

Clinical Testing Virology

Special requirements for Virus Isolation/Identification

Requests for virology laboratory tests should be based on clinical symptoms. Be specific. Use of vague terminology such as “Viral Studies” should be avoided since this only delays the examination until the laboratory can obtain the required specific clinical information. Do not ship specimens immediately before a weekend or holiday. **All virus isolation/identification testing is performed at the Illinois Department of Public Health’s Chicago laboratory.** Ship specimens directly to the Department’s Chicago laboratory.

Information Required

Completed “Communicable Diseases Test Requisition” form

Virus Isolation

Specimen Requirements for Viral Isolation

Collect the specimen within one to three days after onset of symptoms. For optimal virus recovery, assure receipt at the laboratory within 24 hours of collection. Place frozen packs around the specimen and transport specimens to the laboratory in a leak-proof outer container. If delivery will take longer than 24 hours, store the specimen(s) at -70 C and ship on dry ice.

Enteroviruses (Coxsackie Virus, Echovirus, Enterovirus)

Collect specimens (stool, rectal swab, CSF or throat swab) as late as five to seven days after onset of symptoms. Ship specimens overnight on cold packs.

Respiratory Viruses (Influenza A & B, Parainfluenza 1 – 4, RSV and Adenovirus)

See instructions for “Respiratory/Influenza Virus Specimen Submission” in this manual. Transport to the laboratory according to the instructions included with respiratory kit. For outbreaks, contact the regional health officer.

Autopsy Specimens

Collect specimens aseptically as soon as possible after death. Separate in tubes according to tissue type. Label each tube with the contents and patient identifier. Forward the specimens to the laboratory immediately. If a delay in transport is anticipated, store the specimens at -70° C and ship on dry ice.

Referred Tissue Culture Fluid (TCF) Specimens

Hospital labs may submit TCF specimens for identification and typing. Submit only actively growing TCF specimens showing 2+ or greater CPE. **Do not fill the tube with media when transporting to the lab.** See the “Instructions for Influenza Isolates Submission as Part of the W.H.O. Surveillance Program” section of this manual for packaging and shipping guidelines.

Collection Materials

See Appendix C for supplies.

Criteria for Rejection

Preservatives added; received without refrigerant; submitted on expired media; dry swab; excessive delay in transit; specimen not labeled

Test Methodology

Virus isolation in tissue culture, identification by fluorescent antibody (IFA/DFA) and influenza subtyping by PCR and hemagglutination-inhibition (HAI)

Interfering Substances

Bacterial, yeast or fungal contamination

Table 10. Virology

Disease or Agent	Specimen	TAT (Days)
Coxsackie viruses	CSF, throat, stool, rectal swab	7
Echoviruses	CSF, throat, stool	7
Enteroviruses 70 & 71	CSF, throat, stool	7
Adenoviruses	Throat, nasal wash, eye, CSF	7
Influenza viruses, A & B	Throat, nasopharyngeal, bronchial wash	7
Parainfluenza viruses, 1-4	Throat, nasopharyngeal, bronchial wash	7
Respiratory syncytial virus (RSV)	Throat, nasopharyngeal, bronchial wash	7
Measles virus (Rubeola)	Throat, urine, CSF, brain tissue	7
Varicella-zoster (V-Z) virus	Lesion swab, vesicle fluid, CSF, brain tissue, throat, bronchial wash	7

Performed at the Chicago Laboratory

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Instructions for the Submission of Specimens for Virus Culture

Specimen Collection

Please follow these guidelines to maximize the recovery of virus from specimens:

1. The optimal time for collecting specimens for virus isolation studies is during the early acute phase of the illness, preferably within three days of onset. The probability of recovery of virus from asymptomatic patients is slight.
2. The choice of specimens collected must be determined by a physician, a person acting under the direction of the physician or public health professional familiar with the clinical syndrome. The choice of specimens should be made with the objective of maximizing the amount of virus in the specimen. The highest concentrations of viruses are associated with infected tissues, organs and body fluids or body wasted products derived from or in contact with infected tissues and organs.
3. The optimum volume is 2.0 mL of fluid specimens or a sufficient quantity of solid specimens is 0.2 gm. Sub-optimum specimen volumes may reduce the probability of virus isolation. Follow universal handling precautions and use standard aseptic medical procedures for the collection of all specimens.
4. Place specimen in a sturdy, leak-proof, plastic container with a screw cap and seal with Parafilm® or tape.
5. Label the specimen vial with the patient's name and collection date. Refrigerate immediately after collection. Complete all the demographic information on the communicable disease laboratory test requisition form.

Specimen Transport

1. Store at 4 C until shipping is possible. When packaging for shipment, add cold pack to the **shipping container**.
2. a. By ground transport
 - i. Wrap specimen(s) individually in absorbent material.
 - ii. Place wrapped specimen(s) into a biohazard labeled bag and seal securely.
 - iii. Place the test requisition(s) in the biohazard bag outside pouch so that it does not come in contact with the specimen sealed inside the bag.
 - iv. Place the sealed biohazard bag and test requisition(s) inside the shipping container.
 - v. The shipping container must be rigid such as a cooler and labeled with the UN 3373 Biological Substance Category B marking.
 - vi. Close securely.
- b. Commercial carrier by ground/air transport
 - i. Wrap specimen(s) individually in absorbent material.
 - ii. Place the wrapped specimen(s) inside a biohazard labeled 95 kPa bag and seal following the instructions on the bag.
 - iii. Place the test requisition(s) in the 95 kPa bag outside pouch so that it does not come in contact with the specimen sealed in the bag.
 - iv. Place the sealed 95 kPa bag and completed test requisitions(s) inside the outer shipping container and close securely.
 - v. Label the outer shipping container with the appropriate Illinois Department of Public Health laboratory address.
 - vi. Complete the return address section to include the name of the person shipping the package, business name and address and a business phone number.
 - vii. The shipping container must include the UN3373 Biological Substance Category B marking.

Note: When shipping liquid specimens by commercial carrier or by ground air transport, wrap specimens individually in absorbent material. Place the specimens in a biohazard labeled 95 kPa bag and seal according to the instructions on the bag.

3. It is preferred that specimens be shipped within 24 hours of collection. Avoid shipping specimens over weekends or holidays.
4. Ship specimens by fastest delivery to the attention of the Infectious Disease Unit at the Chicago laboratory. This can be accomplished by use of local courier, shipping corporations or U.S. Postal Service Overnight.

Ship to:

Illinois Department of Public Health Laboratory
Infectious Disease Unit
2121 W. Taylor St.
Chicago, IL 60612-4224
Phone: 312-793-4760
Fax: 312-793-1322

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Instructions for Influenza Virus Specimen Submission Based on Interim Guidance on Specimen Collection, Processing and Testing for Patients With Suspected Novel Influenza A (H1N1) Virus Infection from the U. S. Centers for Disease and Control and Prevention <http://www.cdc.gov/h1n1flu/specimencollection.htm>

This document is intended to assist you with the collection and shipping of specimens for influenza testing. *Note: The Illinois Department of Public Health has been provided polymerase chain reaction (PCR) testing materials from the U.S. Centers for Disease Control and Prevention (CDC) for surveillance, rather than diagnostic testing.* Currently, this includes specimens from hospitalized patients with acute febrile respiratory illness, patients who have recently died while experiencing such symptoms, and certain individuals of congregate groups that are of interest to local health department investigations. For the most current Illinois Department of Public Health acceptance criteria, go to www.idph.state.il.us/h1n1_flu/index.htm and click on the “Health Care Providers” link. **Testing for diagnostic purposes, rather than surveillance, should be through a private laboratory.** A list of laboratories that provide PCR-based influenza testing also can be found at the above Internet link.

Preferred Respiratory Specimens

The following should be collected as soon as possible after illness onset: nasopharyngeal swab, nasal aspirate or a combined nasopharyngeal swab with oropharyngeal swab. If these specimens cannot be collected, a nasal swab or oropharyngeal swab is acceptable. For patients who are intubated, an endotracheal aspirate also should be collected. Bronchoalveolar lavage (BAL) and sputum specimens are also acceptable. Specimens should be placed into sterile viral transport media (VTM), labeled, and immediately placed at 2 C to 8 C (refrigerator). **Do not send ribonucleic acid (RNA) extracts.** In the case of deceased patients, a postmortem respiratory specimen like those just described should accompany any other autopsy specimens.

Following a Rapid Influenza Test

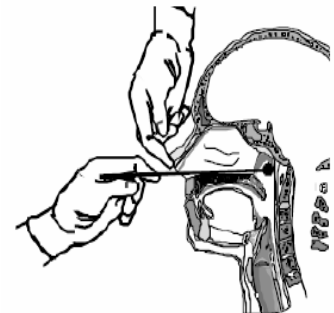
If a specimen has been tested by a rapid influenza test, and it is decided to follow this up with a PCR assay, it is extremely important to send a second specimen, not the remaining material from the rapid test, which will inhibit PCR and culture testing. Regardless of the rapid test result, submission to the Illinois Department of Public Health for PCR testing is still restricted to the acceptance criteria described above.

Swabs

Swab specimens should be collected using swabs with a synthetic tip (e.g. polyester or Dacron®) and an aluminum or plastic shaft. Swabs with cotton tips and wooden shafts are not recommended. Specimens collected with swabs made of calcium alginate are not acceptable. The swab specimen collection vials should contain 1 mL to 3 mL of viral VTM. Store VTM as recommended by the manufacturer, and allow it to come to room temperature before use.

Instructions for Throat and Nasopharyngeal Swab Collection

1. Collection of a throat swab and/or a nasopharyngeal swab from each patient is optimal for culture of all respiratory viruses. If collecting both a throat and a nasopharyngeal swab, both swabs should be combined in the same vial of viral transport medium.
2. Aseptically remove the sterile swab from the package. Collect throat swabs by vigorous swabbing of the posterior pharynx and tonsil regions, using a rigid shaft swab. Aseptically remove the cap from the VTM. Place the swab in a tube of transport medium, being careful to avoid touching the sides or rim of the tube. Break the shaft. Close tightly. Refrigerate immediately after collection.
3. Collect nasopharyngeal swabs by inserting a flexible swab through each nostril deeply into the nasopharynx (aiming toward the ear). Rotate, remove and place the swab in a tube of transport medium. Break the shaft. Close tightly.
4. Label the specimen vial with the patient’s name and collection date. **Refrigerate immediately.** Complete all the information on the “Request for Respiratory Influenza Testing” requisition form.



Instructions for Specimen Transport

1. Ship specimens within 24 hours of collection. Avoid shipping specimens over weekends and holidays.
2. a. By ground transport
 - i. Wrap specimen(s) individually in absorbent material.
 - ii. Place wrapped specimen(s) into a biohazard labeled bag and seal securely.
 - iii. Place the test requisition(s) in the biohazard bag outside pouch so that it does not come in contact with the specimen sealed inside the bag.
 - iv. Place the sealed biohazard bag and test requisition(s) inside the shipping container.

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- v. The shipping container must be rigid such as a cooler and labeled with the UN 3373 Biological Substance Category B marking.
- vi. Close securely.
- b. Commercial carrier by ground/air transport
 - i. Wrap specimen(s) individually in absorbent material.
 - ii. Place the wrapped specimen(s) inside a biohazard labeled 95 kPa bag and seal following the instructions on the bag.
 - iii. Place the test requisition(s) in the 95 kPa bag outside pouch so that it does not come in contact with the specimen sealed in the bag.
 - iv. Place the sealed 95 kPa bag and completed test requisitions(s) inside the outer shipping container and close securely.
 - v. Label the outer shipping container with the appropriate Illinois Department of Public Health laboratory address.
 - vi. Complete the return address section to include the name of the person shipping the package, business name and address and a business phone number.
 - vii. The shipping container must include the UN3373 Biological Substance Category B marking.
3. **Ship specimens by the fastest delivery method possible.** This can be accomplished by use of a local courier or commercial shipping corporations. If approved for testing at the Illinois Department of Public Health (see section above), ship to the attention of the Molecular Diagnostics Lab at the closest Illinois Department of Public Health laboratory.

Illinois Department of Public
Health Laboratory
1155 S. Oakland Ave.
P.O. Box 2797
Carbondale, IL 62902-2797
Phone: 618-457-5131
Fax: 618-457-6995

Illinois Department of Public
Health Laboratory – Molecular
Diagnostics Lab
2121 W. Taylor St.
Chicago, IL 60612-7260
Phone: 312-793-4760
Fax: 312-793-1322

Illinois Department of Public
Health Laboratory – Molecular
Diagnostics Lab
825 N. Rutledge St.
Springfield, IL 62702
Phone: 217-782-6562
Fax: 217-524-7924

Note: Shipping containers will not be returned. See Appendix L. Request for Respiratory Influenza Testing

Instructions for Influenza Isolates Submission as Part of the W.H.O. Surveillance Program

Specimen Collection

1. The optimal time for collecting specimens for influenza virus isolation is during the early acute phase of the illness, preferably within three days of onset. The probability of recovery of virus from asymptomatic patients is slight.
2. The choice of clinical specimen collected should be determined by a physician, a person acting under the direction of the physician or a public health professional familiar with the clinical syndrome. Influenza viruses can be readily isolated from a variety of respiratory specimens, including sputum, nose and throat swabs, and nasal aspirates or washes. The nasopharyngeal swab is the preferred specimen.
3. A processed shell vial aliquot (RMIX™ or RMK shell vial) is **not an acceptable specimen** for recovering virus for HAI subtyping. **Processed shell vials will be accepted for influenza PCR testing only.**
4. Inoculate a RMK culture tube using the original clinical specimen or shell vial that was identified as containing influenza virus. Incubate the RMK tube at 37 C until CPE of 2+ or greater is present. CPE usually occurs within three to five days.
5. Label the RMK culture tube with the patient's name and collection date. Complete all the demographic information on the communicable disease laboratory test requisition form.
6. Ship the RMK culture tube with 1mL to 2 mL of media. **Do not fill the culture tube with media.** Wrap the cap with parafilm or tape to provide additional insurance against leakage. Wrap each tube with absorbent material.

Specimen Transport

1. Maintain the isolate(s) at 37 C until shipping is possible. **When packaging for shipment, add cold packs to the shipping container.**
2. a. By ground transport
 - i. Wrap specimen(s) individually in absorbent material.
 - ii. Place wrapped specimen(s) into a biohazard labeled bag and seal securely.
 - iii. Place the test requisition(s) in the biohazard bag outside pouch so that it does not come in contact with the specimen sealed inside the bag.

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- iv. Place the sealed biohazard bag and test requisition(s) inside the shipping container.
- v. The shipping container must be rigid such as a cooler and labeled with the UN 3373 Biological Substance Category B marking.
- vi. Close securely.
- b. Commercial carrier by ground/air transport
 - i. Wrap specimen(s) individually in absorbent material.
 - ii. Place the wrapped specimen(s) inside a biohazard labeled 95 kPa bag and seal following the instructions on the bag.
 - iii. Place the test requisition(s) in the 95 kPa bag outside pouch so that it does not come in contact with the specimen sealed in the bag.
 - iv. Place the sealed 95 kPa bag and completed test requisitions(s) inside the outer shipping container and close securely.
 - v. Label the outer shipping container with the appropriate Illinois Department of Public Health laboratory address.
 - vi. Complete the return address section to include the name of the person shipping the package, business name and address and a business phone number.
 - vii. The shipping container must include the UN3373 Biological Substance Category B marking.
3. **Ship to the laboratory on cold packs as quickly as possible** by messenger courier, commercial carrier or United States Postal Service overnight. Avoid shipping specimens over weekends or holidays.

Ship to:

Illinois Department of Public Health Laboratory

Virology Unit

2121 W. Taylor St.

Chicago, IL 60612-4224

Phone: 312-793-4760

Fax: 312-793-1322