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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 ship on dry ice.

Respiratory Specimens

Each primary specimen container should be labeled with a unique ID # (date of birth, SSN, or CNDS if Local Health Department) and the patient's first and last name, 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.



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Location: 306 N. Wilmington Street • Bath Building • Raleigh, N.C. 27601

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.

Transport medium is available from the NCSLPH Mailroom (919-733-7656). Upon receipt, place the transport media in refrigerator. Commercially available viral transport media can be used as well.

Required Submission Form

The laboratory submission form for influenza testing can be downloaded at <http://slph.ncpublichealth.com/Forms/DHHS-3431.pdf>. 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:

- Symptom onset date
- First and Last name of patient, date of birth, and SSN (or CNDS if local health department)
- Date of collection
- Sample source
- Return address, including telephone number and submitter's EIN number
- Epidemiologic risk factors including epi links, travel history, and vaccination history [please include date and type of vaccine given, i.e., LAIV (live attenuated influenza vaccine) or TIV (trivalent inactivated vaccine)]
- Date of patient death (if applicable)

Shipping Instructions

Mailers and transport media may be ordered online at the NCSLPH [North Carolina State Laboratory Public Health - Forms, Newsletters & Documents](#). **Only Sentinel Network Providers** can 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.

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. All samples must be shipped cold in insulated containers.***

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, i.e. paper towel, and place into a leak proof secondary container (50 ml conical tubes).
- 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.

If extra boxes, and/or cold packs are needed, sentinel sites can call the NCSLPH mailroom at 919-733-7656 to place an order. All other submitters need to request boxes by ordering online at [North Carolina State Laboratory Public Health - Forms, Newsletters & Documents](#).

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.*

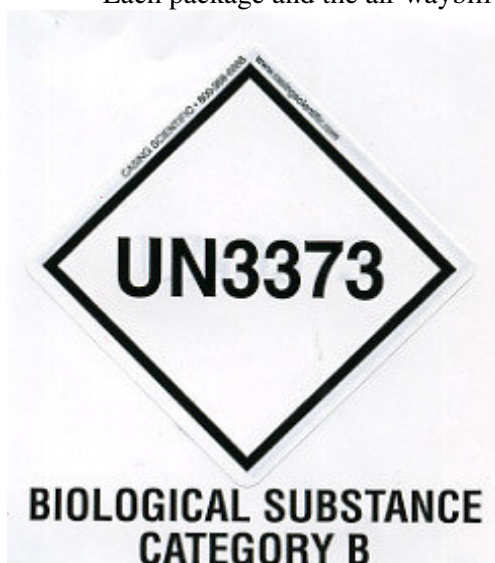
Secondary Packaging

- 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, e.g. 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 websites:

<http://www.fedex.com/us/services/options/dangerousgoods/regulatory.html>

<http://www.fedex.com/us/service-guide/our-services/index.html>

Note: FedEx requires someone to sign for packages. Please contact the NCSLPH before sending a package that needs to be delivered at a time other than normal business hours. (Mon-Friday 8-4:30)

Shipping Address: North Carolina State Laboratory of Public Health
306 N. Wilmington Street
Raleigh, NC 27601

NCSLPH Standard Operating Procedures

Notification

There is no need to notify NCSLPH personnel when submitting routine influenza specimens. However, when specimens from suspected novel or avian H5N1 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, and Viral Culture/Rabies Supervisor. All samples submitted for avian influenza testing must receive prior approval by an Epidemiologist at the Communicable Disease Branch (919-733-3419).

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 H5N1 influenza, the primary specimen will be taken directly to the BSL-3 laboratory for nucleic acid isolation and subsequent RT-PCR for H5N1 detection. If H5N1 detection by RT-PCR is negative, and positive for influenza A, then additional RT-PCR subtyping will be performed.

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 is being performed, the sample will be finalized as “Virus NOT DETECTED”. A computer-generated report will 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, or Influenza 2009 A/H1 virus detected. A computer-generated report will be sent out 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.” A computer-generated report will 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 _____.” A computer-generated report will be sent out 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.

For avian H5N1 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 avian H5N1 influenza viral RNA (H5N1 influenza viral RNA not detected by RT-PCR), the specimen is assumed negative for avian H5N1 influenza and testing for other respiratory pathogens may be indicated. The specimen will be finalized as “H5N1 virus NOT DETECTED”. The submitter will be notified by phone and a computer-generated report will be issued to the submitter via the mail.
- If the RT-PCR is positive for the presence of H5N1 influenza viral RNA (H5N1 influenza viral RNA detected by RT-PCR), a preliminary report is issued as “Presumptive positive for Influenza A/H5 (Asian lineage) Virus”. The submitter will be notified by phone and a computer-generated report will be sent out via the mail. The primary specimen will be forwarded to CDC for further analysis.
- If H5N1 influenza antibody is detected by serologic testing, CDC will notify the NCSLPH. In either situation, the Communicable Disease Branch Epidemiology 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>.