

**Level 0* Guidelines for Collection of Specimens
from Potential Cases of SARS in Michigan**

Key Messages

- Consult your state or local health department to determine the appropriateness and details of SARS testing before submitting samples for testing.
- It is preferable to collect multiple specimens from different sites and at different times during illness.

*(These guidelines may change when a new SARS case is documented anywhere in the world. Please continue to check this website for the latest recommendations.)

Please contact the MDCH Bureau of Epidemiology (BOE) at 517-335-8165 (517-335-9030 after hours/weekends) for consultation to determine whether patients potentially meet the SARS case definition before collecting and shipping specimens for SARS testing. Specimens will not be tested by MDCH Bureau of Laboratories (BOL) without approval by BOE. When possible, collect multiple specimens for testing. For example, collect specimens from two different body sites on the same day (e.g. one nasopharyngeal swab and a stool specimen or another respiratory specimen and serum) and additional specimens later during the illness. (See table that follows for details.)

NOTE: Positive results from testing at laboratories outside of the public health system will not be used to determine if the patient meets the epidemiological case definition and be included in the state and national disease statistics. In the absence of SARS cases worldwide, positive results are most likely to be false-positives. Clinical laboratories should save an aliquot of samples sent to commercial or reference laboratories or alternatively collect multiple new samples from the patient to submit to MDCH BOL. Preliminary positive results received from non-public health labs must be retested at MDCH BOL, which uses reagents and methods validated at the Centers for Disease Control and Prevention.

**Priority specimens to collect during the course of illness for
evaluation of potential cases of SARS* in Michigan**

Specimen	Submit Using	< 1 week post symptom onset	1-3 weeks post symptom onset	> 3 weeks post symptom onset
Serum ** (collect in serum separator tube)	MDCH Unit 45	++	++	++
Respiratory (deep cough sputum preferred sample)	MDCH Unit 45	++	++	+
Stool	MDCH Unit 45	+	++	++

*Priority is based on the likelihood that the specimen will be positive in a SARS-CoV-infected person.

**To rule out SARS serologically, it is important to collect serum > 28 days post onset.

++Priority specimen(s)

In the absence of SARS worldwide (Level 0), patients hospitalized with radiographic evidence of pneumonia should be evaluated for the most likely alternative diagnoses. Agents/tests which might be included in this process:

- CBC with differential
- Pulse oximetry
- Blood cultures
- Sputum culture and Gram's stain
- Chlamydia pneumoniae*
- Human metapneumovirus (MDCH is implementing this CDC-developed test)
- Viral respiratory pathogens (influenza A and B, RSV, Adenovirus, Parainfluenza virus, rhinovirus)
- Legionella and pneumococcal urinary antigen
- Mycoplasma pneumoniae*

Laboratory Biosafety Guidelines for Michigan Laboratories Handling And Processing Specimens Associated with SARS

Key Messages

- Laboratories performing routine hematology and clinical chemistry studies may handle potential SARS specimens similarly to specimens containing other blood borne pathogens (e.g. hepatitis or HIV, see specific biosafety guidelines at www.cdc.cio/od/ohs/biosfty/bmbl4/bmbl4s7f.htm).
- Laboratories performing serology or RT-PCR testing should handle potential SARS specimens using Standard Precautions (previously Universal Precautions).
- A detailed description of recommended facilities, practices, and protective equipment for the various laboratory biosafety levels (BSLs) can be found in the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories Manual at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm

It is estimated that several thousand diagnostic specimens from patients with SARS have been processed in routine clinical laboratories throughout the world and to date there have been no reported cases of SARS illness among laboratory workers performing diagnostic assays; however, there has been one reported case in a research laboratory setting where the SARS-CoV was being propagated. Until more information about the transmission of the SARS agent in the laboratory setting is known, precautions should be taken in handling these specimens. Effective and timely communication between clinical and laboratory staff is essential in minimizing the risk incurred in handling specimens from patients in whom SARS is suspected. Specimens from patients who may have SARS should be labeled accordingly and the laboratory should be alerted to insure proper specimen handling. Listed below are biosafety guidelines for handling these specimens.

Blood Specimens (blood, serum, plasma)

These specimens may be handled using Standard Precautions (previously Universal

Precautions). Careful attention should be given to hand hygiene after removal of gloves and especially before touching the eyes or mucosal surfaces.

Any procedure with the potential to generate fine particulate aerosols (e.g. vortexing or sonication of specimens in an open tube) should be performed in a biological safety cabinet (BSC). The use of sealed centrifuge rotors or sample cups, if available, should be employed for centrifugation. Ideally, these rotors or cups should be loaded and unloaded in a BSC. Procedures performed outside of a BSC should be performed in a manner that minimizes the risk of exposure to an inadvertent sample release.

Work surfaces and equipment should be decontaminated after specimens are processed. Standard decontamination agents (e.g., bleach) that are effective against lipid-enveloped viruses should be sufficient.

Other Specimens (e.g., respiratory secretions, stool, urine, tissue)

The following activities should be performed in BSL-2 facilities with standard BSL-2 work practices:

- Pathologic examination and processing of formalin-fixed or otherwise inactivated tissues.
- Molecular analysis of extracted nucleic acid preparations.
- Electron microscopic studies with glutaraldehyde-fixed grids.
- Routine examination of bacterial and mycotic cultures.
- Routine staining and microscopic analysis of fixed smears.
- Final packaging of specimens for transport to diagnostic laboratories for additional testing. Specimens should already be in a sealed, decontaminated primary container.

The following activities involving manipulation of untreated specimens should be performed in BSL-2 facilities and in a Class II biological safety cabinet:

- Aliquoting and/or diluting specimens.
- Inoculation of bacterial or mycological culture media.
- Performing diagnostic tests that don't involve propagation of viral agents in vitro or in vivo.
- Nucleic acid extraction procedures involving untreated specimens.
- Preparation and chemical- or heat-fixing of smears for microscopic analysis.

Laboratory workers should wear protective equipment including disposable gloves and laboratory coats. Work surfaces should be decontaminated on completion of work with appropriate disinfectants (e.g., 10% bleach solution) and all disposable waste autoclaved or disposed of as regulated medical waste.

Any procedure or process that cannot be conducted within a biological safety cabinet requires use of the appropriate combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors). Acceptable methods of respiratory protection include a properly fit tested NIOSH approved filter respirator (N95 or higher); or powered air-purifying respirators (PAPRs) equipped with high efficiency particulate air (HEPA) filters. Accurate fit testing is a key component of effective respirator use. Personnel who cannot wear fitted respirators because of facial hair or other fit-limitations should wear loose fitting hooded or helmeted PAPRs. Centrifugation should be carried out using sealed centrifuge cups or rotors that are unloaded in a biological safety cabinet.

When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors) **must** be used.

The following activities require BSL-3 facilities and BSL-3 work practices:

- SARS-CoV propagation in cell culture.
- Initial characterization of viral agents recovered in cultures of specimens from confirmed or highly suspect SARS cases.

Specimen Collection Procedures for Michigan

Before collecting specimens, review infection control precautions at:

<http://www.cdc.gov/ncidod/sars/infectioncontrol.htm> and
<http://www.cdc.gov/ncidod/sars/aerosolinfectioncontrol.htm>.

NOTE: SARS test results are significantly impacted by the handling of specimens in the pre-analytical phase. Viral titer is very low early in disease, so poor specimen quality or timing may result in false-negative results. Likewise, improper storage may lead to degradation of the low virus titer present in the early stage of disease. False-positives may result from cross-contamination between patients/samples, so exceptional care is required in collecting, handling and labeling samples, especially those for PCR.

Informed Consent

MDCH has fully validated the SARS serologic assay with protocol and reagents supplied by CDC. However, due to the lack of positive samples with which to validate the PCR assay, its use is considered to be investigational at this time and requires a signed patient consent form. These forms are available on the MDCH website; their use will be coordinated by local public health personnel with the attending physician or infection control personnel. Once testing has been approved by BOE, specimens should be forwarded expeditiously to MDCH for testing. The consent form can follow later.

Respiratory Tract Specimens

Respiratory specimens should be collected as soon as possible in the course of the illness for most respiratory pathogens. The likelihood of recovering most viruses diminishes markedly >72 hours after symptom onset. In contrast, for SARS-CoV, the amount of virus may increase later in the course of the illness.

Seven types of respiratory specimens may be collected for viral and/or bacterial diagnostics. These include: 1) nasopharyngeal wash/aspirates; 2) nasopharyngeal (N/P) swabs; 3) oropharyngeal swabs; 4) bronchoalveolar lavage; 5) tracheal aspirate 6) pleural tap or 7) sputum (see chart above for recommended specimen type). Nasopharyngeal wash/aspirates are the specimen of choice for detection of most respiratory viruses and are the preferred collection method among children aged <2 years. However, sputum is the specimen of choice for detection of SARS when it can be obtained.

Upper respiratory tract

- Collection of nasopharyngeal wash/aspirate
Have the patient sit with the head tilted slightly backward. Instill 1 - 1.5 ml of nonbacteriostatic 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. Each specimen container should be labeled with patient identifier and the date collected. Ship with cold packs to keep sample at 4^oC.
- Collection of nasopharyngeal or oropharyngeal swabs
Use only sterile 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 inhibit PCR testing.
 - o Nasopharyngeal swabs - Insert swab into nostril parallel to the palate and leave in place for a few seconds to absorb secretions. Swab both nostrils.
 - o Oropharyngeal swabs - Swab both posterior pharynx and tonsillar areas, avoiding the tongue. Place swabs immediately into sterile vials containing 2 ml of viral transport media. Break applicator sticks off near the tip, avoiding creation of aerosols, to permit tightening of the cap. Each specimen container should be labeled with patient identifier and the date collected. Ship with cold packs to keep sample at 4^oC.

Lower respiratory tract

- Collection of bronchoalveolar lavage, tracheal aspirate, pleural tap
If these specimens have been obtained, half should be centrifuged and the cell-pellet fixed in formalin. Remaining unspun fluid should be placed in sterile 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®. Each specimen container should be labeled with patient identifier and the date the sample was collected. Ship with cold packs to keep sample at 4^oC.
- Collection of 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. Label with patient identifier. Ship with cold packs to keep sample at 4^oC.

Blood Components

Collection of serum for antibody or PCR testing

Acute serum specimens should be collected and submitted as soon as possible. If the patient meets the case definition, convalescent specimens should be collected > 28 days after the onset of illness.

Collect 5-10 ml of whole blood in a serum separator tube. Allow blood to clot, centrifuge briefly and transfer all resulting sera to vials with external caps, seal tightly with the cap and secure with Parafilm®. A minimum of 2.0 ml of serum, which can easily be obtained from 5 mL of whole blood, is preferred for each test.

Pediatric patients: a minimum of 1cc of serum is needed for testing; use a serum separator tube for collection.

Each specimen container should be labeled with patient identifier and the date the specimen was collected. Ship with cold packs to keep sample at 4^oC.

Stool

Collection of stool for PCR

Begin collecting stool specimens as soon as possible in the course of the illness. Although collecting earlier specimens is ideal, SARS-CoV has been detected in stool as late as one month post symptom onset.

Collect each stool specimen, (collect at least 10 cc), in a leak-proof, clean, dry container, transfer to a 50 ml conical centrifuge tube, and refrigerate at 4⁰C.

Turn-around Time for SARS Tests

Serology and PCR results can normally be expected in 2-3 workdays, depending upon testing volume. Any positive results will require confirmation by repeat testing, and possibly retesting at CDC.

Holding and Shipping Specimens

In the absence of SARS cases anywhere in the world (Level 0), specimens should be collected as early in the course of disease as possible (as soon as SARS is considered in the differential diagnosis) and held at 4⁰C during the 72 hour observation period of the patient. Once testing is approved by MDCH BOE, samples should be expeditiously transported on cold packs to MDCH BOL for testing. Complete a test requisition, **adding the approval number supplied by BOE in the ‘Submitter’s Patient Number’ space.** In the absence of SARS cases worldwide, samples will not be tested without this number. Contact the MDCH BOL if assistance is needed to expedite shipment 517-335-8063 (517-335-9030 after hours).

Packaging, shipping and transport of specimens from suspect and probable SARS cases must follow the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations at (www.iata.org/dangerousgoods/index) and US DOT 49 CFR Parts 171-180 (hazmat.dot.gov/rules.htm).

Step-by-step instructions on appropriate packaging and labeling are available at: www.cdc.gov/ncidod/sars/pdf/packingspecimens-sars.pdf.

NOTE: Specimens shipped by commercial couriers, which may utilize air transport even when delivering within the state of Michigan, must be packed in 6.2 packaging as “diagnostic specimens”.

**Recommended specimens for
evaluation of potential
cases of SARS in Michigan**

Outpatient

In patient

Fatal

Upper Respiratory:

1. Nasopharyngeal wash/aspirate
2. Nasopharyngeal and oropharyngeal swabs

Lower Respiratory:

Sputum

Blood:

Serum: Acute and Convalescent (> 28 days post onset)

Stool

Upper Respiratory:

1. Nasopharyngeal wash/aspirate
2. Nasopharyngeal and oropharyngeal swabs

Lower Respiratory:

1. Bronchoalveolar lavage (BAL), tracheal aspirate or pleural tap
2. Sputum

Blood:

Serum: Acute and Convalescent (> 28 days post onset)

Stool

Tissue:

1. Fixed tissue from all major organs (e.g. lung, heart, spleen, liver, brain, kidney, adrenal s)
2. Frozen tissue from lung and upper airway (e.g. trachea, bronchus)

Upper Respiratory:

1. Nasopharyngeal wash/aspirate
2. Nasopharyngeal and oropharyngeal swabs

Lower Respiratory:

Bronchoalveolar lavage (BAL), tracheal aspirate or pleural tap

Blood:

Serum

Stool

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). For information specific to Michigan response, call MDCH BOE at 517-335-8165 or Dr. Hema Kapoor at MDCH BOL at 517-335-8099.

