Children's Hospital Colorado

CONTAGIOUS COMMENTS
Department of Epidemiology

COVID-19 Testing

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Coronavirus Disease 2019 (COVID-19) is the disease caused by the novel SARS Coronavirus 2 (SARS-CoV-2) that emerged in China in December 2019 and is currently causing a worldwide pandemic. This Contagious Comments addresses the two general approaches to laboratory diagnosis of COVID-19:

- Active infection: Detection of SARS-CoV-2 RNA by PCR of respiratory specimens
- Host response to infection: Detection of SARS-CoV-2 antibodies (IgM, IgG) in blood

PCR Testing

The gold standard for clinical diagnosis of COVID-19 remains PCR testing of respiratory specimens during the acute phase of illness. Nasopharyngeal washes or swabs are preferred specimen types because of the high viral load detected at these sites early in the course of disease. In children progressing to more severe disease, lower respiratory tract specimens (tracheal aspirates, bronchoalveolar lavages) can be tested if upper respiratory specimen testing is negative and COVID-19 is suspected.

The Children's Hospital Colorado Clinical Microbiology Laboratory is currently running four different platforms that detect viral RNA of SARS-CoV-2: CDC SARS-CoV-2 PCR assay, DiaSorin/Simplexa COVID-19 Direct, Abbot m2000 Real-Time SARS-CoV-2, and Cepheid Xpert Xpress SARS-CoV-2. Workflow processes are in place to provide optimal turnaround time to guide isolation and medical decision-making for patients, and results are guaranteed within 24 hours of specimen receipt in the lab. SARS-CoV-2 PCR and the Respiratory Pathogen Panel (RPP) are separate tests and must be ordered separately but can be run on the same collected specimen. Of note, the RPP detects four previously recognized seasonal coronaviruses (HKU1, NL63, 229E, OC43), but does not detect SARS-CoV-2 and there is no cross-reactivity between these targets.

All four SARS-CoV-2 assays in use at CHCO are able to detect very small amounts of virus in a sample, with a limit of detection of 100 – 500 viral genomic RNA copies/mL. Because all SARS-CoV-2 PCR assays are currently approved under an FDA Emergency Use Authorization and there is no gold standard for comparison, overall clinical sensitivity and specificity of these assays has not been reported. When considering performance of SARS-CoV-2 tests, it is important to distinguish the difference between analytic sensitivity and clinical sensitivity. Analytic sensitivity assesses the ability of the test to detect viral RNA when it is present within the sample in the laboratory. All assays utilized at CHCO had 100% agreement when tested on over 100 spiked and clinical samples by the

manufacturer, suggesting high analytical sensitivity (ie, when a sample contains viral load above the limit of detection, risk of a false negative is low). Clinical sensitivity assesses the ability of the test to diagnose a patient who has the disease, which is influenced by the type, quality, and timing of specimen collection. With COVID-19, clinical false negatives have been reported in patients who have been re-tested with another sample from a different site and/or at a different point in time but are not usually a result of test performance.

Antibody Testing

Serological tests for COVID-19 detect antibodies made by the host response against SARS-CoV-2. Different assays may detect different types of antibodies (ie, IgM, IgG, and IgA) and antibodies to different viral antigens (ie. full-length spike protein, the S1 subunit or the receptor binding domain of the spike protein, or the nucleocapsid protein). Given delays from infection to antibody production, the timing of specimen collection greatly influences test performance. Though timing and presence of a serological response may vary greatly between individuals, IgM and IgG antibodies are detectable in blood in around half of patients 1-2 weeks after SARS-CoV-2 infection. Cross-reactivity of antibodies against SARS-CoV-2 and other seasonal coronaviruses (eg, HKU1, NL63, 229E, OC43) can affect specificity of serologic testing results and varies between assays.

As a multitude of serological tests have been more recently developed, issues with test quality and interpretation remain challenges to their implementation. The Colorado Department of Public Health and Environment and several other state health departments, Infectious Disease Society of America, and World Health Organization have raised concerns about implementation of serological testing for individual patient-level clinical diagnosis of COVID-19 until more evidence is available to guide their use. Most importantly, until further evidence of protective immunity is available, serology results should not be used to make decisions regarding health care worker staffing, need for personal protective equipment, or personal decisions on preventive measures such as social distancing and face coverings.

The Children’s Hospital Colorado Immunology Laboratory has internally validated a serologic assay to identify individuals with a history of COVID-19 who have antibodies to SARS-CoV-2 and can provide plasma donation for therapeutic use. The assay being utilized is the Epitope Diagnostics ELISA assay which detects IgM and IgG against the SARS-CoV-2 nucleocapsid antigen with sensitivity of 64% (IgM) and 81% (IgG) and specificity of 97% (IgM) and 99% (IgG) based on the laboratory’s extensive validation studies and internal analysis of serum samples from SARS-CoV-2 PCR positive individuals, SARS-CoV-2 PCR negative individuals who were positive for one of the four non-SARS-CoV-2 human coronaviruses, and pre-pandemic serum samples. In addition to donor screening, the primary utility of SARS-CoV-2 serologic assays at this time is to provide epidemiologic data to guide the public health response to COVID-19, and other assays are being evaluated for this purpose. Seroprevalence data (determining what proportion of the population has been exposed to an infection) is important to understanding the scale of the epidemic in differing geographic and organizational settings to help tailor preventative measures moving forward.

For clinical diagnosis, antibody tests for infectious diseases have typically been most useful to diagnose patients who present late or with a very low viral load below the detection limit of RT-PCR assays. There is limited utility of serologic testing early in the course of disease, as it is difficult to determine whether a negative test result signifies that the patient does not have COVID-19 or has been infected with SARS-CoV-2 but not made an antibody response at that time. Given an estimated

prevalence of COVID-19 in Colorado currently well below 5% and the reported specificity of some FDA-authorized SARS-CoV-2 antibody tests, a positive test result may more likely be a false positive than a true positive. Over time as the prevalence of COVID-19 increases in Colorado, the positive predictive value of SARS-CoV-2 serologic assays will increase. There remains limited data on the extent to which antibodies to SARS-CoV-2 convey protective immunity against COVID-19 and the durability of that antibody response.

Summary

SARS-CoV-2 PCR testing of respiratory specimens as early in the course of disease as possible remains the gold standard for diagnosis of COVID-19. Serologic testing to detect antibody response can provide epidemiologic seroprevalence data to guide public health response, but further evidence is needed to guide result interpretation for patient-level serologic diagnosis for clinical purposes.

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