This document is a compilation of Centers for Disease Control and Prevention (CDC) and Ohio Department of Health (ODH) recommendations and procedures to aid in the collection of influenza diagnostic specimens. Under the guidance of Director Alvin Jackson, ODH staff compiled this document as information for Ohio colleagues including local public health, hospitals and practitioners. Please consult the CDC web site as the guideline recommendations may change: [http://www.cdc.gov/swineflu/](http://www.cdc.gov/swineflu/).

Individuals with mild illness should stay at home and call their physician if they have questions about care or treatment. CDC is not encouraging people with mild illness to visit their doctor or other healthcare facilities to avoid clogging the healthcare system. Severe symptoms which would indicate a need to go to a healthcare facility are listed on the CDC web site, but include high fever, difficulty breathing, discoloration of the lips, and unresponsiveness.

Patients who meet these criteria should be tested for influenza and clinical specimens positive for influenza should be sent to public health laboratories for further characterization. Clinicians who suspect swine influenza virus infections in humans should obtain a nasopharyngeal swab from the patient, place the swab in a viral transport medium, refrigerate the specimen, and then contact their local health jurisdiction to facilitate transport to the Ohio Department of Health Laboratory for timely diagnosis. For the current swine influenza A (H1N1) virus investigation, testing should focus on acute illness. Thus, although serology is a methodology that can be used for diagnosis, it is not recommended as a routine primary diagnostic tool at this time.

For the current swine influenza A (H1N1) virus infections, to distinguish these influenza sample submissions from seasonal influenza sample submissions, please note “swine influenza” as a tentative diagnosis on the ODH Laboratory Virus Isolation specimen submission form.
Interim Guidance on Case Definitions to be Used for Investigations of Swine Influenza A (H1N1)  
April 26, 2009 08:30 EDT

This document provides interim guidance for state and local health departments conducting investigations of human cases of swine influenza A (H1N1) virus. The following case definitions are for the purpose of investigations of suspected, probable, and confirmed cases of swine influenza A (H1N1) virus infection.

Definitions of Respiratory Illness

1. Acute respiratory illness  
   Recent onset of at least two of the following:  
   1. rhinorrhea or nasal congestion  
   2. sore throat  
   3. cough  
   4. fever or feverishness  
2. Influenza-like illness: fever >37.8°C (100°F) plus cough or sore throat

Case Definitions for Infection with Swine Influenza A (H1N1) Virus

1. A Confirmed case of swine influenza A (H1N1) virus infection is defined as a person with an acute respiratory illness with laboratory confirmed swine influenza A (H1N1) virus infection at CDC by one or more of the following tests:  
   1. real-time RT-PCR  
   2. viral culture  
   3. four-fold rise in swine influenza A (H1N1) virus specific neutralizing antibodies

2. A Probable case of swine influenza A (H1N1) virus infection is defined as a person with an acute respiratory illness with an influenza test that is positive for influenza A, but H1 and H3 negative.

3. A Suspected case of swine influenza A (H1N1) virus infection is defined as:  
   1. A person with an acute respiratory illness who was a close contact to a confirmed case of swine influenza A (H1N1) virus infection while the case was ill OR  
   2. A person with an acute respiratory illness with a recent history of contact with an animal with confirmed or suspected swine influenza A (H1N1) virus infection OR  
   3. A person with an acute respiratory illness who has traveled to an area where there are confirmed cases of swine influenza A (H1N1) within 7 days of suspect case's illness onset.

Infectious period for confirmed cases = 1 day before onset to 7 days after onset of illness  
Day before onset = Day -1  
Onset day = Day 0  
Days after onset = Days 1-7

* These definitions are intended for establishing the epidemiology of swine influenza in humans.
Ohio Department of Health Guidelines for Collecting and Shipping Specimens for Influenza Diagnostics
April 27, 2009

The following guidelines have been adapted from the U.S. Department of Health and Human Services (HHS) Influenza Plan, Appendix 5, dated November 2005. Please note that appropriate specimens for influenza testing vary by type of test. Before collecting any specimens it is important to review your facility’s infection control practices. The Ohio Department of Health Laboratory has developed a table titled “Ohio Department of Health Laboratory (ODHL) Testing for Respiratory Viruses” for information on appropriate specimens to obtain for detection of other respiratory viruses.

I. Respiratory Specimens
Eight types of respiratory specimens may be collected for viral and/or bacterial diagnostics: 1) nasopharyngeal wash/aspirates, 2) nasopharyngeal swabs, 3) oropharyngeal swabs, 4) bronchoalveolar lavage, 5) tracheal aspirate, 6) pleural fluid tap, 7) sputum, and 8) autopsy specimens.

Nasopharyngeal wash/aspirates are the specimen of choice for detection of most respiratory viruses and are the preferred specimen type for children aged <2 years.

Respiratory specimens for detection of most respiratory pathogens, and influenza in particular, are optimally collected within the first 3 days of the onset of illness.

A. Collecting specimens from the upper respiratory tract
1. Nasopharyngeal wash/aspirate
   - Have the patient sit with head tilted slightly backward.
   - Instill 1 ml–1.5 ml of non-bacteriostatic saline (pH 7.0) into one nostril. Flush a plastic catheter or tubing with 2 ml–3 ml of saline. Insert the tubing into the nostril parallel to the palate. Aspirate nasopharyngeal secretions. Repeat this procedure for the other nostril.
   - Collect the specimens in sterile vials. Label each specimen container with the patient’s ID number and the date collected.
   - See shipping information below.

2. Nasopharyngeal or oropharyngeal swabs
   - Use only sterile dacron or rayon swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden sticks, as they may contain substances that inactivate some viruses and inhibit PCR testing.
   - To obtain a nasopharyngeal swab, insert a swab into the nostril parallel to the palate. Leave the swab in place for a few seconds to absorb secretions. Swab both nostrils.
   - To obtain an oropharyngeal swab, swab the posterior pharynx and tonsillar areas, avoiding the tongue.
   - Place the swabs immediately into sterile vials containing 2 ml of viral transport media. Break the applicator sticks off near the tip to permit tightening of the cap.
Label each specimen container with the patient’s ID number and the date the sample was collected.

- See shipping information below.

B. Collecting specimens from the lower respiratory tract
1. Bronchoalveolar lavage, tracheal aspirate, or pleural fluid tap
   - During bronchoalveolar lavage or tracheal aspirate, use a double-tube system to maximum shielding from oropharyngeal secretions.
   - Centrifuge half of the specimen, and fix the cell pellet in formalin. Place the remaining un-spun fluid in sterile vials with external caps and internal O-ring seals. If there is no internal O-ring seal, then seal tightly with the available cap and secure with Parafilm®. Label each specimen container with the patient’s ID number and the date the sample was collected.
   - See shipping information below.

2. Sputum
   - Educate the patient about the difference between sputum and oral secretions.
   - Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile screw-cap sputum collection cup or sterile dry container.
   - See shipping information below.

II. Blood Components
CDC can perform serology testing for influenza. Both acute and convalescent serum specimens should be collected for antibody testing. Collect convalescent serum specimens 2–4 weeks after the onset of illness. To collect serum for antibody testing:
   - Collect 5 ml–10 ml of whole blood in a serum separator tube. Allow the blood to clot, centrifuge briefly, and collect all resulting sera in vials with external caps and internal O-ring seals. If there is no internal O-ring seal, then seal tightly with the available cap and secure with Parafilm®.
   - The minimum amount of serum preferred for each test is 200 microliters, which can easily be obtained from 5 ml of whole blood. A minimum of 1 cc of whole blood is needed for testing of pediatric patients. If possible, collect 1 cc in an EDTA tube and in a clotting tube. If only 1cc can be obtained, use a clotting tube.
   - Label each specimen container with the patient’s ID number and the date the specimen was collected.
   - See shipping information below.

III. Autopsy Specimens
CDC can perform immunohistochemical (IHC) staining for influenza A (H5) viruses on autopsy specimens. Viral antigens may be focal and sparsely distributed in patients with influenza, and are most frequently detected in respiratory epithelium of large airways. Larger airways (particularly primary and segmental bronchi) have the highest yield for detection of influenza viruses by IHC staining. Collection of the appropriate tissues ensures the best chance of detecting the virus by (IHC) stains.
If influenza is suspected, a minimum total of 8 blocks or fixed-tissue specimens representing samples from each of the following sites should be obtained and submitted for evaluation:

- Central (hilar) lung with segmental bronchi
- Right and left primary bronchi
- Trachea (proximal and distal)
- Representative pulmonary parenchyma from right and left lung

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In addition, representative tissues from major organs should be submitted for evaluation. In particular, for patients with suspected myocarditis or encephalitis, specimens should include myocardium (right and left ventricle) and CNS (cerebral cortex, basal ganglia, pons, medulla, and cerebellum). Specimens should be included from any other organ showing significant gross or microscopic pathology. Specimens may be submitted as:

- Fixed, unprocessed tissue in 10% neutral buffered formalin, or
- Tissue blocks containing formalin-fixed, paraffin-embedded specimens, or
- Unstained sections cut at 3 microns placed on charged glass slides (10 slides per specimen)
- Specimens should be sent at room temperature (NOT FROZEN).
- Fresh-frozen unfixed tissue specimens may be submitted for RT-PCR.
- Include a copy of the autopsy report (preliminary or final if available), and a cover letter outlining a brief clinical history and the submitter’s full name, title, complete mailing address, phone, and fax numbers, in the event that CDC pathologists require further information.

Referring pathologists may direct specific questions to CDC pathologists. The contact number for the Infectious Disease Pathology Activity is 404-639-3133, or the pathologists can be contacted 24 hours a day, 7 days a week through the CDC Emergency Response Hotline at 770-488-7100.

IV. Shipping Instructions

- Specimen submission must be coordinated with local health departments (LHDs). LHDs should call the Ohio Department of Health before arranging specimen submission for influenza A reference testing. ODH is available 24/7. LHDs are to use the contact information for Class A reportable conditions: During business hours contact 614-466-4643; after business hours local health jurisdictions contact the ODH Infectious Disease On-Call at 614-722-7221.
- Specimens should be sent by Priority Overnight Shipping for receipt within 24 hours. Samples (such as fresh-frozen autopsy samples for RT-PCR or other clinical materials) may be frozen at –70°C if the package cannot be shipped within a specified time (e.g. if the specimen is collected on a Friday but cannot be shipped until Monday).
- When sending clinical specimens, include the specimen inventory sheet (see below), and note “Influenza surveillance” on all materials and specimens sent. Follow protocols for standard interstate shipment of etiologic agents, and are

- For delivery of packages using DHL, UPS, US Cargo and other carriers, ship specimens to Ohio Department of Health Laboratory, 8995 E. Main Street, Building 22, Reynoldsburg, Ohio 43068.
This document provides interim guidance on the use of antiviral agents for treatment and chemoprophylaxis of swine influenza A (H1N1) virus infection. This includes patients with confirmed or suspected swine influenza A (H1N1) virus infection and their close contacts.

Case Definitions for Infection with Swine Influenza A (H1N1) Virus

1. A Confirmed case of swine influenza A (H1N1) virus infection is defined as a person with an acute respiratory illness with laboratory confirmed swine influenza A (H1N1) virus infection at CDC by one or more of the following tests:
   a. Real-time RT-PCR
   b. Viral culture

2. A Suspected case of swine influenza A (H1N1) virus infection is defined as:
   a. A person with an acute respiratory illness who was a close contact to a confirmed case of swine influenza A (H1N1) virus infection while the case was ill OR
   b. A person with an acute respiratory illness who traveled to or resides in an area where there are confirmed cases of swine influenza A (H1N1) virus infection

3. Close contact is defined as: within about 6 feet of an ill person who is a confirmed or suspected case of swine influenza A (H1N1) virus infection.

Definitions of Acute respiratory illness: Recent onset of at least two of the following: rhinorrhea or nasal congestion, sore throat, cough (with or without fever or feverishness).

Clinicians should consider swine influenza A (H1N1) virus infection in the differential diagnosis of patients with febrile respiratory disease and who 1) live in areas in the U.S. with confirmed human cases of swine influenza A (H1N1) virus infection or 2) who traveled recently to Mexico or were in contact with persons who had febrile respiratory illness and were in the areas of the U.S. with confirmed swine influenza cases or Mexico in the 7 days preceding their illness onset.

Antiviral Treatment

Suspected Cases

Empiric antiviral treatment is recommended for any ill person suspected to have swine influenza A (H1N1) virus infection. Antiviral treatment with either zanamivir alone or with a combination of oseltamivir and either amantadine or rimantadine should be initiated as soon as possible after the onset of symptoms. Recommended duration of treatment is five days. Recommendations for use of antivirals may change as data on antiviral susceptibilities become available. **Antiviral doses and schedules recommended for treatment of swine influenza A (H1N1) virus infection are the same as those recommended for seasonal influenza:**

http://www.cdc.gov/flu/professionals/antivirals/dosagetable.htm#table
Confirmed Cases
For antiviral treatment of a confirmed case of swine influenza A (H1N1) virus infection, either oseltamivir or zanamivir may be administered. Recommended duration of treatment is five days. These same antivirals should be considered for treatment of cases that test positive for influenza A but test negative for seasonal influenza viruses H3 and H1 by PCR.

Pregnant Women
Oseltamivir, zanamivir, amantadine, and rimantadine are all “Pregnancy Category C" medications, indicating that no clinical studies have been conducted to assess the safety of these medications for pregnant women. Only two cases of amantadine use for severe influenza illness during the third trimester have been reported. However, both amantadine and rimantadine have been demonstrated in animal studies to be teratogenic and embryotoxic when administered at substantially high doses. Because of the unknown effects of influenza antiviral drugs on pregnant women and their fetuses, these four drugs should be used during pregnancy only if the potential benefit justifies the potential risk to the embryo or fetus; the manufacturers' package inserts should be consulted. However, no adverse effects have been reported among women who received oseltamivir or zanamivir during pregnancy or among infants born to such women.

Antiviral Chemoprophylaxis
For antiviral chemoprophylaxis of swine influenza A (H1N1) virus infection, either oseltamivir or zanamivir are recommended. Duration of antiviral chemoprophylaxis is 7 days after the last known exposure to an ill confirmed case of swine influenza A (H1N1) virus infection. Antiviral dosing and schedules recommended for chemoprophylaxis of swine influenza A (H1N1) virus infection are the same as those recommended for seasonal influenza:
http://www.cdc.gov/flu/professionals/antivirals/dosage_table.htm#table

Antiviral chemoprophylaxis (pre-exposure or post-exposure) with either oseltamivir or zanamivir is recommended for the following individuals:
1. Household close contacts who are at high-risk for complications of influenza (persons with certain chronic medical conditions, elderly) of a confirmed or suspected case.
2. School children who are at high-risk for complications of influenza (persons with certain chronic medical conditions) who had close contact (face-to-face) with a confirmed or suspected case.
3. Travelers to Mexico who are at high-risk for complications of influenza (persons with certain chronic medical conditions, elderly).
4. Border workers (Mexico) who are at high-risk for complications of influenza (persons with certain chronic medical conditions, elderly).
5. Health care workers or public health workers who had unprotected close contact with an ill confirmed case of swine influenza A (H1N1) virus infection during the case’s infectious period.
Antiviral chemoprophylaxis (pre-exposure or post-exposure) with either oseltamivir or zanamivir can be *considered* for the following:

1. Any health care worker who is at high-risk for complications of influenza (persons with certain chronic medical conditions, elderly) who is working in an area with confirmed swine influenza A (H1N1) cases, and who is caring for patients with any acute febrile respiratory illness.

2. Non-high risk persons who are travelers to Mexico, first responders, or border workers who are working in areas with confirmed cases of swine influenza A (H1N1) virus infection.

**Adverse events and contraindications**

For further information about influenza antiviral medications, including contraindications, and adverse effects, please see the following:

http://www.cdc.gov/flu/professionals/antivirals/side-effects.htm

http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5707a1.htm
For collecting respiratory specimens from an ill confirmed or suspected swine influenza virus (SIV) case, the following is recommended:

1. Personal protective equipment: fit-tested, disposable N95 respirator [if unavailable, wear a medical (surgical mask)], disposable gloves, gown and goggles.
2. When completed, place all personal protective equipment (PPE) in a biohazard bag for appropriate disposal.
3. Wash hands thoroughly with soap and water or alcohol-based hand gel.