North Carolina State Laboratory of Public Health
Specimen Collection Guidance

Test Methods

The North Carolina State Laboratory of Public Health (NCSLPH) routinely performs a multiplexed real-time reverse-transcription polymerase chain reaction (rRT-PCR) assay that simultaneously detects and differentiates between influenza A, and/or influenza B viruses, along with SARS-CoV-2 in upper or lower respiratory specimens. Specimens positive for influenza virus(es) are further characterized using a FDA-approved CDC sponsored Human Influenza rRT-PCR that allows for the detection of Influenza A types H1, H1N1, H3, H5N1, and H7, and Influenza B types Victoria and Yamagata.

If there is strong reason to suspect infection with a novel or highly pathogenic avian influenza virus (such as an epidemiological link to an individual infected with a novel influenza virus or an individual exposed to poultry infected with highly pathogenic avian influenza) the Communicable Disease Branch (CDB) must be immediately contacted (919-733-3419) to obtain testing approval at the NCSLPH. Once approved, the CDB Flu Coordinator, the NCSLPH Virology/Serology Unit Manager, the submitting physician, and the local health department in which the patient resides will coordinate specimen collection, shipping and testing to ensure a timely response.

The NCSLPH routinely works closely with the CDB to provide guidance for specimen submissions during annual influenza epidemics, where passive and active surveillance strategies are used to monitor for the emergence of novel viruses. When a novel flu A virus emerges and causes a global outbreak, the Pandemic Severity Assessment Framework can be used to assess clinical severity of illness in infected persons and the transmissibility of the infection in the population. This information, along with the Pandemic Intervals Framework (investigation, recognition, initiation, acceleration, peak, deceleration, and resolution) will guide influenza pandemic response, providing recommendation for risk assessment, data driven decision-making, and appropriate public health response.

During influenza pandemic response, an incident command structure will be established that includes executive leadership from the NCSLPH to ensure a robust, statewide laboratory response that provides guidance for the equitable distribution of supplies and testing kits to enhance influenza diagnostic testing throughout the state. DHHS provider memos distributed to all North Carolina clinicians and laboratories will include testing and reporting guidelines for pandemic influenza viruses. The NCSLPH will distribute these memos to all local health department laboratory managers and to clinical diagnostic laboratory managers through the use of various distribution lists including Micronet@lists.ncmail.net, and postings on websites that include the NCSLPH webpage (https://slph.dph.ncdhhs.gov/) and the DPH influenza websites (https://flu.ncdhhs.gov/).

The pandemic intervals will guide statewide testing strategies and testing algorithms. The highest burden of testing for the NCSLPH is expected to occur during the recognition and initiation intervals when the novel virus demonstrates efficient human-to-human transmission. During the acceleration and subsequent peak intervals, laboratory testing at the NCSLPH is expected to decrease as more commercial laboratory testing is available and patients are being treated without laboratory confirmation. During the peak interval, the highest number of newly identified cases is expected and the NCSLPH will be providing testing services to support local health department clinics. Once the peak infection rate has been reached, cases will begin to decline, and the state will enter into the deceleration interval followed by the resolution interval in which cases become sporadic and the NCSLPH will continue testing to support passive and active surveillance strategies to monitor for pandemic strain variants and detect other circulating influenza viruses.

Guidance for clinicians on the use of rapid influenza diagnostic tests can be found on the CDC website. This guide provides detailed information on rapid flu diagnostic assay performance, including their limitations, that aims to assist clinicians when making clinical decisions based on diagnostic testing.Specimen Collection for Influenza Testing at NCSLPH

Acceptable Respiratory Specimens

Each primary specimen container/tube must be labeled with a unique ID # (date of birth, or medical record number), the patient's first and last name, and the collection date. The following specimens are acceptable for testing:

Use only Dacron or rayon swabs with plastic or metal shafts. Calcium alginate swabs and cotton swabs with wooden shafts are **unacceptable**.

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

Nasal Swab - Insert dry swab into nasal passage and allow it to absorb secretions. Rotate swab several times against the nasal wall and repeat in the other nostril using the same swab. Place swabs 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.

Just-in-time validation strategies may be considered for alternative specimen types depending on the pandemic strain and/or supply chain dynamics during response. During pandemic response, please check the NCSLPH SCOPE document for recent updates that may include:

Nasal Aspirate – Approximately 3-7 mls 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), and lung tissue. The aspirates and washes 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.

All specimens must be received cold (2-8°C) within 72 hours of specimen collection or frozen on dry ice (≤ 70°C). It is recommended that specimens ship within 24 hours of collection to accommodate for transit delays. All specimens being transported by a commercial courier must be shipped with ice packs in insulated containers. If a shipment is delayed, i.e., holidays, weekends, or severe weather, freeze and hold specimens at -70ºC, and ship on dry ice as soon as possible.

For local health department laboratories, viral transport medium (VTM), that has been validated for use, can be ordered from the NCSLPH Mailroom Portal, 5 VTM for ~$14, which includes the fee for testing. If there has been personnel or address changes at the facility, please complete the Client Change of Information Form. When completed, the form can be faxed to (919) 807-0730. For further assistance, call the NCSLPH Mailroom (919-733-7656).

Serological Testing (CDC HPAI Confirmatory Testing)

If serological testing is warranted for suspect or presumptive highly pathogenic avian influenza (HPAI) cases, please contact the Virology/Serology Unit Manager, 919-807-8868, to facilitate testing at CDC. The CDC should be consulted prior to submitting specimens for testing. Collection and shipping requirements can be reviewed at the CDC Directory of Services. Each primary specimen container should be labeled with a unique ID # (date of birth or medical record number), the patient's first and last name, and the collection date.

NCSLPH ETOR Specimen Submission

The online electronic test orders and results (ETOR) portal: https://lwp-web.aimsplatform.com/nc/#/auth/login, should be used to submit specimens for SARS-CoV-2 and circulating influenza virus testing at the NCSLPH.

NCSLPH and CDC Submission Forms

The NCSLPH Special Serology Form can be used to submit a specimen for HPAI serological testing, using the Single Agent Diagnostic Tests section, checking "Other", and adding "HPAI" in the open space. Additionally, the CDC 50.34 DASH form is required to submit serum for Influenza Serology (CDC-10424) that includes HPAI testing.

Please fill out all forms 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 and date of birth**
- **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**

Information on the form must match the specimen label exactly or the specimen will be rejected.

Specimen Shipping Guidance

Shipment to NCSLPH:

- If the shipment is to be received at NCSLPH within 72 hours of collection, specimens must be received cold (2-8°C, packaged with frozen cold packs) to be acceptable for testing. For delays exceeding 72h, freeze specimens at -70°C or lower and ship on dry ice to be received at NCSLPH frozen (-70°C or lower).
- Packages should be shipped to NCSLPH as an IATA Category B (UN3373) shipment.

Category B shipping instructions can be found using the following link: Cat B Poster_v3 (dot.gov). If you have questions regarding Category B packaging, please contact the NCSLPH Duty Phone Officer at 919-807-8600.

- The following supplies are necessary for Cat B packaging: a rigid package with insulation, frozen ice pack(s), appropriate Category B labels (UN3373), and a leakproof container that specimens can be placed into (i.e. a larger sample container or a specimen bag). All specimen submissions must have a completed NCSLPH submission form (described above) and a CDC 50.34 DASH Form, if testing will be conducted at the CDC.

For the DOA Medical Courier:	**For UPS, FedEx, and other courier services:**
Specimen pickup at local health department (LHD) laboratories for evening delivery at the NCSLPH will occur Monday – Friday. Specimens will be transported at the proper temperature and placed in the vestibule at appropriate holding temperatures. If frozen specimens are being transported, please notify the DOA Medical Courier and NCSLPH using the following email address: slph.courier@dhhs.nc.gov. The DOA Medical Courier Dispatch can be reached at 984-236-7160 for assistance.	Cat B packages should be addressed to: North Carolina State Laboratory of Public Health Attn: Virology/Serology Unit 4312 District Drive Raleigh, NC 27607 Ship packages for overnight delivery. You **must** specify Saturday delivery if shipping on Friday.

NCSLPH Standard Operating Procedures

Notification

There is no need to notify NCLSPH personnel when submitting routine influenza specimens. However, when specimens from a suspected or presumptive H5N1 case or other novel influenza variant case are to be submitted to NCSLPH during the investigation or recognition phase of the outbreak, advance notification will occur between the Epidemiologist on-call and the Virology/Serology Unit Manager.

Laboratory Handling

All samples requesting routine influenza testing will be processed using the rRT-PCR assay under BSL 2 conditions.

If the specimen is from a suspected or presumptive H5N1 case or other novel influenza variant case, the primary specimens will be processed and added to lysis buffer in a BSL 2 laboratory using BSL 3 work practices. Once specimens have been inactivated in lysis buffer, the specimen extraction and rRT-PCR used for further characterization may continue under BSL 2 conditions using with BSL 2 work practices. Specimens will be tested as described in the North Carolina State Laboratory of Public Health Influenza Plan, Test Methods section above.

Reporting Results – see Figure 1: NCSLPH Result Reporting Structure

For seasonal influenza and SARS-CoV-2 specimens

For rRT-PCR results, allow 2-3 working days for specimen processing, extraction, rRT-PCR testing, and reporting results.

1) If the rRT-PCR is negative for the presence of influenza and SARS-CoV-2 viral RNA, the

specimen is negative for these agents. The specimen will be finalized as "NOT DETECTED" and CDC Influenza SARS-CoV-2 Multiplex Assay "Negative". A computer-generated report will be issued to the submitter via the ETOR system.

2) If the rRT-PCR is positive for the presence of SARS-CoV-2 viral RNA only, the specimen is positive for SARS-CoV-2 only. The specimen will be finalized as "DETECTED" and CDC Influenza SARS-CoV-2 Multiplex Assay "Positive for COVID-19". A report is issued to the submitter via the ETOR system.

3) If the rRT-PCR is positive for the presence of influenza viral RNA only or both SARS-CoV-2 viral RNA and influenza RNA, the specimen is positive, and a report is issued to the submitter via the ETOR system. In these situations, additional testing is performed to type the viral RNA for surveillance purposes. The CDB is informed when seasonal Influenza A H1, Influenza A H3, Influenza B, or an Influenza A/H?N? pandemic strain is detected. **Influenza typing results will not be reported to the submitter.**

For H5 or H7 influenza specimens

Due to the significant public health implications of a positive result, these samples will be processed as quickly as possible. All results for suspected H5 or H7 cases will be immediately called to the CDB epidemiologist on-call.

1) If the rRT-PCR is negative for the presence of H5 or H7 influenza viral RNA (H5 or H7 influenza viral RNA not detected by rRT-PCR), the specimen is negative for H5 or H7 influenza and testing for other respiratory pathogens may be indicated. The specimen will be finalized as "NOT DETECTED". A computer-generated report will be issued to the submitter via CELR and sent in the mail.

2) If the rRT-PCR is positive for the presence of H5 or H7 influenza viral RNA (e.g. H5N1 influenza viral RNA detected by rRT-PCR), the specimen is considered to be positive and the specimen will be finalized as "DETECTED". The submitter will be notified by phone and a computer-generated report will be available on the CELR site. The primary specimen will be forwarded to CDC for further characterization and confirmation. Positive results will be immediately reported to the NCSLPH RO or ARO, who will document immediate notification to the Federal Select Agent Program (FSAP) via eFSAP. The RO or ARO will be responsible for submitting a Form 4 via eFSAP. If additional clinical specimens are requested by CDC, shipping must occur within 7 calendar days of confirmation, or all materials will be destroyed with destruction confirmation being submitted via eFSAP. This activity will be recorded on Form 4 initiated in the system.

3) If H5N1 influenza antibody is detected by serologic testing, CDC will provide an electronic report to the NCSLPH. The CDB Epidemiologist on-call and the submitter will be notified by phone and CDC report will be sent via an encrypted email.

***All NCSLPH results are available online via the NC State Laboratory's Clinical and Environmental Lab Results (CELR) website ***

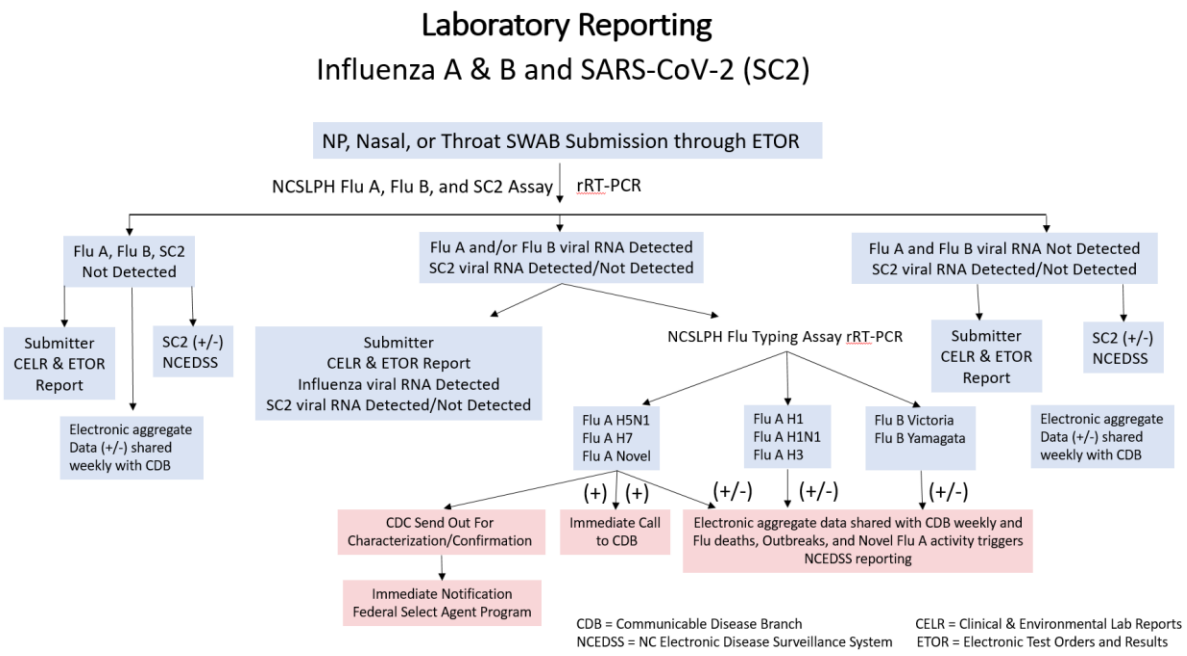

Figure 1: Outline of Flu A, Flu B, and SARS-CoV-2 result reporting structure.