SUPPLEMENT F: LABORATORY DIAGNOSIS

North Carolina Department of Health and Human Services, Division of Public Health

I. Rationale and Goals

The goal of this plan is to be prepared and to ensure a coordinated, timely response to requests for SARS-associated coronavirus (SARS-CoV) laboratory testing.

A. Coordination with CDC

The North Carolina State Laboratory of Public Health (NCSLPH) carries out testing of SARS-CoV samples using the guidelines and reagents procured directly from the CDC. The reagents are in limited supplies, requiring the cooperation of our public health partners in following CDC case definitions to limit unnecessary testing.

B. Coordination with Epidemiology / General Communicable Disease Control Branch

The accurate diagnosis of a SARS-CoV infection is a step-wise process. The first and most important step is for health care providers to be informed of the current risk of encountering patients who may have SARS-CoV and to recognize signs and symptoms. The next step is to report all suspected SARS-CoV cases to the General Communicable Disease Control (GCDC) Branch Epidemiology staff at 919-733-3419. If GDCD staff concur that laboratory testing is appropriate for the patient, then specimens may be sent to NCLSPH for testing. If specimens arrive without prior approval of the GCDC staff, the specimens will not be tested until approval is obtained. All laboratory results will be reported to GCDC staff to assist submitters with interpretation of SARS-CoV test results.

II. Test Methods

CDC developed two laboratory methods used in the diagnosis of SARS-CoV infections that have been shared with state public health laboratories. NCSLPH performs a reverse transcriptase polymerase chain reaction (RT-PCR) assay to detect SARS-CoV viral RNA in a number of specimens types. In addition, an enzyme immunoassay (EIA) is used to detect total antibodies (IgA, IgG and IgM) to SARS-CoV in serum specimens. At this time, these assays are not licensed by the FDA and are under CDC Institutional Review Board (IRB) and FDA Investigational Device-Experimental (IDE) oversight. The majority of specimen types detailed below will be tested by staff at NCSLPH, although initial specimens that show positive results will be sent to the CDC for confirmation.

A. Acceptable Specimens

The likelihood of recovering most respiratory pathogens diminishes significantly after 72 hours from symptom onset. For SARS-CoV, however, the amount of virus may increase later in the course of the illness. Because of the way SARS-CoV virus appears to behave, **taking specimens from multiple sites and sampling at different time points will be crucial for laboratory confirmation of infection** (be it SARS-CoV or other respiratory virus).

Before collecting specimens, please review infection control precautions at:
http://www.cdc.gov/ncidod/sars/guidance/I/index.htm

Each specimen container should be labeled with either a unique ID # or the patient's first and last name, and the collection date. Ship all specimen types with cold packs to keep sample at 4°C.

B. Respiratory Tract Specimens

In general, lower respiratory tract samples are more suitable specimens than those from the upper respiratory tract. The preferred choice of specimens in decreasing order of suitability are the following: **Sputum or BAL > NP aspirates > NP/OP Swabs**

1. **Lower Respiratory Tract Samples:**
   a. **Sputum**

Educate the patient about the difference between sputum and spit. Have the patient rinse the mouth with water then expectorate deep cough sputum directly into a sterile screw-cap sputum collection cup or sterile dry container.

b. **Broncheoalveolar Lavage, Tracheal Aspirate, and Pleural Tap**

If these specimens have been obtained, half should be centrifuged and the cell-pellet fixed in formalin. The remaining un-spun fluid should be placed in sterile vials with external caps and internal O-ring seals. If there are no O-ring seals, then seal tightly with the available cap and secure with Parafilm®.

2. Upper Respiratory Tract Samples:

a. **Nasopharyngeal wash/aspirate**

Have the patient sit with the head tilted slightly backward. Instill 1-1.5 mL of non-bacteriostatic saline (pH 7.0) into one nostril. Flush a plastic catheter or tubing with 2-3 mL of saline. Insert the tubing into the nostril parallel to the palate. Aspirate nasopharyngeal secretions. Repeat this procedure for the other nostril. Collect specimens in sterile vials.

b. **Nasopharyngeal/ Oropharyngeal Swabs**

Use only Dacron or rayon swabs with plastic shafts. Do NOT use calcium alginate swabs or swabs with wooden sticks, as they may contain substances that inactivate some viruses and/or inhibit PCR testing. Place swabs immediately into sterile vials containing 2 mL of viral transport medium. Break applicator sticks off near the tip to permit tightening of the cap.

C. Blood Components

1. Serum for serology and RT-PCR testing

a. **Adults:** Acute serum specimens should be collected and submitted as soon as possible. Convalescent specimens should be collected greater than 28 days after the onset of illness. Collect 5-10 mL of whole blood in a serum separator tube. Allow blood to clot for 30 minutes, then centrifuge 5-10 minutes at 1000-1500 *xg*. Transfer serum to plastic vials with external caps and internal O-ring seals. If there are no O-ring seals, then seal tightly with the available cap and secure with Parafilm®. A minimum of 0.5 mL is required for each test which should be obtained easily from 5 mL of whole blood.

b. **Pediatric Patients:** A minimum of 1 mL of whole blood is needed for testing. If possible, collect 1 mL in both an EDTA tube and clotting tube. However, if only 1 mL can be obtained, please use a clotting tube for collection.

2. EDTA blood/plasma for RT-PCR

Collect 5-10 mL of blood in an EDTA (purple top) tube. Transfer to vials with external caps and internal O-ring seals. If there are no internal O-ring seals, then seal tightly with the available cap and secure with Parafilm®.

D. Stool

Begin collecting stool samples as soon as possible in the course of the illness. Obtain as large a quantity as possible (at least 10 mL in a leak-proof, clean, dry container such as a sterile urine cup or a 50mL blue cap sputum tube). Patients may drape plastic kitchen wrap across the back half of the toilet, under the toilet seat, to facilitate collection of specimens.

Specimens for SARS-CoV Testing:
Priority Specimens and Timing for Collection

The likelihood of detecting infection is increased if multiple specimens, e.g., stool, serum, and respiratory tract specimens, are collected during the course of illness.

Specimen, by test type	<1 week after symptom onset	1-3 weeks after symptom onset	>3 weeks after symptom onset
RT-PCR[1] for viral RNA detection			
Sputum[2]	√[3]	√√	√
Bronchoalveolar lavage, tracheal aspirate, or pleural fluid tap[4]	√	√√	√
Nasopharyngeal wash/aspirate	√	√√	√
Nasopharyngeal and oropharyngeal swabs	√	√√	√
Serum (serum separator tube)	√√	√	not recommended
Blood (plasma) (EDTA/purple top tube for plasma)	√√	√	not recommended
Stool (minimum 10 cc specimen)	√	√√	√√
EIA[1] for antibody detection			
Serum[5] (serum separator tube)	√√	√√	√√

[1] Because of the investigational nature of both the SARS RT-PCR (reverse transcription-polymerase chain reaction) and the SARS EIA (enzyme immunoassay), it is recommended that the clinician obtain a signed informed consent form from the patient. The consent form for the RT-PCR test can be found at: http://www.cdc.gov/ncidod/sars/lab/rtpcr/consent.htm. The consent form for the EIA test can be found at: http://www.cdc.gov/ncidod/sars/lab/eia/consent.htm.

[2] A sputum specimen is preferred if the patient has a productive cough.

[3] The more checks, the better the results from a particular specimen at a specific point in the illness.

[4] Consider these specimen types if sputum is not available.

[5] Also collect a convalescent specimen >28 days post onset.

For more information, visit www.cdc.gov/ncidod/sars or call the CDC public response hotline at (888) 246-2675 (English), (888) 246-2857 (Español), or (866) 874-2646 (TTY)

Source: CDC *Public Health Guidance for Community-Level Preparedness and Response to Severe Acute Respiratory Syndrome (SARS) Version 2 Supplement F: Laboratory Guidance* January 8, 2004

E. Required Submission and Recommended Consent Forms

All SARS specimens will be logged in using Virology/Serology accession numbers. Links to the forms are shown below. The patient's health care provider must complete the submission (requisition) forms and the patient should complete the appropriate consent forms.

Forms for performing RT-PCR analysis (performed on ALL specimens):
Submission form: Appendix F-1 http://www.dhhs.state.nc.us/dph/sars/sars_state_plan_docs/app_F1_vir.pdf
Consent form: Appendix F-2 http://www.cdc.gov/ncidod/sars/lab/rtpcr/pdf/rtpcrparticipant.pdf

Forms for Serology (performed on SERUM only):
Submission form: Appendix F-3 http://www.dhhs.state.nc.us/dph/sars/sars_state_plan_docs/app_F3_ser.pdf
Consent form: Appendix F-4 http://www.cdc.gov/ncidod/sars/lab/eia/pdf/eiaconsent.pdf

Please fill out all forms as completely as possible. Be sure to include an onset date, a collection date, a return address, symptoms and travel history or specimen may be considered UNSATISFACTORY for testing. Finally, be sure to specify on each form (DHHS-3431 and DHHS-3445) that SARS-CoV testing is requested. It may need to be handwritten in "Other" category.

III. Shipping Instructions

Any suspect SARS specimen should be shipped as a DIAGNOSTIC SPECIMEN. The shipper (hospital or clinic) – not the transport company – is responsible for the shipment until the package reaches the consignee (NCSLPH). For more complete instructions, please see either of the following websites:

http://www.fedex.com/us/services/packaging/diagnosticbrochure.pdf
or
http://www.cdc.gov/ncidod/sars/pdf/packingspecimens-sars.pdf

If using the State Courier system for transportation, the viral culture kits from the NCSLPH may be used.

A. Primary Packaging

The primary receptacle(s) must be water-tight. 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 entire contents of the primary receptacle is the diagnostic specimen.

B. Secondary Packaging

1. 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 diagnostic specimens including 1.2 meter (3.9 feet) drop test procedure. Since infectious substance packaging surpasses the requirements for diagnostic specimen packaging, the IATA Packing Instruction 602 can be used as well. Infectious substance packaging will have the required specification markings on packaging:

   ("UN" will be in a circle): e.g.,  **4G/CLASS 6.2/99/GB/2450**

2. Secondary packaging must be watertight. 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. Must be large enough for shipping documents, e.g. air waybill.

C. Outer Packaging

1. The outer packaging must not contain more than 4 L or 4 kg. Both dry ice and cold packs must be placed outside the secondary packaging.

   a. Dry Ice**:** Packaging must permit the release of carbon dioxide gas and not allow a build-up of pressure that could rupture the packaging.

   b. Cold Packs**:** The cold packs must be leak-proof.

   c. Each package and the air waybill must be marked with the following exact wording:

      UN 3373 DIAGNOSTIC SPECIMEN
      PACKED IN COMPLIANCE WITH
      IATA PACKING INSTRUCTION 650

   d. An itemized list of contents must be enclosed between the secondary packaging and the outer packaging. Place in a sealed plastic bag to protect from moisture. A Shipper's Declaration for Dangerous Goods is **NOT** required for Diagnostic Specimens.

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

Please coordinate shipment of specimens for SARS-CoV testing through General Communicable Disease Control Branch **(919-733-3419)** or with NCSLPH **(919-733-7834)** directly.

IV. NCSLPH Standard Operating Procedures

A. Notification

When the specimens from first group of suspected SARS-CoV cases arrive at NCLSPH, the following internal staff will be notified by phone, email, or verbally: Laboratory Director, Assistant Laboratory Director, Microbiology Supervisor, Virology/Serology Supervisor, Serology Team, and Bioterrorism and Emerging Infectious Disease Team.

B. Laboratory Handling

Once in the laboratory, the specimens will either be split into aliquots and assayed here (upper/lower respiratory samples and NP/OP swabs, blood components and stool) or sent directly to the CDC (tissue and autopsy specimens). For those specimens to be split into aliquots, the splits should take place in BSL-3 laboratory room number 511C, and subsequently processed in room number 511D. The specimens will be aseptically split into 4 separate, sterile, fully sealed containers. All personnel working with SARS-CoV specimens must have baseline serum drawn.

1. **Molecular Testing:** For all specimens to be assayed in the laboratory, RT-PCR will be performed first (except for convalescent serum). If sample is an acute serum sample, it will be taken to the Serology Team immediately following negative RT-PCR results. For RT-PCR results, allow 2-3 working days for sample preparation, RT-PCR testing and reporting of results.
    a. The four aliquots will be used for a) Test for detection of SARS-CoV viral RNA b) Repeat testing c) Confirmatory Testing and d) To test for other agents.
    b. If control guidelines are not met with the initial sample, a new aliquot will be tested.
    c. If initial result is NEGATIVE, specimen is considered negative for SARS-CoV and testing for other respiratory pathogens may be indicated.
    d. If initial result is POSITIVE from one source, a new aliquot will be tested for reproducibility on the next day.
    e. If new aliquot shows negative results, CDC will be consulted.
    f. If new aliquot shows positive results, THIS IS A PRESUMPTIVE POSTIVE RESULT FOR SARS. NCSLPH will send an untested aliquot to the CDC for confirmation (include copy of data).
    g. If initial result shows positives from two clinical specimens from different sources, or two clinical specimens collected from the same source on different days, THIS IS A PRESUMPTIVE POSITIVE.
2. **Serological Testing:** Acute serum will be tested by EIA, but should be paired with convalescent serum taken >28 days after onset of illness. Some people do not test positive for antibodies to SARS-CoV until three weeks or more post-onset of illness. Therefore, a negative serology result is only considered to be a final negative result if serum specimen was collected more than 28 days post-onset of illness. For EIA results, allow 5 working days (logging in specimens, antibody screening, antibody quantification and reporting of results).
    a. The four aliquots will be used for i) Test for antibodies to SARS-CoV ii) Repeat testing iii) Confirmatory Testing and, iv) To test for other agents.
    b. If acute serum result is NEGATIVE, specimen is negative for SARS-CoV and convalescent serum should be collected >28 days post onset of illness.
    c. If initial result is POSITIVE, test will be repeated for reproducibility AND an aliquot will be sent to the CDC for confirmation (include copy of data). A follow up serum will be requested as well.
    d. If repeat testing shows negative results, additional testing or consultation with CDC may be required.
    e. If repeat and/or follow up serum testing shows positive results, THIS IS A PRESUMPTIVE POSITIVE RESULT FOR SARS. Standard serological interpretations of positive results will apply as follows: serum antibodies detected to SARS-CoV in a single serum specimen; OR a four-fold or greater increase in SARS-CoV antibody titer between acute and convalescent phase serum specimens tested in parallel; OR negative SARS-CoV antibody test result on an acute-phase serum and a positive SARS-CoV antibody test result on a convalescent-phase serum tested in parallel.

PLEASE NOTE: All presumptive positives from RT-PCR or serological testing performed at SLPH will be confirmed at the CDC until the CDC guidance changes.