FACT SHEET

SARS Laboratory Diagnostics

When to Test for SARS

In the absence of documented SARS transmission, diagnostic testing for SARS-associated coronavirus (SARS-CoV) should NOT be considered unless the clinician and health department have a high index of suspicion for SARS (e.g., a hospitalized pneumonia patient has a possible SARS exposure during travel and no other explanation for their pneumonia).

Successful SARS Diagnosis

A successful laboratory diagnosis of SARS infection requires obtaining the correct type of specimen(s), based on the stage of the illness, using validated assay methods, and understanding the strengths and limitations of the diagnostic assays used. All laboratory results should be interpreted in the context of the clinical and epidemiologic findings.

Current Diagnostic Methods for SARS-CoV

Laboratory assays for SARS-CoV are based on either the detection of the virus or virus products, or detection of an antibody response to viral infection. Isolation in Vero E6 cells and electron microscopy played a critical role in the early identification of SARS-CoV, however, these methods are not suitable for routine diagnoses because they lack sensitivity and viral culture requires biosafety level III containment. Current detection methods for SARS-CoV include real-time reverse transcription polymerase chain reaction (RT-PCR) assay for detection of viral RNA and enzyme immunoassay (EIA) for detection of antibodies to SARS-CoV. The real-time RT-PCR assay is highly sensitive, detecting between 1 and 10 RNA transcript copies per reaction, and utilizes primer & probe sets to three independent sites along the SARS-CoV genome to assure specific detection of SARS CoV. Serology is the "gold-standard" for diagnosis of SARS-CoV infection. The SARS EIA uses a lysate of SARS-CoV infected Vero E6 cells as antigen. Serosurveys with the SARS EIA have demonstrated low or undetectable levels of antibody to the SARS-CoV in the general population. No cross-reactivity has been observed in validation studies with serum specimens containing antibodies to other human coronaviruses.

Specimen Selection for RT-PCR

Detection of SARS-CoV infection by RT-PCR within the first few days following onset of symptoms has been problematic because of extremely low virus titers in respiratory specimens during the early stages of infection. To increase the rate of detection of SARS positive cases, it is recommended that more specimens and multiple specimen types be collected. Respiratory tract specimens are valuable, with lower respiratory tract specimens (e.g., sputum, bronchial alveolar lavage) preferred over upper respiratory tract specimens (e.g., washes, aspirates, swabs) when available. New evidence suggests that blood collected within the first few days of onset of illness may be useful for detection of SARS-CoV RNA. Stool may be the best specimen for detection of SARS-CoV beginning about one week after onset of illness and may continue to be positive for several weeks thereafter.

For the patient to be considered positive for SARS-CoV infection by RT-PCR, current recommendations specify that a positive result on one specimen should be confirmed in a reference laboratory and a second specimen, either from another site or collected at a different time, should also be confirmed positive.

Special Considerations for Interpreting SARS RT-PCR Results

The CDC real-time RT-PCR assay has proven both sensitive and specific for detection of SARS-CoV. However, as with all PCR assays, there is potential for both false positive and false negative results. False positive results can occur from contamination with previously amplified DNA during specimen processing or preparation of the amplification reaction. Cross-contamination between patient specimens can also occur during the course of collection, transport, storage, and processing. Careful monitoring of all steps between specimen collection and RT-PCR should be made to minimize the possibility of false positive results. All positive results should be confirmed by repeat testing from a new extraction of the original sample AND having the sample retested in a second qualified laboratory (e.g., CDC) before reporting. False negative results can occur from insufficient SARS-CoV RNA in the sample or degradation of RNA during storage and transport of the specimen. Optimal specimen collection as described above and careful handling of the specimen can help minimize false negative results.

Serology Testing

Detecting SARS-CoV antibodies by EIA is a less ambiguous approach to diagnosing SARS-CoV infection than is RT-PCR, but antibodies are often not be detectable early in the course of illness or if the patient is immune suppressed and unable to mount a good antibody response. Seroconversion from negative to positive or a four-fold rise in antibody titer from acute to convalescent serum specimens confirms recent infection. At the present time, when prior infection is exceedingly rare, a positive serology result is also considered indicative of acute infection with SARS-CoV in a patient with a SARS-like illness. Although many SARS patients develop antibodies to SARS-CoV within as few as 8 to 10 days, some patients do not test positive until more than 28 days after the onset of illness. For patients with a negative antibody test result with specimens collected $\leq$28 days after illness onset, an additional serum specimen collected >28 days after onset should be tested. A negative antibody test after 28 days from onset of illness can be used to rule out SARS-CoV infection.

How CDC is Improving SARS Diagnostics

CDC is actively working to improve existing assays and to develop new tools for diagnosis of SARS-CoV infection. Serologic EIAs using recombinant viral antigens to replace cell culture derived virus and to detect SARS-CoV IgM antibodies are currently under evaluation. CDC is also preparing new protocols to guide the type and timing of specimen collection and processing to try to improve the ability to detect low levels of SARS-CoV early in infection and is developing a quality assessment program to monitor the accuracy and reliability of the tests. By working with partners, CDC will continue to disseminate the most up to date assay protocols and reagents to the laboratory response network (LRN) and state and local public health laboratories.

CDC WEB Resources

Interim Guidelines for Laboratory Diagnosis of SARS-CoV Infection
http://www.cdc.gov/ncidod/sars/labdiagnosis.htm
Handling & Processing Specimens Associated with SARS
http://www.cdc.gov/ncidod/sars/sarslabguide.htm
Guidelines for Collection of Specimens from Potential Cases of SARS
http://www.cdc.gov/ncidod/sars/specimen_collection_sars2.htm
Specimen Submission Form for Potential Cases of SARS
http://www.cdc.gov/ncidod/sars/pdf/specimensubmissionform-sars.pdf
Guidelines for International Specimens Associated with SARS
http://www.cdc.gov/ncidod/sars/intspecimens.htm
Packing Diagnostic Specimens for Transport
http://www.cdc.gov/ncidod/sars/packingspecimens-sars.htm
Emerging Infectious Diseases (Journal)
http://www.cdc.gov/ncidod/EID/sars_links.htm