New Jersey Department of Health
Instructions for Collection, Testing, and Shipping of Influenza Specimens

The New Jersey Department of Health Public Health and Environmental Laboratories (PHEL) has the ability to conduct PCR testing for both seasonal and novel influenza viruses. The following is a guide on appropriate collection, testing and shipping of influenza specimens to PHEL.

General Considerations

- Appropriate infection control procedures should be followed when collecting samples: [http://www.cdc.gov/flu/professionals/infectioncontrol/healthcaresettings.htm](http://www.cdc.gov/flu/professionals/infectioncontrol/healthcaresettings.htm)
- Detection of influenza viruses is more likely from specimens collected within the first 3-4 days of illness onset.
- The following should be collected as soon as possible after illness onset: nasopharyngeal swab, nasal aspirate or wash 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 should also be collected. Bronchoalveolar lavage (BAL) and sputum specimens are also acceptable. Collection instructions can be found below.
- Ideally, swab specimens should be collected using swabs with synthetic tips (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 1-3 ml of viral transport medium (e.g., containing protein stabilizer and antibiotics to discourage bacterial and fungal growth; buffer solution).
- Specimens should be placed into sterile viral transport media and immediately placed on refrigerant gel-packs or at 4°C (refrigerator) for transport to PHEL.
- The positive predictive value increases as the disease incidence increases. However, test results on samples collected early in the season are important to understand which strains of influenza are circulating. It would be impossible for health care providers to test every person presenting with influenza-like illness; however, health care providers are encouraged to submit samples early in the influenza season (initial patients presenting with influenza like illness regardless of rapid test result), during the peak of the season, and towards the end of the season. This will help to characterize influenza strains throughout the influenza season.
- Rapid influenza diagnostic tests (RIDTs) have unknown sensitivity and specificity to detect novel influenza viruses such as Influenza A 2009 H1N1 or Influenza A (H3N2)v virus in clinical specimens. Negative results from these tests do not exclude influenza virus infection in patients with signs and symptoms suggestive of influenza. Therefore, a negative test result could be a false negative and should not preclude further diagnostic testing such as PCR.
- All specimens collected and sent to PHEL should be labeled with a minimum of two unique patient identifiers. Patient identifiers can include: patient's first and last name, date of birth, medical record number, date of collection, and specimen type. Ideally every specimen should include all of this information.
Specimen Collection

Nasopharyngeal (NP)

- Materials
  - Sterile Dacron/nylon swab
  - Viral transport media tube (3 ml)

- Procedure
  - Collect specimen with a sterile Dacron/nylon swab with a non-wooden shaft (do NOT use calcium alginate swabs or swabs with wooden sticks as they may contain substances which can inactivate viruses or interfere with PCR testing).
  - Insert the swab through the nostril, parallel to the palate to the posterior nasopharynx (distance from the nostrils to the external opening of the ear). The swab should be left in place for a few seconds to absorb secretions. Slowly withdraw the swab with a rotating motion. Swab both nostrils with the same swab.
  - Put the tip of the swab into the plastic vial containing 3 ml of viral transport media and cut off the applicator stick.

Nasopharyngeal aspirates/wash

- Materials
  - Suction apparatus
  - Sterile suction catheter
  - Sterile saline
  - Viral transport media

- Procedure
  - Aspirate nasopharyngeal secretions through a catheter connected to a mucus trap and fitted to a vacuum source.
  - For NP wash, have the patient sit with head tilted slightly backward. Instill 1ml-1.5ml of nonbacteriostatic saline (pH 7.0) into one nostril. No saline is used for an aspirate.
  - Insert the catheter into the nostril parallel to the palate. Apply the vacuum and slowly withdrawn the catheter with a rotating motion. Mucus from the other nostril should be collected the same way. Specimen should be placed in a sterile vial.

Nasal swab

- Materials
  - Dry polyester swab
  - Viral transport media tube (3 ml)

- Procedure
  - Insert a dry polyester swab into the nostril. Using a gentle rotation, push the swab until resistance is met at the level of the turbinates (less than 1 inch into the nostril). Rotate the swab a few times against the nasal wall. Repeat in the other nostril using the same swab.
  - Put the tip of the swab into the plastic vial containing 3 ml of viral transport media and cut off
the applicator stick.

Combined nasopharyngeal and oropharyngeal (throat) swab

- **Materials**
  - Dry polyester swab
  - Sterile Dacron/nylon swab
  - Viral transport media tube (3 ml)

- **Procedure**
  - Collect specimens with sterile Dacron/nylon or polyester swabs with a non-wooden shaft (do NOT use calcium alginate swabs or swabs with wooden sticks as they may contain substances which can inactivate viruses or interfere with PCR testing).
  - Insert the swab through the nostril, parallel to the palate to the posterior nasopharynx (distance from the nostrils to the external opening of the ear). The swab should be left in place for a few seconds to absorb secretions. Slowly withdraw the swab with a rotating motion. Swab both nostrils with the same swab.
  - Put the tip of the swab into the plastic vial containing 3 ml of viral transport media and cut off the applicator stick.
  - For oropharyngeal specimen collection, swab the posterior pharynx and tonsillar areas, avoiding the tongue using the second swab.
  - Put the tip of the swab into the same plastic vial containing the nasopharyngeal swab and break or cut off the applicator stick.

**Bronchoalveolar lavage or tracheal aspirate**

- During bronchoalveolar lavage or tracheal aspirate, use a double-tube system to maximize shielding from oropharyngeal secretions.
- Centrifuge half of the specimen, and fix the cell pellet in formalin. Place the remaining unspun 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®.

- For fatal cases associated with possible influenza infection, autopsy and collection of appropriate postmortem specimens should be performed. Additional information is available at: [http://www.hhs.gov/pandemicflu/plan/sup2.html#app5](http://www.hhs.gov/pandemicflu/plan/sup2.html#app5).

**Use of Rapid Antigen Test Kits**

If a rapid antigen test is positive or if a rapid antigen test was not performed but influenza is suspected, a second sample should be sent to PHEL for additional testing. Each site can send a maximum of 3 specimens per week to PHEL. In order to ensure that consistent testing is performed at both the physicians’ office and reference laboratories, PHEL recommends collecting two samples at the same time as indicated in the instructions above. All samples should be labeled, stored and packaged appropriately as described below.

**Storage, Packaging and Shipping**

- The vial containing the collected specimen should be labeled with a minimum of two unique patient identifiers. Patient identifiers can include: patient's first and last name, date of birth, medical record
number, date of collection, and specimen type. Ideally every specimen should include all of this information. **Samples which are not labeled correctly will not be accepted for testing.**

- Respiratory specimens should be kept at 4°C for no longer than 3 days. Specimens can alternatively be frozen at ≤-70°C. Avoid freezing and thawing specimens if at all possible.
- The SRD-1 form (available at [http://www.state.nj.us/health/forms/srd-1.pdf](http://www.state.nj.us/health/forms/srd-1.pdf)) should be completely filled out for each specimen that is sent.
- Commercial carriers can be used to ship samples, which should be handled as Biologic Substance, Category B. Samples should be packaged in accordance with DOT regulation 49 CFR 178.199 utilizing packaging meeting DOT specifications for biological substances. Please include a frozen cold pack with the specimens to maintain the cold chain during shipment. Information on shipping regulations for these carriers can be found at [www.iata.org](http://www.iata.org) or [www.hazmat.dot.gov](http://www.hazmat.dot.gov).
- Facilities should ensure that the specimen will be received at PHEL during normal business hour Monday through Friday. Samples collected on Friday or Saturday should be held in refrigeration and shipped on Sunday or Monday.
- Specimens should be mailed to the following address:
  
  New Jersey Public Health, Environmental and Agricultural Laboratories  
  Health and Agriculture Building  
  3 Schwarzkopf Drive  
  Ewing, NJ 08628  
  Attn: Margaret Kirkuff

**Resources**

**General guidance**  
(http://www.cdc.gov/flu/swineflu/influenza-variant-viruses-h3n2v.htm)

**Specimen collection**  
(http://vimeo.com/7748371)  
(http://www.youtube.com/watch?v=DVJNWefmHjE)  
(http://www.cdc.gov/pertussis/clinical/diagnostic-testing/specimen-collection.html)

**Directions to NJDOH PHEL**  
(http://www.state.nj.us/health/forms/vir-16inst.shtml)  
(http://nj.gov/health/phel/documents/contact.pdf)