Serology for SARS-CoV-2 Diagnosis and Surveillance – Current State and Future Directions

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KEY POINTS:

- **SARS-CoV-2 is a novel pathogen with many unknown virological and pathological characteristics.**
- **IgM, IgG and IgA antibody responses occur a few days to a few weeks after exposure to SARS-CoV-2 in both RT-PCR positive and negative individuals.**
- **Antibody responses occur in most, but likely not all, asymptomatic individuals and symptomatic patients. Patients with the most severe disease have been reported to have higher levels of antibody compared to patients with mild disease.**
- **The clinical accuracy (sensitivity and specificity) and utility of serological assays is emerging and will be defined by future well-designed clinical studies.**
- **Given the important unknown characteristics of antibody immunity to SARS-CoV-2, laboratory-based antibody detection methods are likely to provide accurate, clinically meaningful information compared to rapid, point-of-care devices.**

Abstract

A global pandemic is underway due to SARS-CoV-2, a novel coronavirus causing the disease known as Coronavirus Disease 2019 (COVID-19). Molecular detection is established as the primary method for direct detection of this virus in the early stages of infection. Serology (antibody) testing offers the promise for enhanced detection of infected or previously infected individuals although key questions remain about the significance of serological results. A large number of antibody tests are available, both laboratory-based methods and rapid, point-of-care (POC) tests. Current antibody tests are minimally validated with little clinical information regarding result interpretation. Given our present state of knowledge, reliance on the most accurate, reproducible diagnostic methods available will provide the most effective approach to control of this virus. Serology can be an important part of SARS-CoV-2 diagnosis and control, although it is important to understand that the level and specificity of antibodies to prevent re-infection of individuals and prevent viral transmission to others has not yet been identified.

SARS-CoV-2 Pathobiology and Diagnosis

Late in 2019, a novel respiratory coronavirus emerged and quickly spread worldwide resulting in a global pandemic which is unprecedented in modern times. This virus, named SARS-CoV-2, causes the disease known as COVID-19. Although related to other human and animal coronaviruses, SARS-CoV-2 is truly novel with many unknown virological and pathological characteristics. In the early stages of the COVID-19 pandemic, molecular testing by real-time reverse transcriptase polymerase chain reaction (RT-PCR) tests became the main method for rapid diagnosis of SARS-CoV-2 infection in symptomatic patients (Corman et al., 2020). These assays can be developed based on viral genomic sequence information, which was available during the earliest days of the pandemic, and quickly validated. Detection of the virus by RT-PCR is the most accurate method of diagnosis in the early stages of infection and has strong clinical utility. However, viral loads decline to undetectable levels in the first week(s) following infection. When this occurs, detection of antibody responses to SARS-CoV-2 potentially provides an accurate diagnostic tool.

When individuals are exposed to infectious agents such as SARS-CoV-2 an innate immune response initiates within hours. After several days post-infection, an adaptive immune response to SARS-CoV-2 typically becomes detectable. Typical adaptive responses have both cellular (e.g. antigen-specific T cells) and antibody components. Serology tests measure antibodies that specifically recognize SARS-CoV-2 proteins. Antibodies important for responses to pathogens are classified as IgM, IgG and IgA. Antibodies in the IgM subclass are the first to emerge post-infection, typically within 5 – 7 days. IgM responses are followed by IgG responses which are typically detected at 10 – 14 days post-onset of symptoms, although one SARS-CoV-2 study reported earlier detection of IgG compared to IgM in some patients (To et al., Lancet). Antibodies classified as IgA, which are common following respiratory and gastrointestinal infections, emerge along the same timelines as IgM. Both IgA and IgM titers decline relatively quickly and are often undetectable in SARS-CoV-2 patients by 21 – 30 days post-infection (Guo et al., 2020). Conversely IgG levels tend to be present for many months although given that the COVID-19 pandemic started very recently, the full length of IgG duration following SARS-CoV-2 infection is not yet defined. While SARS-CoV-2 virus can be detected by RT-PCR in most infected individuals, studies have shown that antibodies can be detected even in exposed individuals that never had a positive RT-PCR result (Zhao et al., 2020; Pan et al., 2020). Interestingly, studies have shown that SARS-CoV-2 can be detected in some patient's respiratory secretions via PCR at the same time as SARS-CoV-2 antibodies can be detected in serum (Guo et al., 2020; Zhao et al., 2020) suggesting that antibody immunity may not fully (or rapidly) eliminate viral shedding.

Although numerous studies document the presence of IgM, IgG and IgA responses along typical timelines, examination of individual results reveal that some SARS-CoV-2 infected patients have either no antibody responses or responses below typical cutoff values and therefore would be reported as negative by most serological assays (To et al., 2020) especially in the first 30 days post-infection (Zhao et al., 2020). Studies have also shown that patients with more severe infection typically have higher antibody levels detected in serum when compared to those with mild disease (To et al., 2020).

SARS-CoV-2 Serology – Methods and Applications

Broadly speaking, two types of antibody assays have been developed and placed into use for SARS-CoV-2 serology. The first type are rapid, designed for POC use, and typically based on lateral flow assay designs. The convenience, cost and immediacy of rapid tests are appealing although the accuracy and appropriate interpretation of these tests has not be defined beyond the manufacturer's claims. It is important to note that the World Health Organization (WHO) currently does not recommend the use of antibody-detecting rapid tests for patient care, although they do encourage research in this area (WHO Scientific Brief, 2020). The second category of antibody tests use a laboratory-based analysis based on ELISA or chemiluminescence techniques. Laboratory-based methods have the advantage of more sophisticated and sensitive detection techniques and provide a numerical value which allows assessment of the consistency of testing. To date, a single comparative study of laboratory-based and POC methods has been reported, with an ELISA assay demonstrating the highest levels of sensitivity and specificity (Lassauniere et al., 2020).

For both the rapid and laboratory-based methods, a number of different antigens have been used which has led to further difficulty in comparing methods. Assays using spike (S) protein, the receptor binding domain (RBD) of the spike protein and the nucleocapsid (N) protein are the most common. Importantly, virus neutralization has been correlated with ELISA detection of antibodies to both the RBD and N proteins (To et al., 2020; Okba et al., 2020).

Given that SARS-CoV-2 is related to other coronaviruses, the specificity of serological assays is important for result interpretation. The genetic relatedness of the seasonal (common) respiratory coronaviruses OC43, HKU1, NL63 and 229E viruses has led to concerns of cross-reactivity with SARS-CoV-2 antibody responses. However, the seasonal respiratory coronaviruses only have 36% or lower identity in S and N protein sequences (Okba et al., 2020) which are common targets for serology assays. Additionally, the prevalence of the seasonal coronaviruses in the US is low (0.6% to 2.2%, depending on the strain) in one large study of over 800,000 samples collected in the US from 2014 to 2017 (Killerby et al., 2018). Studies with serum known to be antibody positive to seasonal coronaviruses has shown minimal cross-reactivity and false positivity rates to SARS-CoV-2 (Guo et al., 2020, Okba et al., 2020). Additionally, testing serum collected prior to the SARS-CoV-2 pandemic from random patient populations have shown very low false positivity rates (Okba et al., 2020). Based on a combination of these lines of investigation, the potential for false positive results due to seasonal coronavirus for well-designed SARS-CoV-2 serology assays appears low.

Potential applications of SARS-CoV-2 antibody assays:

- Confirmation of a clinical diagnosis for recent infection with SARS-CoV-2.
- An indication that the patient or individual possibly has current immunity to SARS-CoV-2 and therefore may be a lowered risk for re-infection.
- Screening of large communities of people for past exposure to SARS-CoV-2, particularly in groups that do not have documented cases but likely exposure.
- When multiple samples from the same individual are tested, an indication of changes in immune status to SARS-CoV-2 over time.

Questions about the appropriate use of serology in SARS-CoV-2 diagnosis and surveillance remain. Based on current knowledge, a conservative approach is warranted. Most importantly, reasonable use of serology and interpretation of results will vary depending on the population in question, history of disease or exposure, and presence of other risk factors for disease or consequences of transmission. For example, patients with a recent history of compatible clinical signs and a positive RT-PCR result, the presence of IgM, IgG and/or IgA confirms the diagnosis and is a promising indication that patient either has or is developing immunity. In situations in which exposure to SARS-CoV-2 is suspected but not proven, antibody testing of multiple individuals from the same group may provide a reliable answer to such a question. One certainty is that the presence of SARS-CoV-2 antibodies does not eliminate an individual's potential for infection, disease or transmission to contacts although the likelihood may be reduced.

Conclusions

In summary, significant challenges remain in efforts to control COVID-19. Until results are published from well-designed studies, the most powerful use of serology tests will be on a population basis and in conjunction with sensitive and specific molecular detection techniques such as RT-PCR. Combining molecular (RT-PCR) and serology methods will provide physicians the greatest opportunity to accurately diagnose patients and prevent transmission.

FUTURE DIRECTIONS - Key questions to be answered:

- **What specificity and level of antibodies is protective against infection (or re-infection) and recovery from SARS-CoV-2 disease?**
- **Which subclass(es) of antibody, IgA or IgG or both, confers immunity most effectively?**
- **What is the duration of antibody responses and antibody-mediated immunity?**
- **What percentage of people who become infected will develop a detectable antibody response and is this dependent on the severity of clinical signs?**

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