SPECIMEN COLLECTION GUIDELINES

Purpose of this document

The purpose of this document is to provide general specimen collection guidelines for healthcare providers and public health staff during a respiratory disease outbreak when the pathogen is unknown. The specimens listed in this document are those that may need to be collected to detect the etiologic agent during a respiratory disease outbreak. When a specific pathogen is known or very strongly suspected, specimen collection should be tailored to the pathogen (Appendix A).

Note: Consult your local or state health department about the potential respiratory outbreak as soon as possible.

Reference Testing

Testing may be conducted by clinical laboratories, reference laboratories or city, county or state public health laboratories. Only State Health Departments and other Federal Agencies may submit specimens for reference testing to CDC. All specimen submissions to CDC require first approval by the individual State Health Department and CDC prior to shipment.

Private citizens, health practitioners and hospitals must contact their local (city or county) health department about how and when to submit specimens. If the local health department is unable to make a determination, they will forward the specimen to their State Health Department.

A list of State and Local Health Departments can be found at http://www.cdc.gov/mmwr/international/relres.html.

The State list of the Association of State and Territorial Public Health Officials can be found at http://www.astho.org/index.php?template=regional_links.php.

Special arrangements will be made for specimens collected for studies/projects by collaborators of CDC investigators. Contact the Principle Investigator for specific instructions.

General principles

1. **Integrated approach**. These guidelines are designed for use in an outbreak setting where the etiologic agent is unknown. Sensitive assays should allow for an efficient and coordinated approach to specimen collection and diagnostic testing to evaluate multiple potential viral and bacterial etiologies.

   Each respiratory pathogen requires a unique set of specimen types, collection methods and handling conditions to optimize diagnostic yield. Because these guidelines are designed for detection of multiple pathogens, the sensitivity of detection of any one agent may be compromised. If a particular agent is strongly suspected, please refer to

pathogen-specific materials. To rule out other pathogens, multiple specimens may be necessary.

2. **Recommended specimens.** Please refer to Appendix A.
3. **Timing of specimen collection**. Respiratory tract specimens should be collected as soon as possible in the course of the illness and before antimicrobic therapy begin, if possible. The likelihood of recovering most viruses and many bacteria diminishes markedly >72 hours after symptom onset and after the initiation of appropriate antimicrobial therapy. If possible, respiratory specimens should be collected within 72 hours of symptom onset and no later than 7 days after onset.
4. **Interpretation of results**. The interpretation of laboratory test results should take into account whether proper specimen collection and handling occurred prior to receiving the specimen in the laboratory and pathogen specific test sensitivities and concurrent treatment. Also, some pathogens colonize the upper respiratory tract (***e.g. S. pneumoniae*** and Hib), or can cause asymptomatic or symptomatic infection (***e.g.*** rhinovirus or coronavirus). Therefore, each laboratory result needs to be interpreted individually for each pathogen. Combining results from selected cases may significantly improve the overall specificity for identifying the predominant cause or causes of an outbreak.

Collection of Upper Respiratory Tract Specimens

1. **Oropharyngeal (OP) and nasopharyngeal (NP) swabs**

    a. Optimal timing. Specimens should be collected within 3 days of symptom onset and no later than 7 days from all patients meeting the case definition identified during the outbreak, ideally prior to the initiation of antimicrobial chemoprophylaxis or therapy.

    b. Swab types. Use only sterile dacron or rayon swabs with plastic shafts or if available, flocked swabs. DO NOT use calcium alginate swabs or swabs with wooden sticks, as they may contain substances that inactivate some viruses and inhibit some molecular assays.

    c. Collecting the OP swab. Insert swab into the posterior pharynx and tonsillar areas. Rub swab over both tonsillar pillars and posterior oropharynx and avoid touching the tongue, teeth, and gums.

    d. Collecting the NP swab. Insert flexible wire shaft swab through the nares parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient indicating contact with the nasopharnyx. Gently, rub and roll the swab. Leave the swab in place for several seconds to absorb secretions before removing.

    e. Specimen handling. Place NP and OP swabs immediately into a sterile vial containing 2 ml of viral transport media **without** antibiotics. Both swabs can be

placed in the same vial, if desired. Aseptically, cut or break applicator sticks off near the tip to permit tightening of the cap. Label the vial with the patient's name, ID number, specimen type, and date collected. If specimens will be examined within 48 hours after collection, keep specimen at 4ºC and ship on wet ice or refrigerant gel-packs, otherwise store frozen at ≤-70ºC and ship on dry ice. Avoid freezing and thawing specimens. Viability of some pathogens from specimens that were frozen and then thawed is greatly diminished and may result in false-negative test results.

2. **Nasopharyngeal wash/aspirate.** This specimen is commonly collected in children <5 years old.
    a. Optimal timing. Specimens should be collected within 3 days of symptom onset and not later than 7 days from all patients meeting the case definition identified during the outbreak, ideally prior to the initiation of antimicrobial chemoprophylaxis or therapy.

    b. Specimen collection. Have the patient sit with head tilted slightly backward. Instill 1 ml-1.5 ml of nonbacteriostatic 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 (not upwards). Aspirate nasopharyngeal secretions. If permitted, repeat this procedure for the other nostril.

    c. Specimen handling. Collect the specimens in sterile vials. Label each specimen container with the patients name, ID number, specimen type, and the date collected. If specimens will be examined within 48 hours after collection, keep specimen at 4ºC and ship on wet ice or refrigerant gel-packs, otherwise store frozen at ≤-70ºC and ship on dry ice. Avoid freezing and thawing specimens. Viability of some pathogens (*e.g.* respiratory syncytial virus) from specimens that are frozen and then thawed is greatly diminished and may result in false-negative test results.

Collection of Lower Respiratory Tract Specimens

1. **Sputum, tracheal aspirate, broncheoalveolar lavage (BAL) fluid, pleural fluid.** Due to the increased technical skill and equipment needs, collection of specimens other than sputum from the lower respiratory tract may be limited to patients presenting with more severe disease, including persons admitted to the hospital and/or fatal cases.

    a. Optimal timing. These specimens may be obtained at any time during the clinical course, but ideally prior to initiation of antimicrobial therapy.

    b. Specimen types. Acceptable lower respiratory tract specimens include sputum, tracheal aspirate, BAL fluid, pleural fluid, or lung biopsy. Specimens with less chance for upper airway contamination (i.e., BAL fluid, pleural fluid, lung biopsy) are preferred.

c. Specimen collection.

i. **BAL fluid, tracheal aspirate, pleural fluid**
Collect specimens in sterile containers. Centrifuge half of the specimen, and fix the cell pellet in formalin. Place the remaining uncentrifuged fluid into 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 name, ID number, the specimen type, and the date the specimen was collected.

ii. **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 collection cup or sterile dry container.

d. Specimen handling. Label the vial or container with the patient's name, ID number, specimen type, and date collected. Store fixed cells at room temperature. If unfixed specimens will be examined within 48 hours after collection, keep specimen at 4ºC and ship on wet ice or refrigerant gel-packs, otherwise store frozen at ≤-70ºC and ship on dry ice. Avoid freezing and thawing specimens. Viability of some pathogens from specimens that were frozen and then thawed is greatly diminished and may result in false-negative test results.

Collection of Blood Components

1. **Acute and convalescent serum specimens.** If possible, acute **and** convalescent sera should be obtained from all patients identified during the outbreak. For most respiratory pathogens, both acute and convalescent sera must be collected to permit a definitive diagnosis. Antibody titers against suspected bacteria or viruses may be measured in sera and provide an important adjunct to or confirmation of PCR and culture results. However, these results are not timely enough to guide clinical care.

a. Optimal timing.

i. Acute. Acute serum specimens should be collected within one week of symptom onset and submitted as soon as possible.

ii. Convalescent. Convalescent specimens should be collected and submitted at 3-6 weeks after the acute specimen was collected.

b. Collecting the sera. For each serum specimen, collect 5 ml of whole blood into a serum separator tube (marble or tiger top SST). A minimum of 1 ml of whole blood is needed for testing of pediatric patients.

c. Specimen handling. Allow whole blood to clot at room temperature for a

minimum of 30 minutes and centrifuge. Label the tube with the patient's name, ID number, specimen type, and date collected. Store refrigerated at 4ºC or frozen, and ship on refrigerant gel packs or dry ice.

d. Aliquoting sera. If aliquoting is performed, divide the sera into 0.5 ml aliquots in sterile containers. Label each vial with the patient's name, ID number, specimen type, and date collected. Store refrigerated at 4ºC or frozen, and ship on refrigerant gel packs or dry ice.

2. **Whole blood for Culture.** This specimen may be limited to patients with more severe disease including persons admitted to the hospital.

   a. Optimal timing. Whole blood should be collected as soon as possible after illness onset and ideally before initiation of antimicrobial chemoprophylaxis or therapy. For fatal cases, postmortem whole blood should always be obtained at autopsy.

   b. Collection. Collect whole blood in bottles according to clinical laboratory guidelines.

   c. Specimen handling. Label the bottle with the patient's name, ID number, specimen type, and date collected. Store and ship specimens with cold packs to keep the specimen at 4ºC.

3. **Whole blood plasma for PCR.** For selected situations, whole blood may be obtained for PCR.

   a. Optimal timing. Whole blood should be collected as soon as possible after illness onset and ideally before initiation of antimicrobial chemoprophylaxis or therapy. For fatal cases, postmortem whole blood should always be obtained at autopsy.

   b. Collection. Collect 5-10 ml of whole blood in an EDTA (purple-top) tube.

   c. Specimen handling. Label the tube with the patient's name, ID number, specimen type, and date collected. Store and ship specimens with cold packs to keep the specimen at 4ºC.

Collection of Tissue Specimens

1. **Fixed tissues**

   a. Target population. A complete autopsy should be performed on all fatal cases associated with a respiratory disease outbreak. Lung tissue should also be received from any non-fatal case where a biopsy is performed.

   b. Specimen types. On all fatal cases, tissues should be collected from all major organs and fixed in formalin or embedded in paraffin. The following tissues are

particularly important:

1. Central (hilar) lung with segmented bronchi

2. Right and left proximal and distal bronchi, upper airways (*e.g.* epiglottis, larynx, trachea)

3. Representative pulmonary parenchyma from right and left lung

Representative tissues from all major organs should also be submitted for evaluation. In particular, for patients with suspected myocarditis, encephalitis, or rhabdomyolysis, specimens should include heart (right ventricle, septum, and left ventricle), CNS (cerebral cortex, thalamus, basal ganglia, midbrain, pons, medulla, cerebellum, and spinal cord), and skeletal muscle. Specimens should be included from any other organ showing significant gross or microscopic pathology.

c. Specimen handling. **Since prolonged fixation may interfere with certain immunohistochemical or molecular diagnostic assays, the original paraffin blocks (tissues prepared for initial pathologic evaluation prior to fixation in formalin) are preferred for analysis if the fixed tissues have been stored in formalin > 2 weeks.** Fixed tissues from various organs may be stored and shipped in one or separate containers. Label the container(s) with the patient's name, ID number, specimen type(s), and date collected. Store and ship at room temperature. Paraffin blocks are usually shipped at room temperature; they should not be frozen. However, if the weather is extremely hot, shipping with a cold pack might prevent incidental melting of the paraffin. **DO NOT FREEZE FIXED TISSUES.** For fatal cases, a preliminary autopsy report should be provided with the tissues.

2. **Non-fixed tissues from lung and upper airway (*e.g.* trachea, bronchus)**

a. Target population. A complete autopsy should be performed on all fatal cases associated with a respiratory disease outbreak. Lung tissue should also be received from any non-fatal case where a biopsy is performed.

b. Specimen types. On all fatal cases, tissues should be collected from lung and upper airways (*e.g*. epiglottis, trachea, bronchi), and any other primarily affected organs.

c. Specimen collection. On all fatal cases specimens should be collected aseptically as soon as possible after death since technique and time will impact risk of post-mortem contamination. Use a separate sterile instrument for each collection site. Place each specimen in separate sterile containers containing small amounts of saline.

d. Specimen handling. Label each container with the patient's name, ID number,

specimen type(s), and date collected. If specimens will be examined within 48 hours after collection, keep specimen at 4ºC and ship on wet ice or refrigerant gel-packs, otherwise store frozen at -70ºC and ship on dry ice. **On all fatal cases, a preliminary autopsy report should be provided with the tissues**.

Collection of Other Specimens

1. **Urine**

    a. Optimal timing. Urine may be collected within 7 days of symptom onset from every patient identified during a respiratory disease outbreak for antigen detection.

    b. Specimen collection. Collect 10-20 ml of urine in a sterile container.

    c. Specimen handling. Label the container(s) with the patient's name, ID number, specimen type(s), and date collected. Store refrigerated at 4ºC and ship on wet ice or refrigerant gel packs.

2. **Stool**

    a. Optimal timing. Stool may be collected within 14 days of symptom onset from patients hospitalized as part of a respiratory disease outbreak (*e.g.* from SARS CoV suspect cases for RT-PCR).

    b. Specimen collection. Collect 10-20 ml stool in a clean, dry, leak-proof container.

    c. Specimen handling. Label the container(s) with the patient's name, ID number, specimen type(s), and date collected. If specimens will be examined within 48 hours after collection, they can be refrigerated at 4ºC; otherwise store frozen at -70ºC and ship on dry ice.

Appendix A

LISTS OF RECOMMENDED CLINICAL SPECIMENS TO COLLECT FROM OUTPATIENTS, INPATIENTS, AND FATAL CASES IN THE SETTING OF AN UNEXPLAINED RESPIRATORY DISEASE

The specimens are listed in order of priority; those listed first are those most useful for testing for the greatest number of different pathogens with a single clinical specimen.

OUTPATIENTS

Upper Respiratory
- Nasopharyngeal (NP) and oropharyngeal (OP)
- Nasopharyngeal wash/aspirate

Lower Respiratory
- Sputum

Blood
- Serum: Acute (at onset) and convalescent (3-6 weeks post onset)
- Blood (plasma)

Urine

Stool

INPATIENTS

Lower Respiratory
- Bronchoalveolar lavage, tracheal aspirate, pleural fluid
- Sputum

Upper Respiratory
- Nasopharyngeal (NP) and oropharyngeal (OP) swabs
- Nasopharyngeal wash/aspirate

Blood
- Serum: Acute (at onset) and convalescent (3-6 weeks post onset)
- Whole blood (plasma)

Tissue (e.g., lung)

Urine

Stool

FATAL CASES

All available premortem specimens

Tissue
- Fixed tissue from all major organs (e.g., lung, heart, spleen, liver, brain, kidney, adrenals)
- Non-fixed tissue from lung and upper airways (e.g., trachea, bronchus)

Lower Respiratory
- Bronchoalveolar lavage, tracheal aspirate, pleural fluid
- Sputum

Blood
- Serum
- Blood (plasma)

Deep lung swab for bacterial culture