CORE DIAGNOSTICS

Clinical Assessment of COVID-19: Is there an advantage to combining serology testing with viral RNA or Antigen?

What studies are showing about combining serology testing with Viral RNA or Antigen tests

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Summary

The COVID-19 pandemic continues to rage throughout the world with new outbreaks and community spread occurring even in areas previously thought to have contained the virus. Clinicians and healthcare workers have been challenged by COVID-19 since its emergence. Beginning with the selection of which tests to use to diagnose COVID-19 together with the timing of when they should be used, extending throughout the course of patient management, clinicians and scientists have experienced a steep learning curve due to the rapid and global progression of the disease. However, uncertainty remains, particularly when considering the diagnostic assessment and identification of asymptomatic patients or patients who present later in the course of their disease.

The SARS-CoV-2 virus that causes COVID-19 is a new Human SARS-CoV virus that was unknown prior to the start of the 2020 pandemic. Since it first appeared and its genomic sequence was determined in January of 2020, several different types of diagnostic tests have been developed to identify the virus and the body's response to it. These tests are used to make the pathogenic diagnosis of COVID-19, initiate treatment and manage patients through the disease course, including establishing the time needed for isolation or quarantine, as well as time for release from the hospital. The rapidity with which the tests were developed combined with the pace at which data about the virus and the disease state it caused were reported has provided much information from essentially real time studies. Because the impact of the SARS-CoV-2 virus in terms of the severity of symptoms and impact on health varies greatly between individuals with up to 45% presenting as asymptomatic infections[1], identifying individuals with infections is essential, but can be challenging. In addition, it has been difficult to conduct thorough contact tracing to identify the pattern of spread of COVID-19 within communities. One study conducted in India found that while 70% of infected individuals caused little spread, there were some 'super spreaders' with high risk behaviors - defined as travel exposures and close proximity to an infected individual for > 6 hours - who had a risk of transmission from an index case to an exposed contact of almost 80%. Even in this study, however, the successful tracing was limited at approximately 19-20%. A finding of interest was that there was a larger rate of spread in the community (2.6%) compared to the healthcare setting (1.2%) with the greatest rate of spread being within the home (9.0%)[2].

The need to identify acute but asymptomatic infections while assessing and tracking a patient's immune response and recovery from infection has become of paramount importance because of the increasing desire to keep society functioning. Studies have been conducted since the start of the pandemic to assess ways to best utilize different types of tests (e.g. RNA/Antigen, serology/antibody) to identify acute infections, together with previous and asymptomatic infections, while assessing and tracking a patient's immune response and recovery from the viral infection. Several studies have concluded that combining an antibody test with an RNA test may help identify patients with acute infections who may be missed with viral RNA testing alone. The purpose of this paper is to provide clinicians who are tasked with evaluating and treating patients with COVID-19 a summary of studies that evaluated antibody testing and considered its use together with a viral RNA test.

The primary tool currently used to confirm the clinical suspicion of COVID-19 is a viral RNA test, most commonly a RT-PCR molecular test. More recently, viral antigen tests have become available. Both tools identify the virus itself and although the sensitivity and specificity of the tests that are currently available is excellent, infections are still often missed. Studies have shown that SARS-CoV-2 RNA is present at the sample site, most often the nasopharyngeal area, for only a relatively short time after symptoms begin, typically up to 7 days after symptom onset[3]. Studies have shown that viral antigen is present at the sample site for an even shorter time and the CDC warns that antigen levels in specimens collected beyond 5-7 days of the onset of symptoms may drop below the limit of detection of the test[4]. Because symptom severity varies and people may not recognize early on that they are becoming ill, they may not present for testing early enough during their illness when the SARS-CoV-2 virus is still present to be captured on a sample swab. Other individuals who fail to develop symptoms and are unaware that they have had exposure to the virus may never present for testing while still shedding the virus and potentially infecting others.

A report published in ***Annals of Internal Medicine*** by Kucirika, et al, estimated the rate of false negative SARS-CoV-2 RT-PCR results based on the time since infection by reviewing seven previously published studies that reported RT-PCR performance data based on time since symptom onset. It was noted that the false negative rate on the day symptoms started was 38%, which dropped to 20% at three days after symptom onset but then increased again. Other studies indicated repeat testing using multiple swabs for sampling may be required to capture and identify the virus. Fortunately, these challenges were recognized early on and numerous studies have been carried out at multiple sites using a variety of different commercial assays to work to determine if adding serology testing might enhance the ability to identify recent as well as prior SARS-CoV-2 infections. Because SARS-CoV-2 is a human virus, the human antibody response to this virus was studied and assessed. The earliest studies assessing antibody response were undertaken in Asia, which is not surprising as this was the site with the first evidence of a COVID-19 outbreak with submissions for publication received as early as February 2020[5].

Guo, et al, reported some of the earliest findings in ***Clinical Infectious Diseases.*** The authors noted that the detection of viral RNA requires presence of the viral genome in sufficient amounts at the sample site for collection and that missing the time window of viral replication could provide a false-negative result. An incorrect sample collection technique could further impact the capture of the viral genome for testing and the ramifications of a false-negative were large with the potential for continued spread of the virus. In this early study of the humeral (immune) response to SARS-CoV-2, the authors reported their findings of the time kinetics of IgA, IgM and IgG response. Guo proposed conducting an antibody test in patients who had a negative PCR (RNA) despite other findings suggesting antibody testing could aid in the detection of recent or prior COVID-19 even in subclinical (asymptomatic) cases[6].

Antibody responses to SARS-CoV-2 were also studied early on by Zhao, et al and reported in ***Clinical Infectious Diseases.*** These authors similarly noted that the timely and accurate diagnosis of SARS-CoV-2 infection (e.g. COVID-19) is essential to provide appropriate treatment and limit spread with PCR RNA detection as the only way to confirm the diagnosis. It was stated that the performance of RNA PCR tests depends on many factors, including the sample type and collection and stage of infection of the patient. The authors noted one benefit of serological testing was the faster turnaround time, but that to ascertain the value for clinical use, the host antibody response needed to be understood. They investigated and reported on the dynamics of antibody response to SARS-CoV-2 throughout the course of COVID-19. The authors stated that antibody testing may play several vital roles, and in their opinion, this included use in the evaluation of patients at the initial visit who have not been tested with or confirmed by RNA because the antibody result increased their confidence with their assessment. They concluded that combining RNA with antibody tests significantly raised their ability to identify patients with recent or prior SARS-CoV-2 infection[3].

In a similar early study, Jin, et al, reported the findings of their study in ***International Journal of Infectious Diseases.*** These authors noted that RT-PCR testing of viral RNA has been the primary means of diagnosis of COVID-19, but the risk of false-negative results exists in part because of low viral loads. Their study aimed to investigate the value of serologic detection of SARS-CoV-2 (e.g. antibodies) to assess patients with recent or prior infection. In addition, this study aimed to assess the dynamic variance of viral antibodies to SARS-CoV-2. Based on their results, the authors concluded that viral serological testing is an effective means of identifying recent or prior SARS-CoV-2 infection and that COVID-19 should be considered when serum IgM or IgG are positive. They also noted, however, that in early stages, viral antibodies may not yet have increased such that a negative antibody result in the early stage of infection cannot exclude COVID-19 (SARS-CoV-2) infection[7].

All of these early studies demonstrated that when the SARS-CoV-2 virus was no longer sufficiently present for collection and detection, the use of antibody serology testing increased the ability to identify recent as well as prior SARS-CoV-2 infections. This included those that had been asymptomatic (subclinical), presented later in the course following infection, or where patients had recovered.

Additional studies have been completed and others are still in progress that are evaluating the use of serology together with RNA testing to evaluate patients with suspected COVID-19. The studies reported continue to support the benefit of conducting serology testing when making the assessment of recent SARS-CoV-2 infections. Serology testing may be most beneficial when the patient presents to the medical provider for evaluation in the course of their disease when the viral RNA is likely present in diminishing quantities at the nasopharyngeal sample site, typically at 7 days or more after the start of symptoms.

In a study reported in ***Journal of Virological Methods***, Wang reported an investigation of the feasibility of combining serology testing with RT-PCR testing to identify SARS-CoV-2 when evaluating patients for suspected COVID-19. As noted by others, Wang again presented the impact on RNA detection by the quality of the sample, the sample type and the fluctuation of viral loads throughout different infection phases. Based on his study results, Wang concluded that the joint method of conducting serology (antibody) together with RT-PCR (RNA), was found to be more sensitive for detection of SARS-CoV-2 infection. It was recommended that although the standard for identification of SARS-CoV-2 infection and COVID-19 diagnosis is RNA RT-PCR, that serology testing may be used as a complementary testing method[8].

Caturegli, et al, published a study in the ***Annals of Internal Medicine*** assessing the clinical validity and utility of SARS-CoV-2 antibodies. It was noted that a possible use of serologic testing would be to complement the RT-PCR testing (also referred to as nucleic acid amplification testing or NAAT). Serology was recommended because of the limitations of RT-PCR testing secondary to the time interval between viral exposure to time of testing and nasopharyngeal swab collection techniques. It was proposed that serology would be most useful in cases with high clinical suspicion of COVID-19 but repeated negative RT-PCR results. The study demonstrated the potential contribution of serology in the assessment of COVID-19 as an adjunct to symptom surveillance, radiographic findings and NAAT results. The authors stressed the importance of identifying SARS-CoV-2 infection in those who present with false-negative RT-PCR results to help prevent further viral disease spread. They noted the useful role that serology testing can play to accomplish this as highlighted in their study where in 5% of patients who had repeated NAAT testing (up to 5 times) all but one patient, who had limited serum collected, had recent or prior infections confirmed by serology[9].

A study reported in ***Cell Reports Medicine***, further assessed SARS-CoV-2 detection methods by nucleic acid amplification testing (NAAT) collected by nose/throat swabs with supplemental secondary serology antibody testing. The authors noted that the lack of detectable virus in the upper airway samples presents a barrier to timely and safe decisions as multiple swab samples may be required. They commented that multiple factors may lead to a negative NAAT result, not just the test sensitivity but also the sampling technique and the timing of the sample in the disease course. The authors proposed that because the antibody response becomes detectable later in the course of the infection at a time when the NAAT result on swabs from the nose or throat are more likely to become negative, the identification of the infection may benefit from a strategy to utilize both tests. The study findings supported their conclusion that NAAT testing with supplemental serology testing significantly improved the identification of SARS-CoV-2 infection and development of COVID-19[10].

As the COVID-19 pandemic has progressed, many hospital systems have made their own assessments and recommendations for testing strategies to evaluate patients with suspected COVID-19. The Veterans Health Administration (VHA) has produced a "VA SARS-CoV-2 Testing Consensus Statement" to assist clinicians with decision making when assessing patients for COVID-19. The VA statement recommends Nasopharyngeal swab sampling for detection of viral gene sequences by RT-PCR as the best test for confirming the acute diagnosis of COVID-19, but also states that serologic testing can be more sensitive in detecting patient exposures or missed infections. The VA consensus paper also notes the shortcomings of RT-PCR testing because of the timing of the specimen collection relative to the clinical presentation, issues with specimen collection and storage or viral distribution at the sampled site. Serologic testing is noted to have the advantage of identifying individuals with recent infections or previously infected individuals who have negative PCR test results[11].

These studies present information gathered across many global sites and are shared to provide information that may be valuable in clinical practice. As the COVID-19 pandemic continues, additional information will become available as the world tries to find pathways to diagnose, treat, and ultimately overcome this virus and the harm it has caused to communities throughout the globe. Although these studies are not randomized controlled studies or even prospective studies, the data collected amid this pandemic presents useful evaluations that can help with decision making when evaluating and managing patients who have had exposure to or infection with SARS-CoV-2 and developed COVID-19.

At the start of the pandemic only RT-PCR "COVID" tests were available. There are now several different types of diagnostic laboratory tests to evaluate and manage patients with COVID-19. These include those that test for the virus directly, the RNA test which identifies the viral genome or the Antigen test which tests for a viral protein, and antibody tests that pick up the presence of IgM and IgG antibodies. The table below provides an overview of what tests may be useful at different times during the course of a patient's COVID-19 infection to help manage patients and to help provide guidance as they recover. It may prove useful to you as we enter the flu season.

The table presents information related to some potential patient scenarios that may present at your health system and ideas of what types of testing you might consider to help stem the spread of COVID-19 and manage patient care.

This paper and the information below present a general survey and overview of the various tests available to identify SARS-CoV-2 infections throughout the stages of infection and provide an explanation of the utility of the available diagnostic tests.

Possible Patient Presentation Scenarios:	**Combination of tests to consider:**
As flu season approaches, consideration must be given for diagnostic testing to help distinguish the flu from COVID-19 in symptomatic patients. Deciding what your site considers to be a complete COVID panel and ordering it can help do this as it improves the pathogenic diagnostic sensitivity in the early phase of a new COVID infection (0-14 days). [2, 5, 11-16] It is important to correctly identify and treat COVID-19 patients and to control the spread of infection in the community. COVID-19 (SARS-CoV-2) RNA or Antigen identify acute infection, SARS-CoV-2 IgM indicates recent infection and IgG indicates recent or distant infection.	Flu Test + COVID-19 RNA or Antigen + SARS-CoV-2 IgM and IgG
In symptomatic patients, using a complete COVID-19 panel improves the identification of SARS-CoV-2 in the early phase of a new infection (0-14 days). [3, 6, 12-17] It is important to correctly identify and treat the patient and to control the spread of infection in the community. **Additional Benefits:** IgM and IgG results can often be provided quicker than RNA results, which may help to identify some recent infections sooner. RNA results arrive at a median of 3 days.[18]	COVID-19 RNA or Antigen + SARS-CoV-2 IgM and IgG
Reflex testing with a SARS-CoV-2 IgM test after a negative RNA or Antigen result can improve the identification of SARS-CoV-2. [3, 6, 12-17] This is important to correctly identify and treat patients and control the spread of infection in the community.	COVID-19 RNA or Antigen **negative** result with **reflex** to SARS-CoV-2 IgM
Reflex testing with a SARS-CoV-2 IgM and IgG test after a positive RNA or Antigen result will provide clinical information on the patient's immune response to the infection. Having this information can help in the follow-up treatment or monitoring plan.	COVID-19 RNA or Antigen **positive** with **reflex** to SARS-CoV-2 IgM + IgG
When RNA or Antigen testing may not be available, a SARS-CoV-2 IgM positive result can help provide an indication of a recent or prior COVID-19 infection.	SARS-CoV-2 IgM
New data is being published on the long-term health complications associated with COVID-19 infections. Adding a SARS-CoV-2 IgG test to wellness panels can help identify past infections that can be linked to future health concerns. Additional Benefit: Offering the SARS-CoV-2 IgG test as part of a wellness program may encourage patients to participate in wellness programs.	**Annual Wellness Testing:** Add SARS-CoV-2 IgG to wellness testing panels to identify past exposures
Pandemic management using COVID-19 RNA or Antigen + Antibody (serology) tests can give a full picture of the current state of active and past infections.	**Population Surveillance:** COVID-19 RNA or Antigen + SARS-CoV-2 IgM and IgG.

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The Abbott SARS-CoV-2 IgG and AdviseDx SARS-CoV-2 IgM assays have not been FDA cleared or approved. These tests have been authorized by FDA under EUA for use by authorized laboratories. These tests have been authorized only for the detection of IgG and the presence of IgM antibodies against SARS-CoV-2, not for any other viruses or pathogens. These tests are only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

** AdviseDx SARS-CoV-2 IgM is not yet commercially available*

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