Public Health
HEALTH AND HUMAN SERVICES

DANIEL STALEY
Director, Division of Public Health

SCOTT J. ZIMMERMAN, DrPH, MPH, HCLD (ABB)
Laboratory Director

Specimen Collection for Influenza Testing at the North Carolina State Laboratory of Public Health

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Note: **Testing guidance for NC clinicians is available at www.flu.nc.gov.**

The CDC package insert for RT-PCR requires samples to be stored at 2-8°C for no more than 72 hours between specimen collection and analysis. For this reason, ship specimens within 24 hours of collection to accommodate transit delays. All samples must be shipped with ice packs in insulated containers. If a shipment will be delayed because of holidays or weekends or distance/carrier considerations, freeze and hold specimens at -70°C, and then ship on dry ice.

Respiratory Specimens

Each primary specimen container must be labeled with the patient's first and last name, either date of birth, SSN, or other unique identifier, and the collection date.

Use only Dacron-tipped, rayon-tipped, or flocked swabs with plastic or metal shafts. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are **not recommended**.

Samples acceptable for influenza culture, routine RT-PCR, and novel/Avian influenza RT-PCR testing:

Nasopharyngeal Swab – Carefully swab the posterior nasopharyngeal area via the external nares with a dry sterile tipped swab. Break off the swab tip into a vial containing 2 ml of viral transport medium. Screw the cap on tightly.

Nasal Swab - Insert dry swab into nasal passage and allow it to absorb secretions. Place swab in viral transport medium and break off at the neck of vial. Screw the cap on tightly.

Throat Swab – Vigorously rub the posterior wall of the pharynx with a dry, sterile swab. The swab should not touch the tongue or buccal mucosa. Break off the swab tip into a vial of viral transport medium. Screw the cap on tightly.

Nasal Aspirate/Nasal Washing – Approximately 3-7 ml of sterile PBS is aspirated into a rubber bulb. The patient should be placed on their side in a supine position. Gently press one nostril closed with finger pressure. Use the point of the bulb to completely occlude the other side. The PBS is then squeezed into the nose and quickly aspirated. Secretions are then placed into a sterile vial. Screw the cap on tightly.

Lower Respiratory Tract Specimens - These specimens include bronchoalveolar lavage fluid (BAL), bronchial aspirates (BA), bronchial washes (BW), endotracheal aspirates (EA), endotracheal washes (EW), tracheal aspirates (TA), lung tissue, and sputum. Fluids, aspirates, washes, or sputum should be placed into a sterile vial; lung tissue should be placed into viral transport medium. In both cases, ensure that the cap is screwed on tightly. **Note: Lower respiratory tract specimens are not acceptable for Influenza B lineage typing.**

Please note: 'YouTube' videos showing how to properly collect a variety of respiratory specimens can be accessed at www.jointcommission.org/siras.aspx under "Specimen Collection Videos".

Supplies

Shipping mailers and transport media may be ordered online at the NCSLPH North Carolina State Laboratory Public Health – Order Supplies. **Sentinel Network Providers should only order mailers and transport media by calling the NCSLPH Mailroom at 919-733-7656.** Upon receipt, open and place transport media in refrigerator and store ice packs in freezer. Commercially available viral transport media can be used as well.

Required Submission Form

The laboratory submission form for influenza testing (DHHS #3431) can be downloaded at SLPH Virology Form. The patient's health care provider must complete the submission (requisition) form. Please fill out the form as completely as possible with the following information or the specimen may be considered UNSATISFACTORY for testing:

- First and Last name of patient and either date of birth, SSN, or other unique identifier
- Submitter address, including telephone number and EIN number
- Ordering Provider Name and NPI (National Provider Identifier)
- Specimen source
- Date of collection
- Symptom onset date
- Diagnosis code (ICD-10)
- Epidemiologic risk factors including epi links, travel history, and vaccination history [please include date and type of vaccine given, i.e., RIV (recombinant influenza vaccine) or IIV (inactivated influenza vaccine)]
- Date of patient death (if applicable)

Shipping Instructions

Any suspect influenza specimen should be shipped as a UN3373 BIOLOGICAL SUBSTANCE, CATEGORY B. The shipper (hospital or clinic) – not the transport company – is responsible for the shipment until the package reaches the consignee (NCSLPH). Specimens can be

shipped via the State Courier system, United States Postal Service (USPS), or FedEx. Package should be shipped by the fastest means possible. ***Transit time of less than 24 hours after collection will optimize virus detection. <u>All samples must be shipped cold in insulated containers.</u>***

Shipping Address: North Carolina State Laboratory of Public Health
4312 District Drive
Raleigh, NC 27607

State Courier Shipping or USPS

If using the State Courier system or USPS, viral culture kits and shipping materials from the NCSLPH may be used.

- Ship specimen(s) to the NCSLPH the same day collected. DO NOT DELAY SHIPMENT OF SPECIMENS BY WAITING UNTIL ALL FOUR VIALS OF TRANSPORT MEDIA ARE USED. Although this kit was designed for up to four specimens, the cost of the transport media is negligible and unused media can simply be discarded.
- Wrap the properly labeled (first and last name of patient with a unique identifier such as date of birth) inoculated transport medium (primary container) in an absorbent material such as a paper towel and place into a leak proof secondary container such as a 50 ml conical tube.
- The empty plastic shipping tubes used to transport excess media should be used to maintain a tight pack for the specimens being submitted.
- Place secondary container(s) containing specimen(s) between the ice packs.
- Place completed forms in plastic bag and slide into space at narrow end of ice pack.
- Replace styrofoam lid on the box, seal cardboard box, and attach return pre-addressed shipping label over existing label.

Adherence to the following UN3373 BIOLOGICAL SUBSTANCE, CATEGORY B packaging instructions will ensure that the package containing test samples meets federal requirements for shipment to the laboratory:

Primary Packaging

The primary receptacle(s) must be leak-proof. Multiple primary receptacles must be wrapped individually to prevent breakage. When determining the volume of diagnostic specimens being shipped, include the viral transport media. Primary receptacle(s) must not contain more than 500 mL or 500 g. ***The biological substance consists of the primary receptacle and its contents.***

Secondary Packaging

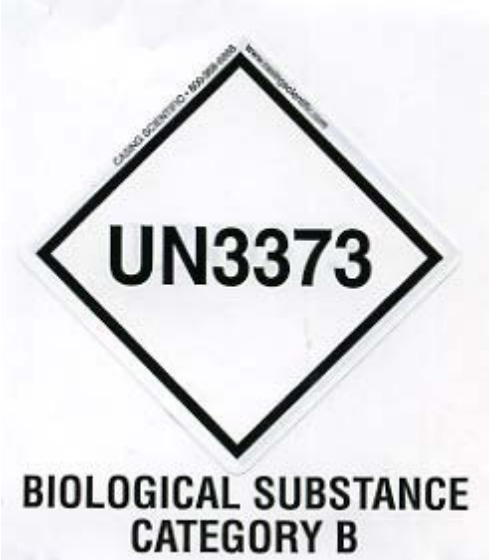
- Use enough absorbent material to absorb the entire contents of all primary receptacles in case of leakage or damage. Secondary packaging must meet the IATA packaging requirements for biological substances, category B including 1.2 meter (3.9 feet) drop test procedure. (IATA packing Instruction 650)
- Secondary packaging must be leak-proof. Follow the packaging manufacturer or other authorized party's packing instructions included with the secondary packaging. Secondary packaging must be at least 100 mm (4 inches) in the smallest overall

external dimension, and must be large enough for shipping documents such as the air waybill.

Outer Packaging

The outer packaging must not contain more than 4 L or 4 kg. Cold packs must be placed outside the secondary packaging.

- The cold packs must be leak-proof.
- Each package and the air waybill must be marked with the following label or exact wording:



- The name and telephone number of the responsible person must be marked on the package or provided on the waybill.
- An itemized list of contents must be enclosed between the secondary packaging and the outer packaging. This can be the requisition form (DHHS #3431). Place in a sealed plastic bag to protect from moisture. A Shipper's Declaration for Dangerous Goods is NOT required for Category B.

FedEx Shipping

For more complete instructions for FedEx shipping, please see the following website:
http://www.fedex.com/us/services/options/dangerousgoods/regulatory.html

Please Note: All shipments (including after-hours shipments) via FedEx will be accepted by State Capitol Police at the Laboratory (24/7/365). The individual delivering the package should come to the loading dock and ring the buzzer at the left side of the loading dock, where State Capitol Police will receive the package (please follow signs).

NCSLPH Standard Operating Procedures

Notification

There is no need to notify NCLSPH personnel when submitting routine influenza specimens. However, when specimens from suspected novel or avian A/H5 influenza cases are submitted to NCSLPH, advance notification of the Laboratory Director is requested (919-733-7834) and the following internal staff will be notified: Assistant Laboratory Director, Virology/Serology Manager, Viral Culture/Rabies Supervisor, and Bioterrorism and Emerging Pathogens Coordinator. **All samples submitted for avian influenza or other novel influenza testing must receive prior approval by an Epidemiologist at the Communicable Disease (CD) Branch (919-733-3419).**

Routine influenza testing at NCSLPH will detect highly-pathogenic avian influenza (HPAI) A/H5 viruses. Testing is available for persons with signs and symptoms consistent with influenza who have been exposed to HPAI H5-infected birds. Prior consultation and approval for testing by the Communicable Disease Branch is required.

Laboratory Handling

All appropriate samples requesting influenza testing will be processed using the RT-PCR assay and/or culture. Specimens will be placed into culture if the RT-PCR assay is negative (if laboratory resources permit) or if the submitter has requested additional testing by writing "culture if negative by PCR" on the submission form. Cell culture will be observed by light microscopy for the presence of CPE which indicates viral replication. If CPE is present, slides will be prepared for subsequent manipulation and fluorescent viewing with the appropriate monoclonal antibodies.

If the specimen is from a suspected case of A/H5 influenza or other novel influenza, the primary specimen will either be taken directly to the BSL-3 laboratory or handled under enhanced BSL-2 conditions (dependent upon CDC biosafety level recommendations). Nucleic acid isolation and subsequent RT-PCR will immediately be performed using A/H5 and/or all available CDC novel influenza primers/probes. If A/H5 or other novel influenza detection by RT-PCR is negative, specimens will be further tested by RT-PCR for the presence of seasonal influenza.

Reporting Results

For seasonal influenza specimens:
For RT-PCR results, allow 2-3 working days for sample preparation, RT-PCR testing, and reporting results.

- If the RT-PCR is negative for the presence of influenza viral RNA (influenza viral RNA not detected by RT-PCR), the specimen is assumed negative for influenza and testing for other respiratory pathogens may be indicated. If no further testing has been requested, the sample will be finalized as "Virus NOT DETECTED". Test results will be available online and a computer-generated report will also be issued to the submitter via the mail.

- If the RT-PCR is positive for the presence of influenza viral RNA, the specimen is assumed positive and no further testing is performed. The specimen is finalized as:

seasonal Influenza A H1, Influenza A H3, Influenza B/Yamagata, Influenza B/Victoria, or Influenza 2009 A/H1 DETECTED. Test results will be available online and a computer-generated report will also be issued to the submitter via the mail.

For culture and typing results, allow 5-7 working days for inoculation, viral propagation, fluorescent staining, and reporting results.

- If no CPE is observed by day 7, the specimen will be finalized as: "Negative-no virus isolated." Test results will be available online and a computer-generated report will also be issued to the submitter via the mail.

- If CPE is observed, slides will be made and the specimen will be finalized as: "Positive- isolate identified as _____." Test results will be available online and a computer-generated report will also be issued to the submitter via the mail.

For A/H5 or other novel influenza specimens:

Due to the significant public health implications of a positive result, these samples will be processed as quickly as possible.

- If the RT-PCR is negative for the presence of A/H5 or other suspected novel influenza viral RNA [A/H5 influenza (or other novel influenza) viral RNA not detected by RT-PCR], the specimen is assumed negative for A/H5 or other novel influenza strains and testing for other respiratory pathogens may be indicated. The specimen will be finalized as "Virus NOT DETECTED". CD Branch staff, Laboratory Director, and the submitter will be notified by phone. Test results will be available online and a computer-generated report will also be issued to the submitter via the mail.

- If the RT-PCR is positive for the presence of A/H5 or other novel influenza viral RNA [A/H5 influenza (or other novel influenza) viral RNA detected by RT-PCR], the specimen is assumed positive and a preliminary report is issued as "Presumptive positive for A/H5 (or other novel influenza)". CD Branch staff, Laboratory Director, and the submitter will be notified by phone. Test results will be available online and a computer-generated report will also be issued to the submitter via the mail. The primary specimen will be forwarded to CDC for further analysis. When CDC results are received, CD Branch staff, Laboratory Director, and the submitter will be notified by phone. Test results will be available online and a computer-generated report will also be issued to the submitter via the mail.

- If A/H5 (or other novel influenza) antibody is detected by serologic testing, CDC will notify the NCSLPH. In either situation, the CD Branch staff and the submitter will be notified by phone and a formal report will be sent via the mail.

Note: **All results are available online via the NCSLPH's secure website at http://slph.ncpublichealth.com.** Influenza results will not be routinely called to submitters. However, results will be called upon request if it is clearly indicated on the request form that results should be phoned, along with the name and phone number of the individual wishing to receive the results.