case corresponding to its virus. In this way, information about possible vaccine breakthrough cases can be viewed and analyzed by virus subtype, mutations, time, and geography. This also provides a way for USAFSAM to identify viruses that are important for further antigenic testing by the CDC.

During the summer of 2012, CDC invited staff from USAFSAM and NHRC to participate in molecular detection, sequencing, and sequence analysis training. A need to standardize methods was recognized; for the 2012-2013 season, USAFSAM has changed its sequence analysis methods, adopting the CDC’s methods for phylogenetic analysis and for displaying results.

Through two types of pyrosequencing instruments, the lab has been able to (1) identify mutations typically associated with influenza antiviral resistance in A(H1N1)pdm09, seasonal A(H1N1) prior to the pandemic, and seasonal A(H3N2) and (2) prepare for full genome sequencing for the 2013-2014 season. Results of antiviral resistance marker testing are forwarded to CDC and have been included in CDC data tables that appear in CDC’s weekly and seasonal results. From all specimens submitted by USAFSAM, CDC selects some for its own in vitro antiviral testing and provides test results for each of these viruses. Pyrosequencing-based antiviral resistance testing was interrupted during part of the 2011-2012 season due to the BRAC-related laboratory move, but was performed for the 2012-2013 season.
3.3 Methods in Relation to Vaccine

Influenza vaccination is the most significant force health protection measure for military personnel and missions; annual vaccination is mandatory. Through surveillance questionnaires and military electronic vaccination registries, vaccination data on individuals are sought for each specimen submitted to USAFSAM. This includes the date of vaccination and type of vaccine, live attenuated influenza vaccine (LAIV) or trivalent inactivated influenza vaccine (TIV). USAFSAM is one of very few laboratory-based surveillance programs that has both a high proportion of vaccinated individuals in its surveillance population and the ability to match vaccination history with clinical and virus sequence data for a significant percentage of its specimens. For years, the program has evaluated genetic mutations, based on sequence analysis, and tracked geographic, temporal, and vaccine “breakthrough” trends in influenza viruses. Within the past 2 years, progress has also been made in vaccine effectiveness analysis.

3.4 Coordination with CDC

There are many areas within the influenza program that are coordinated closely with the CDC. The CDC prefers to have a single entity, a DoD POC, for communications between the CDC Influenza Division and DoD laboratories regarding laboratory aspects of influenza – USAFSAM has been that POC. In preceding sections, as prescribed in PDD-7, a high-level of coordination occurs between the CDC and USAFSAM. In the past, the CDC has played a role in the selection of DoD sentinel sites through participation in JISWG (1999-2010).

All sequences are shared with CDC. This sharing automatically includes sharing with WHO. While much activity is directed toward ensuring a flow of high quality specimens, isolates, and sequence data to the CDC, there are also training, testing, and consultation activities worth mentioning. One example is discussed in 2.2.6 involving USAFSAM staff participating in CDC’s genetic sequence training. In addition, this training is conducted to improve standardization in testing, sequencing, and sequence analysis. At the beginning of the 2009 pandemic, USAFSAM personnel evaluated CDC assays, trained at CDC, and conducted training on the CDC influenza PCR assays at Brooks City-Base for all DoD labs needing it so that they could certify their results in-house.

3.5 Influenza Vaccine Effectiveness

During the 2011-2012 season, USAFSAM calculated vaccine effectiveness using data solely from the sentinel surveillance system and related clinical laboratory operations. A case-control method was used. A case was defined as a patient with a specimen that was laboratory positive by virology, molecular detection, or both. Controls were selected from ILI patients with specimens that were test-negative for influenza. This work was based on a study completed the previous season, performed through a collaboration with the Armed Forces Health Surveillance Center [13]. In that study, the analysis was similar with exception to case ascertainment and control selection. For the 2011-2012 season, we were able to determine that vaccine effectiveness was high, with statistical significance, against influenza overall and for A/H3, the predominant subtype for the season. Also, both LAIV and TIV vaccines conferred similar levels of protection. This method made it possible to evaluate vaccine effectiveness at mid-season and have statistically significant results to report in time for the DoD presentation at VRBPAC, even
in spite of the later onset of flu activity for that season. We found that this method is repeatable and sustainable within the resources of the program. The mid-season 2011-2012 study was submitted to the journal *Vaccine* and recently published [14].

Using the same methodology, analysis was repeated for the 2012-2013 influenza season, and statistically significant results were reported in the DoD presentation at VRBPAC for a second year in a row. Methodology involved the calculation of crude and adjusted (using logistic regression) odds ratios (OR). Vaccine effectiveness was defined as (1-OR)*100. Analysis determined that vaccine effectiveness was moderate, and statistically significant, against influenza overall [44% (28-56)] and for A/H3, again the predominant subtype. During the 2012-2013 season, TIV conferred a marginally higher level of protection than LAIV [47% (29, 60) vs. 40% (18, 56)]. Results for USAFSAM were published in the March 2013 edition of *Medical Surveillance Monthly Report* [15] with analysis from other collaborating study groups.

This is a time when increased attention is being focused on trying to measure influenza vaccine effectiveness. There are at least three large collaborative study groups that were established for this purpose, two in Europe and one in the U.S. Compared to these efforts, this program is less complex and already has an established system for specimen and data collection. Also, the work can be performed without additional cost.

### 3.6 Novel Respiratory Pathogens

The 2012-2013 influenza season revealed the emergence of two novel respiratory pathogens: Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and influenza A(H7N9). MERS-CoV was first identified in patients who lived in or had recent travel to the Saudi Arabian Peninsula, while influenza A(H7N9) was first identified in several individuals in Eastern China. To date, MERS-CoV has shown limited human-to-human transmission, while influenza A(H7N9) has not shown sustained human-to-human transmission. CDC’s case definitions are evolving and updated as more information is available on the viruses.

USAFSAM was one of the first DoD laboratories qualified to perform MERS-CoV testing using the CDC’s “Novel Coronavirus 2012 Real-Time RT-PCR Assay.” An Emergency Use Authorization (EUA) was issued by the FDA on 5 June 2013 enabling this assay to be used for MERS-CoV in vitro diagnostic testing. USAFSAM can test multiple specimen types (i.e., nasal wash, nasopharyngeal swabs, sputum, serum, stool, bronchoalveolar lavage) from DoD beneficiaries meeting the CDC’s case definition for a “Patient Under Investigation” or “Probable Case.” The assay can also be used for testing patients meeting AFHSC’s screening criteria. During the 2012-2013 season, USAFSAM investigated four Patients Under Investigation for MERS-CoV and all patients were determined to be negative. In response to the MERS-CoV novel pathogen, AFHSC/GEIS requested USAFSAM look for the presence of the novel virus among our archived respiratory specimens from calendar year 2012. To accomplish this, USAFSAM Force Health Protection Technology and Global Health Surveillance Division developed an in-house, research use only assay for detection of coronavirus and, specifically, MERS-CoV. The assay developed did not detect any MERS-CoV among specimens submitted during the 2012 calendar year.

The CDC also provided us with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) (Eurasian Lineage). This assay was issued under FDA’s EUA assay for detection of influenza A(H7N9). The EUA assay must be used in conjunction with the CDC’s Human Influenza RT-PCR Diagnostic Panel, an FDA-approved assay for in vitro
diagnostics, which tests for seasonal influenza B, influenza A(H3N2), influenza A(H1N1), and influenza A(H1N1)pdm09. Any specimen that is positive for influenza A by molecular testing and is unsubtypeable will be tested on the CDC influenza A(H7) (Eurasian Lineage) assay. Confirmatory testing must be performed at the CDC. USAFSAM hasn’t identified any influenza A(H7N9) cases to date.

Since our military is at increased risk, due to dispersion and mobility, of contracting novel pathogens, USAFSAM created guidance in the event of a suspected case of a novel respiratory pathogen, such as influenza A(H7N9) or MERS-CoV. In April 2013, general guidelines for suspect novel respiratory pathogen guidance were sent out through public health channels and to all sentinel sites on procedures to be followed should they encounter a patient with a suspect novel respiratory pathogen (Appendix N).

4.0 CONCLUSION

During the 2012-2013 season, the USAFSAM influenza surveillance program continued its global public health surveillance mission for influenza and respiratory viral diseases. Laboratory-based surveillance and disease investigation remain the cornerstones of proper public health preparedness and practice. The goal of the program is to reduce influenza and respiratory disease morbidity and mortality among our active duty and reserve forces, thereby protecting the mission, military personnel and their families, and civilian populations globally. Surveillance data and reports from this system are shared with military and civilian leadership to provide the basis for informed decisions. The USAFSAM program aims to reduce gaps in influenza surveillance, better respond to potential threats, better describe viral respiratory diseases, collect high quality specimens, and ensure their efficient transport and proper analysis with the best diagnostic capability available. In addition, the program will assess vaccine effectiveness annually and during early phases of a pandemic.

5.0 REFERENCES


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APPENDIX A

Health Affairs Policy Memo 99-008

THE ASSISTANT SECRETARY OF DEFENSE
WASHINGTON, DC 20301-1200

03 FEB 99

MEMORANDUM FOR:  
SURGEON GENERAL OF THE ARMY  
SURGEON GENERAL OF THE NAVY  
SURGEON GENERAL OF THE AIR FORCE  
DEPUTY DIRECTOR FOR MEDICAL READINESS, J-4, THE JOINT STAFF

SUBJECT: Policy for DoD Global, Laboratory-Based Influenza Surveillance

The Department of Defense policy shall be to conduct global, operationally relevant, laboratory-based influenza surveillance. Influenza surveillance is essential to preserve readiness, enhance force protection, and support national and international efforts to detect new influenza viruses and to prevent or control endemic and pandemic influenza.

Operation of DoD Influenza Surveillance

The Surgeon General of the Air Force will be the Executive Agent for DoD influenza surveillance and will program and budget for the tri-Service program.

- The Department of Defense Global Emerging Infections Surveillance and Response System (DoD-GEIS) Central Hub will provide professional guidance and direction and will issue periodic and special reports to the ASD(HA), the Surgeons General, and the Centers for Disease Control and Prevention (CDC).
- The Defense Medical Surveillance System will maintain the surveillance database.
- Each service will identify one virology laboratory with expertise in the isolation and identification of influenza viruses to support DoD influenza surveillance. A detailed, annual execution plan will define each laboratory’s support of DoD influenza surveillance.
- A DoD Joint Influenza Surveillance Working Group, including DoD-GEIS Central Hub representatives, will develop a detailed annual plan for conducting the surveillance effort, and will provide the plan and any recommendations to the ASD(HA) and the Surgeons General.
- Influenza surveillance will occur among selected military populations in the United States and stationed or deployed outside of the United States, and among other selected populations.
- The Working Group will review the operation of DoD influenza surveillance at least quarterly and will report to the Armed Forces Epidemiological Board at least annually.
The dynamic nature of the influenza viruses results in the frequent emergence of new strains that are beyond the scope of protection provided by existing vaccines and that may pose a pandemic threat. These viruses have a demonstrated capacity to cause great morbidity and mortality in our military forces and compromise operational readiness. The global dispersion and mobility of our forces and their families place them at risk for acquiring and quickly spreading an emergent virus. Mixing of people from diverse geographic areas, close living conditions and other factors place military training centers at high risk for influenza outbreaks. Additionally, our forces and facilities overseas sometimes are the only medical resources in a region that are capable of obtaining specimens and information on new influenza threats, which may be critical to the international surveillance effort.

The key to countering influenza is the early detection, identification, and characterization of emerging strains so that changes in the vaccine constituents may result in protective immunization. When timely vaccine modification is not possible, disease control still may be achieved through the use of antiviral drugs. Early and accurate assessment of the potential threat to military forces is critical to effective medical intervention and essential when military planners must develop contingencies to deal with large outbreaks when there are no medical interventions.

DoD influenza surveillance will: isolate and identify circulating influenza viruses; detect new virus variants or subtypes for possible vaccine modification; identify influenza outbreaks; and determine the incidence of influenza-like illness among sentinel military populations at high risk, such as basic training populations. Active duty populations will receive highest priority for surveillance. Among other DoD health care beneficiary populations, surveillance will be based upon priorities and available funds. Host nation populations will be studied in high priority geographical areas served by DoD’s overseas medical research activities. DoD influenza surveillance will be coordinated with and will complement the national and international influenza surveillance efforts directed by the CDC and the World Health Organization.

This policy is effective immediately and shall be included in Service and Joint Staff plans and policies for joint medical surveillance and force health protection.

Dr. Sue Bailey

HA Policy 99-008
APPENDIX B

Notable Past Contributions
(seed viruses, reference strains, viruses that impacted seasonal selections)

Seed Viruses and Reference Strains from DoD Surveillance

A/PR8/1945, an A strain isolated from a recruit in May, 1943, plus B/Lee (Army)

A/Texas/1/77(H3N2) (USAF)

A/Philippines/2/82(H3N2) (USAF)

A/Panama/2007/99 (H3N2) (USAF)

A/Wuhan/359/1995 (H3N2) (USAF)

A/South Dakota/06/2007(H1N1) (USAF Base, Army Case)

A/California/4/2005 (H3N2) (USN)

A/Texas/05/2009 (H1N1) (USAF)

A/Iraq/18529/2009 (H1N1) (USAF)

A/California/07/2009 (H1N1) (USN)
APPENDIX C
DoD Policy Memo for Influenza Season 2012-2013

THE ASSISTANT SECRETARY OF DEFENSE
1200 DEFENSE PENTAGON
WASHINGTON, DC 20301-1200

HEALTH AFFAIRS

AUG 13 2012

MEMORANDUM FOR ASSISTANT SECRETARY OF THE ARMY (MANPOWER & RESERVE AFFAIRS)
ASSISTANT SECRETARY OF THE NAVY (MANPOWER & RESERVE AFFAIRS)
ASSISTANT SECRETARY OF THE AIR FORCE (MANPOWER & RESERVE AFFAIRS)
DIRECTOR, HEALTH AND SAFETY, U.S. COAST GUARD
DIRECTOR OF THE JOINT CHIEFS OF STAFF
COMMANDER, JOINT TASK FORCE, NATIONAL CAPITAL REGION MEDICAL

SUBJECT: 2012-2013 Influenza Surveillance Program Sentinel Sites

The Armed Forces Health Surveillance Center, Division of Global Emerging Infections Surveillance and Response Systems (AFHSC/GEIS) Operations provides central coordination for the Department of Defense (DoD) influenza surveillance program. With AFHSC/GEIS support and coordination, the U.S. Air Force School of Aerospace Medicine (USAFSAM) performs global, laboratory-based influenza surveillance through a sentinel system. Surveillance is coordinated with the Services, the Centers for Disease Control and Prevention, and the World Health Organization influenza surveillance programs.

The DoD Global, Laboratory-Based, Influenza Surveillance Program at USAFSAM selects military installations from world-wide locations to participate as sentinel sites. The AFHSC/GEIS Respiratory Pathogens Surveillance Steering Committee (RPSSC) reviews the sentinel site list annually. These installations are selected based on criteria such as mission, population, deployment/operations tempo, and location. The 2012-2013 Sentinel Site listing (Attachment 1) was approved on June 21, 2012.

Sentinel sites are expected to participate starting October 1, 2012, for a period of 1 year. USAFSAM requests sentinel sites submit 6 to 10 respiratory specimens per week obtained from patients meeting the influenza-like-illness (ILI) case definition. The ILI case definition and key points about the program are included in Attachment 2.

The Military Departments are expected to notify each of the military Medical Treatment Facilities listed in Attachment 1 of this requirement. USAFSAM will cover the shipping costs for sentinel samples and will contact sentinel sites to provide necessary program management information and laboratory supplies, such as nasal wash kits for collecting specimens.
All sentinel sites, with some exceptions discussed below, will submit respiratory specimens directly to USAFSAM. U.S. Central Command (USCENTCOM) and USAFSAM will coordinate directly concerning the shipment of specimens from the USCENTCOM area of responsibility to USAFSAM.

All sentinel sites within the U.S. European Command will submit respiratory specimens to the Landstuhl Army Medical Center (LRMC) laboratory in Landstuhl, Germany, for testing. LRMC will coordinate the shipment of specimens and data exchange with USAFSAM. USAFSAM will conduct laboratory testing as needed. Sentinel sites should monitor the USAFSAM Web site (Attachment 2) periodically to verify that specimens and surveillance data sent are reflected in their site-specific surveillance data. If problems are identified, notify the points of contact at LRMC and USAFSAM.

Sentinel sites within the state of Hawaii may submit respiratory specimens to Tripler Army Medical Center (TAMC). TAMC will coordinate the shipment of specimens with USAFSAM. USAFSAM will conduct laboratory testing as needed.

Camp Lemonier may submit samples through the existing surveillance programs run through the Naval Medical Research Unit No. 3.

All other installations not mentioned in the attachment may submit respiratory specimens to USAFSAM and will be considered as participating sites.

Questions regarding the AFHSC/GEIS influenza surveillance program are best directed to CDR Michael Cooper, who may be reached at Michael.Cooper38@us.army.mil. The point of contact for USAFSAM Influenza Surveillance is Lt Col Victor MacIntosh, who may be reached at Victor.Macintosh@us.af.mil. For regular communications with USAFSAM, send e-mails to influenza@wpafb.af.mil. Direct laboratory-specific questions to usafsam.phcussv@wpafb.af.mil. The point of contact for this guidance is COL Scott Stanek, who may be reached at (703) 681-8457 or at Scott.Stanek@tma.osd.mil.

Jonathan Woodson, M.D.

Attachments:
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**Includes Camp Arifjan and Camp Virginia**
DoD-Global Laboratory-Based Influenza Surveillance System: 2012-2013
Program Guidance Overview

- DoD-wide and global program; active surveillance at sentinel sites and overseas DoD research laboratories; partnered with the Centers for Disease Control and Prevention, and the World Health Organization
- Surveillance is year-round due to the mobility of our population
- In-depth guidance and information is provided annually to Public Health/Preventive Medicine offices/departments, laboratory personnel, and physician/clinic staff
- Nasal wash collection kits are provided initially and upon request
  Kits include:
  - Sterile saline
  - Collection cup and bib
  - Viral Transport Media (VTM)
  - Biohazard bag
  - Surveillance Questionnaire
- Nasopharyngeal swabs may also be used as a secondary option to nasal washes
- USAFSAM also:
  - Provides shipping containers
  - Pays for shipment to USAFSAM via commercial carrier
- Target: 6-10 specimens per week per sentinel site
  - Surveillance Questionnaire for the current season version is provided with each collection kit and is also available at https://gumbo2.wpaeb.af.mil/epi-consult/influenza/
  - Hard copy questionnaire must accompany each patient specimen
- Specimens are processed at the USAFSAM laboratory
- Results are reported in CHCS/AHLTA; a summary of results is provided in weekly surveillance reports which are available on the USAFSAM website at: https://gumbo2.wpaeb.af.mil/epi-consult/influenza/ and sent by email upon request

Case definition for influenza-like illness (ILI)
- Fever ≥ 100.5°F & cough or sore throat
- Specimens should be collected within 3 days of onset of symptoms
- If fewer than six patients are seen meeting the ILI case definition in a given week, sites may send fewer than six samples
- If there is an increase in ILI patients, sites are to continue submitting up to 10 specimens per week, giving priority to the sickest or hospitalized patients
APPENDIX D

Map of Sentinel Site Locations 2012-2013
APPENDIX E

Surveillance Questionnaire & Instructions for Specimen Collection/Submission

(Form page)

DOD GLOBAL INFLUENZA SURVEILLANCE QUESTIONNAIRE, 2012-2013
US AIR FORCE SCHOOL OF AEROSPACE MEDICINE (USAFA), WRIGHT-PATTERSON AFB, OH

Influenza-like Illness (ILI) Case Definition: Fever ≥ 100.5°F (38°C), oral or equivalent AND cough and/or sore throat (<72 hours duration)
- Submit 6-10 specimens/week for different patients who meet the ILI case definition
- Medical personnel - please complete the questionnaire in full for each specimen submitted
- Specimens accepted year round for influenza/respiratory surveillance

*PRIVACY ACT STATEMENT: The social security number is required to facilitate documentation of health care received and patient follow-up. The primary use of this information is to aid in preventing and communicable disease control programs. The requested information is voluntary.

PATIENT INFORMATION — PLEASE PRINT LEGIBLY

Patient name: ___________________________ Date of clinic visit (dd/mm/yyyy): ________/_____/_______

*Patient FM/FP/Spouse SSN: ______/_____/_______ Date of birth (dd/mm/yyyy): ______/_____/_______

Height: ______ inches ______ cm Weight: ______ lbs ______ kg Gender: Male □ Female □

Installation: ___________________________ Permanent duty station (if different)

Please indicate if patient belongs to one or more of the following groups by checking the appropriate box:

Trainee □ Academy cadet □ Healthcare worker □ Daycare attendee □ Prisoner □ Pregnant □ ROTC □ Flight crew □ Daycare worker □ Other:

VACCINE INFORMATION (2012 - 2013):

Has patient received the 2012-2013 seasonal influenza vaccine? □ Yes □ No □ Unk

If YES, check type: □ Injection (flu shot) □ Nasal spray (Flumist®)

□ Intramuscular (All DoD clinics provide this type) □ Intradermal

If YES, list date (dd/mm/yyyy): ______/_____/_______ OR Estimated date: Month: ______ & □ 1st half □ 2nd half

SIGNS AND SYMPTOMS

Date symptoms started (dd/mm/yyyy): ______/_____/_______ Temperature taken at clinic: □ °F □ °C

Highest recorded temperature at home (if known): ______/_____/_______ Date temperature taken at home (dd/mm/yyyy): ______/_____/_______

Did patient take fever-reducing meds (acetaminophen/buprofen) within 6 hours prior to temperature taken at the clinic? □ Yes □ No □ Unk

SYMPTOMS — Please check Unk (Unknown) if the presence of symptoms cannot be determined

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OTHER CLINICAL INFORMATION

At this visit, were antivirals prescribed? □ Yes □ No □ Unk

If YES, check antiviral below:

□ Tamiflu (oseltamivir) □ Relenza (zanamivir) □ Symmetrel (amantadine) □ Flumadine (rimantadine) □ Other: ___________________________

Did patient have pneumonia? □ Yes □ No □ Unk

Was patient hospitalized (admitted into inpatient ward of hospital) for ILI symptoms? □ Yes □ No □ Unk

IF NO, complete “Outpatient Visit”. IF YES, complete “Inpatient Visit” below. IF UNKNOWN, complete what information you can.

Outpatient Visit

Was patient seen at the emergency room or a clinic? □ ER □ Clinic

Was patient placed on quarantine? □ Yes □ No □ Unk

If YES, how many hours? ______ hrs

Inpatient Visit

Was patient hospitalized in a civilian or military facility? □ Civ □ MIL

Admission date (dd/mm/yyyy): ______/_____/_______

If applicable, Discharge date (dd/mm/yyyy): ______/_____/_______

CONTACT AND TRAVEL HISTORY INFORMATION

Has patient been in close contact with anyone who was recently ill with ILI symptoms? □ Yes □ No □ Unk

Did patient travel in the past 14 days? □ Yes □ No □ Unk

If YES, where did they travel to/from?

Travel Return Date (dd/mm/yyyy): ______/_____/_______

Questionnaire Submission:

1. Questionnaires can be completed by hand or by computer and printed.
2. Submit a hard copy of the questionnaire with each specimen. See shipping address on reverse side.

Note — Additional questionnaires can be downloaded at https://gumo22.wpafh.af.mil/eip-consult/influenza

Questions? Please email: influenza@wpafh.af.mil

Distribution A: Approved for public release; distribution is unlimited. Case Number: 88ABW-2014-1676, 14 Apr 2014

Revised 2012
To effectively reduce the risk of transmission, use PPE (disposable gloves and surgical mask) while collecting a respiratory specimen. Wash hands before and after specimen collection.

### Nasal Wash Procedural Guidelines (Preferred Method of Collection)

**Instructions:**

1. Have patients blow their nose into a tissue to clear excess mucus.
2. Tuck bib into patients’ shirt collar.
3. Uncap prefilled saline syringe and specimen collection container. Decap, seal on the syringe by gently expressing a small amount of saline into the tip of the tube.
4. Have patients tilt their head back so they are able to look directly at the ceiling while they hold the specimen collection container up to their child’s nose.
5. Encourage patients to not swallow saline by saying “Ka Ka Ka” or making a constant “choking sound” while saline is expressed into their nostrils.
6. Gently express 2-4 mL of sterile saline into right nostril of patient. Saline will drain back into the back of the nasopharynx.
7. After a few seconds, have patients lean their head forward for enough forward so the saline will drain into the specimen collection container. Repeat for second nostril.
8. Offer patients a facial tissue or have them use the bib to wipe away excess saling from their face.

### Nasopharyngeal Swab Collection

Nasopharyngeal swabs can be used to collect an appropriate specimen for influenza testing. Specimens must be immediately placed in 1-3 mL of VTM. Use a flexible fine-ribbed aluminum swab with a polyester (Dacron or rayon, not cotton or calcium alginate) tip. *Note - Specimens in Universal Transport Medium (UTM) will not be tested and are unacceptable to our lab.*

**Instructions:**

1. Have patients blow their nose into a tissue to clear excess mucus.
2. Have patients close their eyes to help them cope with the slight discomfort they are about to experience.
3. With the patient’s head at a 30° angle, insert swab into nostril (straight back, not upwards) until resistance is met by contact with the nasopharynx. The distance from the patient’s nose to ear gives an estimate of the distance the swab should be inserted.
4. Rotate the swab several times (5-6 times) across the mucosal surface to loosen and collect cellular material.
5. Although a contact time of 30 seconds is suggested, a few seconds of contact often induces coughing or patient resistance, either of which is adequate incentive to remove the swab.
6. Withdraw swab and insert into the tube of VTM. Break off the portion of the stem that extends past the opening of the tube, and cap securely.
7. Place specimens in the biohazard bag included in the kit and forward to laboratory for packaging and shipment to USAFSAM. To maintain optimal quality for diagnostics, please be sure to ready the specimen for immediate shipment.

**Video Demonstration:**

Please see the video demonstration for a nasal wash and nasopharyngeal specimen collection at: [https://gumbl.gwafab.af.mil/epl-consult/influenza](https://gumbl.gwafab.af.mil/epl-consult/influenza)

**Storage, Packing, and Shipping**

**Best:** It is best to freeze the specimens at -70°C and ship on dry ice. Please contact USAFSAM if dry ice is not available at your site.

**Note - Specimens frozen at -20°C are not acceptable due to loss of viability of the viruses.**

**Acceptable:** If received at the USAFSAM lab within 48 hours from collection time, a specimen may be shipped on frozen gel packs at refrigerated (2-8°C) temperature. 

**UTM is not acceptable to our lab and will not be tested.**

Viral transport supplies may be ordered by emailing our Customer Service department at usafsam.phe.cit@wpafab.af.mil or by calling 937-938-4140 (DSN: 708-4140).

Please ship to: Fedex number: 425177729 (for respiratory viral panel testing ONLY)

**USAFSAM/PHE Epidemiology Laboratory Service**

2510 Fifth Street, Bldg 20840, Area B, WPAFB, OH 45433-7951

**For additional packing and shipping details (supply order form location), please see the USAFSAM/PHE Lab website (under Shipping/Training):**

[https://kx.atms.mil/Fcpi](https://kx.atms.mil/Fcpi) (CAC required site)
APPENDIX F

Public Health Quick Sheet

DOD GLOBAL INFLUENZA SURVEILLANCE, 2012-2013
U.S. AIR FORCE SCHOOL OF AEROSPACE MEDICINE (USAFSAM)
WRIGHT-PATTERSON AIR FORCE BASE, OH

QUICK SHEET

Public Health/Preventive Medicine
for Influenza Surveillance Program Sentinel Sites

• Ensure all clinic teams are supplied with the 2012-2013 Influenza Surveillance Questionnaire
  Questionnaire available on our website at: https://gumbo2.wpafb.af.mil/epi-consult/influenza

• Ensure clinic teams are supplied with collection kits
  To request collection kits, use the current version of the “Supply Order Form” available (under Shipping/Training) at: https://kx.afms.mil/epi

• Submit 6-10 specimens meeting the ILI case definition each week from your MTF
  ILI Case Definition: Fever ≥ 100.5 °F (38 °C) oral or equivalent AND cough and/or sore throat <72 hours duration

• Verify all specimens submitted for testing have completed questionnaires and are shipped along with the specimens
  Questionnaires are to be completed by clinic staff at the time of specimen collection

• Enter appropriate lab-confirmed influenza case-patient information into your service-specific Reportable Medical Events System (i.e., AFRESS, NDRSi) according to service-specific requirements

• Staff training is recommended at least two times each year: at the start of the influenza season and again at the mid-point
  Please convey the importance of influenza surveillance.

• If you suspect an influenza outbreak or are seeing hospitalized cases due to influenza at your installation, notify us
  Tracking outbreaks and hospitalized cases is important in mitigating the impact of influenza on the military community

We are happy to provide support and assistance.
Contact us at: (937)938-3207; DSN 798-3207
E-mail: influenza@wpafb.af.mil
Website: https://gumbo2.wpafb.af.mil/epi-consult/influenza
DOD GLOBAL INFLUENZA SURVEILLANCE, 2012-2013
US AIR FORCE SCHOOL OF AEROSPACE MEDICINE (USAFSAM)
WRIGHT-PATTERSON AIR FORCE BASE, OH

APPENDIX G

Laboratory Quick Sheet

We are happy to provide support and assistance.
Contact us at: (937)938-3196; DSN 798-3196
E-mail: influenza@wpafb.af.mil
Website: https://gumbo2.wpafb.af.mil/epi-consult/influenza
APPENDIX H

Clinician Quick Sheet

DOD GLOBAL INFLUENZA SURVEILLANCE, 2012-2013
U.S. AIR FORCE SCHOOL OF AEROSPACE MEDICINE (USAFSAM)
WRIGHT-PATTERSON AIR FORCE BASE, OH

QUICK SHEET

Physician or Provider

for Influenza Surveillance Program Sentinel Sites

- Order the “RESPIRATORY CULTURE PNL (EPI)” in AHLTA/CHCS, where available
- Submit 6-10 specimens meeting the ILI case definition each week from your MTF
  - **ILI Case Definition:** Fever ≥ 100.5 °F (38 °C) oral or equivalent AND cough and/or sore throat <72 hours duration
- Ensure specimens are collected within 3 days of onset of symptoms
- If your MTF sees <6 patients with ILI symptoms in a given week, you may send fewer than 6 samples
  - Please do not send samples from asymptomatic patients
- If your MTF sees >10 patients with ILI symptoms in a given week
  - Submit no more than 10 specimens per week
  - Give priority to patients most ill and/or hospitalized with ILI
- If you see a patient with severe ILI (i.e., hospitalized), collect the specimen and notify your Public Health Officer
- Please do not send more than one specimen per patient within 14 days of a previous specimen unless clinically indicated
- Coordinate with your laboratory and public health staff about specimen handling
- Please collect specimens year-round due to seasonal variations among locations and the mobility of military populations
- We recommend the paper copy of the questionnaire be completed by clinic staff at time of specimen collection
- Coordinate with public health to ensure that questionnaires are completed appropriately and submitted with each specimen
- Coordinate with laboratory to ensure that specimens are properly collected and shipped to USAFSAM lab

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## Ongoing Collaborations

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<th>Collaboration</th>
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<td>Armed Forces Health Surveillance Center/Global Emerging Infections Surveillance and Response System (AFHSC/GEIS)</td>
<td>Funding organization &amp; Vaccine effectiveness</td>
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<tr>
<td>Armed Forces Research Institute of Medical Sciences (AFRIMS)</td>
<td>Partner lab: Provide USAFSAM isolates for sequencing</td>
</tr>
<tr>
<td>Association of Public Health Laboratories - Public Health Laboratory Interoperability Project (APHL-PHLIP)</td>
<td>USAFSAM respiratory testing results securely shared with CDC</td>
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<tr>
<td>Centers for Disease Control and Protection (CDC)</td>
<td>Data &amp; specimen sharing</td>
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<tr>
<td>Landstuhl Regional Medical Center (LRMC)</td>
<td>Partner lab: Provide USAFSAM isolates for sequencing</td>
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<tr>
<td>Lovelace Respiratory Research Institute (LRRI)</td>
<td>Provide adenovirus specimens to LRRI</td>
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<tr>
<td>National Center for Biotechnology Information (NCBI)</td>
<td>USAFSAM submits sequences to GENBANK</td>
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<tr>
<td>National Institute of Allergy and Infectious Diseases (NIAID)</td>
<td>Provide data for Influenza Research Database (IRD)</td>
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<tr>
<td>Naval Health Research Center (NHRC)</td>
<td>Partner lab: Provide USAFSAM isolates for sequencing</td>
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<tr>
<td>Naval Medical Research Unit (NAMRU-2, 3, &amp; 6)</td>
<td>Partner lab &amp; sentinel site: Provide USAFSAM isolates for sequencing</td>
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<tr>
<td>Navy and Marine Corps Preventive Medicine Center (NMCPMC)</td>
<td>Provide USAFSAM with inpatient influenza data</td>
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<td>Navy Environmental and Preventive Medicine Unit (NEPMU-2)</td>
<td>Provide Navy sentinel site assistance</td>
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<tr>
<td>Northrop Grumman</td>
<td>Provide data for Influenza Research Database (IRD)</td>
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<td>Tripler Army Medical Center (TAMC)</td>
<td>Partner lab &amp; sentinel Site: Provide USAFSAM isolates for sequencing</td>
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<tr>
<td>U.S. Army Medical Research Unit - Kenya (USAMRU-K)</td>
<td>Partner lab: Provide USAFSAM isolates for sequencing</td>
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<tr>
<td>U.S. Coast Guard (USCG) Commandant</td>
<td>Coordinate influenza program with non-DoD USCG sites</td>
</tr>
<tr>
<td>Walter Reed Army Institute of Research (WRAIR)</td>
<td>Provide specimens for full genome sequencing</td>
</tr>
<tr>
<td>World Health Organization (WHO)</td>
<td>Data &amp; specimen sharing through CDC</td>
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APPENDIX J

Instructions for Specimen Submission

DoD Global, Laboratory-based, Influenza Surveillance Program
Summary of Specimen Collection, Storage, and Shipping Guidelines

United States Air Force School of Aerospace Medicine (USAFSAM)
Department of Public Health & Preventive Medicine

PURPOSE

The purpose of this document is to highlight guidance for physicians, medical staff, and Public Health on collection, storage, and shipping of respiratory specimens related to the DoD Global, Laboratory-based, Influenza Surveillance Program. It is not intended to replace the Wright-Patterson Epidemiology Laboratory Guide. For the most up to date laboratory guidance visit: https://kx.afms.mil/epi

SPECIMEN TYPES

USAFSAM prefers to receive nasal wash specimens. However, nasopharyngeal swabs can be accepted and tested if received in viral transport medium (VTM).

Nasal Wash Collection

1. Have patient blow their nose into a tissue to clear excess mucus.
2. Tuck bib into patients’ shirt collar.
3. Uncap pre-filled saline syringe and specimen collection container. Break the seal on the syringe by gently expressing a small amount of saline into the tip of the hub.
4. Have patient tilt their head back so they are able to look directly at the ceiling while they hold the specimen collection container up to their chin area.
5. Encourage patient not to swallow saline by saying “Ka Ka Ka” or by making a constant “choking sound” while saline is expressed into their nostrils.
6. Gently express 2-4 mL of sterile saline into right nostril of patient. Saline will drain back into the back of the nasopharynx.
7. After a few seconds, have patient lean their head far enough forward so the saline will drain into the specimen collection container. Repeat for second nostril.
8. Offer patient a facial tissue or have them use the bib to wipe away excess saline from their face.
9. Transfer the contents to the M4RT viral transport medium (VTM) vial. Squeezing the rim of the cup will help in pouring the contents into the VTM tube. Freeze at -70°C or colder.
10. Package each specimen individually in the biohazard bag included in the collection kit and transport specimen immediately to the laboratory’s shipping department (See “Shipping” section below for more information).
11. Place specimen in a secondary receptacle with the surveillance questionnaire.

SHIPPING
When shipping specimens, it is essential that each specimen be packaged and shipped properly. To control or eliminate health and financial liabilities (criminal and civil) it is essential MTFs adhere to the regulations set forth by the U.S. Department of Transportation (DOT) and the International Air Transport Association IATA). These are recommendations only. Each laboratory is responsible for implementing the procedures that comply with Federal Regulations. IATA requires that the person who packs/ships materials be a trained person. The following websites, as well as others not listed, can provide information on training.
- SAFTPACK, Inc.: www.saftpak.com

Each packer/shipper must have a training record on file. Certification is valid for 2 years. Each person must be re-trained and re-certified at the end of 2 years period. USAFSAM must maintain a copy of current IATA training for OCONUS personnel who pack specimen shipments. A yearly audit is performed to ensure compliance.

USAFSAM provides IATA training disks to OCONUS sites. Once training is completed, print your certificate and scan to usafsam.phe.cst@wpafb.af.mil. Once your certificate is received, you will be sent the laboratory import permit that is required with each shipment.

SHIPMENT TEMPERATURE AND PACKAGING
If shipments are received out of temperature the specimens will be rejected.

Best: Frozen Box
Specimens frozen immediately at -70°C and shipped to USAFSAM on dry ice is the preferred method. These specimens should be shipped in the following manner:
1. Place individually packed biohazard bags of specimens in box.
2. Place a barrier device (i.e., chuck) between the specimens and the dry ice.
3. Add pellets or block dry ice making sure to fill any “dead” space with packing material, such as newspaper. Do not leave dead air space which leads to faster evaporation of the dry ice.
4. Ensure enough block dry ice is added to keep the specimens frozen for any unexpected delay.
5. Each standard shipping box should contain a minimum of:
   a. 5 lbs of dry ice for CONUS
   b. 15lbs of dry ice for OCONUS
6. Do not use flaked dry ice as it evaporates much faster.

Acceptable: Refrigerated Box
A specimen may be shipped on frozen gel packs at refrigerated (2-8°C) temperature, only if received at the USAFSAM lab within 48 hours of collection from patient. Specimens received over 8°C or over 48 hours from collection cannot be accepted.
1. Place individually packed biohazard bags of specimens in box.
2. Add enough coolant packs to keep specimens cool until arrival. A minimum of six are recommended. Be sure to place gel packs under and on top of specimen.
3. Fill any “dead” space with packing material, such as newspaper.
ADDITIONAL INFORMATION
- Specimens frozen at -20°C are not acceptable. This results in rapid loss of virus which will yield false negative results.
- Please contact USAFSAM if dry ice is not available.
- Viral transport medium (VTM) tube should have at least two identifiers on the label, i.e. SSN/FMP, DOB, or Name.
- Universal transport medium (UTM) is not accepted at USAFSAM and will not be tested.
- Package each specimen individually in biohazard bags.

PREPARING BOX FOR SHIPMENT
1. Place paperwork in box pertaining to that shipment ensuring that only the paperwork for that particular individual box is included.
2. Seal the box and properly identify box with labels, according to IATA/DOT shipping guidelines.
3. Do not ship boxes on a day that will have the shipment arrive on a holiday.
4. Affix courier waybill to the outside of the box, using the following address:
   Epidemiology Laboratory Service
   USAFSAM/PHE
   Bldg 20840
   2510 Fifth Street
   Wright Patterson AFB, OH 45433-7951

FEDERAL EXPRESS (FEDEX) SHIPPING
1. Use the FedEx number 425177729.
2. Ship specimens “Priority Overnight” to arrive Tuesday through Friday. FedEx does not make Sunday deliveries.
3. Specimens sent for Saturday arrival require special handling.
   a. Notify the USAFSAM/PHE Customer Service Team at 937-938-4140 (DSN: 798-4140) that you are sending a Saturday shipment.
   b. Mark the shipment for both “Priority Overnight” AND “Saturday Delivery.” Both boxes must be checked or your package will not be delivered until Monday. Saturday shipments not delivered to the laboratory until Monday are usually rejected because they are out of temp.
   c. Use the FedEx online management tool, “InSight.” FedEx Insight allows you to monitor the status of your shipment in real time. Also, InSight automatically alerts you of critical shipping events.
4. Check the box for dry ice and included the estimated weight of the dry ice. We recommend that you use a minimum of 5 lbs of dry ice for CONUS shipments and 7 lbs for OCONUS.
5. When you print the shipment paperwork out, you will see the dry ice amount listed in the upper right-hand corner of the waybill. The amount on that must be written on the dry ice sticker.
6. If there is not already a pickup scheduled, you can create that under Pickup/Drop Off on the FedEx website.
7. Print 3 copies for the driver.
8. For OCONUS shipments please email the FedEx tracking number to usafsam.phe.cst@wpafb.af.mil.
9. Track your shipments. Tracking your own shipments is critical in preventing shipping problems. When you program your shipment you can select “email notifications” to alert you to delivery status or delays.

CONTACT INFORMATION
For general information about surveillance program guidelines, ordering collection kits, IATA training, or submission of specimens please call: (937) 938-3196, DSN 798-3196 or email influenza@wpafb.af.mil.

If you have laboratory or shipping questions, request your laboratory staff call USAFSAM/PHE customer service at: (937) 938-4140, DSN 798-4140 or email usafsam.phe.cst@wpafb.af.mil. The USAFSAM Epidemiology Laboratory Service Guide can be obtained here: https://kx.afms.mil/epi or https://gumbo2.wpafb.af.mil/epi-lab/labguide/.
APPENDIX L

Molecular Laboratory Section Methods
(from detection through sequencing and analysis)

Molecular Sequencing Process - Culture¹

Possible Inputs

NP Swab or Wash Received (No Isolate)

Culture Isolate Received²

Possible Outputs

Original (if available) and Flu Isolate Archived

Original & any Virus Isolated Archived

NRV or virus
Final CHCS culture result

Not Performed
Final CHCS culture result

Flu A or Flu B
Final CHCS culture result

Pos Flu (A or B)

Viral Culture

NRV or Virus/not Flu³

CHCS Results

Molecular

Molecular

Molecular

1. Any unusual circumstances not covered by this flow sheet should be referred to Dr. Macias
2. With or without original specimen
3. NRV = No respiratory virus isolated. Alternately, a non-flu virus could be detected and entered as such under VSQ/culture result.

Distribution A: Approved for public release; distribution is unlimited. Case Number: 88ABW-2014-1676, 14 Apr 2014
Molecular Sequencing Process - Molecular¹

Possible Inputs

- Culture Isolate Received²
- NP Swab or Wash Received (No Isolate)
- Negative NP Swab or Wash Received

Possible Outputs

- Flu Sub-type Provided
- Flu Sub-type Provided
- Sub-type (rRT-PCR)⁴
- Sub-type (rRt-PCR)⁴

Molecular Sequencing (100% resources permitting)

Ordered by virology as VCH ⁵

1. Any unusual circumstances not covered by this flow sheet should be referred to Dr. Macias
2. Have not received any to date that were not also accompanied by NP swab or wash
3. Unlikely – Notify Dr. Macias
4. Add as ATA to VSQ. If unable to sub-type refer to Dr. Macias
5. Culture added to VSQ to address other viruses (i.e. RSV, Adeno). Also used to address sub-type conflicts between us and submitting source.
NW or NP Swab in VTM

**Ship to Lab on Dry Ice**
- Aliquot for Archive
- Culture

**Ship to Lab on Wet Ice**
- Aliquot for Archive
- Culture

**Molecular Lab**
- Aliquot of original
- All influenza +'s from virology

**NW or NP Swab in VTM**
- Ship to Lab on Dry Ice
- Ship to Lab on Wet Ice

**Molecular Lab**
- Aliquot for Molecular

**PMK SV**
- Examine tubes until day 10
- NO
- Prepare slide for individual viruses
- NO
- Report: No Respiratory Virus Isolated
- NO
- ID by FA
- -Report Result
- -Archive Virus

**PMK Tube**
- Examine tubes until day 10
- YES
- CPE
- NO
- PMK SV
- YES
- FA
- NO
- Follow until day 10
- YES
- Prepare slide for individual viruses
- NO
- Report: No Respiratory Virus Isolated
- NO
- ID by FA
- -Report Result
- -Archive Virus

**Resp. Viruses Routinely Isolated**
- Influenza A
- Influenza B
- Adenovirus
- Parainfluenza 1,2,3
- RSV
- Enteroviruses
- HSV

**NW:** Nasal Wash
**NP:** Nasopharyngeal swab
**VTM:** Viral Transport Media (M4RT in this lab)
**PMK:** Primary Rhesus Kidney cells
**SV:** Shell Vial
**CPE:** Cytopathic Effect
**FA:** Fluorescent Antibody Stain
**HAd:** Hemadsorption
Guidance for Suspected Cases of Novel Respiratory Pathogens

In the event that medical or public health personnel have identified a suspected case of a novel respiratory pathogen, such as novel coronavirus or influenza A (H7N9), we request that you contact the USAFSAM Epidemiology Consult Service immediately. We can help determine if a specimen submission is necessary, and if so, ensure all logistical details are in place to receive and test the specimen as rapidly as possible.

Epidemiology Consult Service Influenza Surveillance
For general questions email or call:
influenza@wpafb.af.mil; Comm: 937-938-3196 DSN: 798-3196

For Preventive Medicine Consultation:
Maj Shauna Zorich, MD, MPH, Influenza Program Lead
Shauna.Zorich@wpafb.af.mil; Comm: 937-938-3206 DSN: 798-3206

Epidemiology Laboratory Support
usafsam.phecuss.v@wpafb.af.mil; Comm: 937-938-4140 DSN: 798-4140

The CDC and WHO are good resources for case definition guidance and additional updates:
Influenza A (H7N9): http://www.cdc.gov/flu/avianflu/h7n9-case-definitions.htm

Steps for Specimen Submission

Collect a respiratory specimen (with a nasal wash collection kit) from the suspect patient and submit to the USAFSAM Epidemiology Laboratory for respiratory virus testing.

Influenza sentinel sites: To request collection kits from USAFSAM, use the current version of the “Supply Order Form” available (under Shipping/Training) at: https://kx.afms.mil/epi.
Other Non-sentinel sites: Please contact Josh Cockerham at Josh.Cockerham.ctr@wpafb.af.mil or by phone at Comm: 937-938-3196 DSN: 798-3196 if you are in need of supplies.

Complete the Influenza Surveillance Questionnaire (2012-2013) and ship with specimen.
Questionnaires can be found on the USAFSAM Influenza website (see below for link) under Program Guidance or ‘Click Here’. They are to be completed by clinic staff at the time of specimen collection.

USAFSAM will conduct both viral culture and molecular testing (RT-PCR) on the specimen and update CHCS with the results within 24-48 hours of receiving the specimen.
Specimens that test positive for Influenza A, but cannot be subtyped will be referred immediately to the CDC. Specimen from a suspect case of any other novel respiratory virus will be referred to the CDC if routine testing protocols do not yield a definitive result.

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<th>Description</th>
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<td>AF</td>
<td>Air Force</td>
</tr>
<tr>
<td>AFB</td>
<td>Air Force Base</td>
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<tr>
<td>AFHSC</td>
<td>Armed Forces Health Surveillance Center</td>
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<tr>
<td>AHLTA</td>
<td>Armed Forces Health Longitudinal Technology Application</td>
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<tr>
<td>ASD(HA)</td>
<td>Assistant Secretary of Defense for Health Affairs</td>
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<tr>
<td>BMT</td>
<td>Basic Military Training</td>
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<td>BRAC</td>
<td>Base Closure and Realignment Commission</td>
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<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<td>CHCS</td>
<td>Composite Health Care System</td>
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<td>CONUS</td>
<td>Contiguous United States</td>
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<td>DoD</td>
<td>Department of Defense</td>
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<td>EID</td>
<td>emerging infectious disease</td>
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<td>ESSENCE</td>
<td>Electronic Surveillance System for the Early Notification of Community-Based Epidemics</td>
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<td>Global Emerging Infections Surveillance and Response System</td>
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<td>International Air Transport Association</td>
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<td>ICD-9</td>
<td>International Classification of Diseases-Ninth Revision</td>
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<td>ILI</td>
<td>influenza-like illness</td>
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<td>JISWG</td>
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<td>LAIV</td>
<td>live attenuated influenza vaccine</td>
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<td>Abbreviation</td>
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<td>LRMC</td>
<td>Landstuhl Regional Medical Center</td>
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<td>NAMRU</td>
<td>Naval Medical Research Unit</td>
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<td>MTF</td>
<td>military treatment facility</td>
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<td>MERS-CoV</td>
<td>Middle East Respiratory Syndrome Coronavirus</td>
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<td>OCONUS</td>
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<td>OPORD</td>
<td>operational order</td>
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<td>OR</td>
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<td>RT-PCR</td>
<td>reverse-transcriptase polymerase chain reaction</td>
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<td>trivalent influenza vaccine</td>
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<td>USAFSAM</td>
<td>U.S. Air Force School of Aerospace Medicine</td>
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<td>VRBPAC</td>
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<td>VTM</td>
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<td>Wright-Patterson Air Force Base</td>
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