

SYNOPSIS

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Serology testing and COVID-19 – What We Know So Far

Introduction

PHO is actively monitoring, reviewing and assessing relevant information related to Coronavirus Disease 2019 (COVID-19) and the virus that causes it, SARS-CoV-2. “What We Know So Far” documents are intended to provide a rapid review of the evidence related to a specific aspect or emerging issue related to COVID-19.

The development of these documents includes a systematic search of the published literature as well as scientific grey literature (e.g., [ProMED](#), [CIDRAP](#), [Johns Hopkins Situation Reports](#)) and media reports, where appropriate. Relevant results are reviewed and data extracted for synthesis. All “What We Know So Far” documents are reviewed by PHO subject-matter experts before posting.

As the COVID-19 outbreak continues to evolve and the scientific evidence rapidly expands, the information provided in these documents is only current as of the date of posting.

Key Points

- Serological assays detect the host antibody response to COVID-19. Tests have been developed to detect immunoglobulin A (IgA), IgM, IgG, and total antibody.
- Independent evaluations of the various COVID-19 serological assays have shown highly variable performance, with sensitivities and specificities ranging from 0%-100%.
- Correlation between COVID-19 serological assays (detecting antibodies) and neutralization assays (measuring functional neutralizing antibodies) varies, based on a limited number of studies.
- It is currently not known whether a COVID-19-specific antibody response correlates with immunity and if so, whether serological assays can adequately measure immunity.
- At this time, serological assays can only determine past exposure to COVID-19. Given the variable test performance and the uncertainty of true disease prevalence, utmost caution needs to be taken when interpreting results from serological assays.

Background

The current gold standard method for diagnosing COVID-19 is real-time reverse transcriptase polymerase chain reaction (RT-PCR), which detects the presence of viral RNA ([World Health Organization](#)). Although RT-PCR generally has high sensitivity and specificity for detecting infection, sensitivity decreases over time from symptom onset and can be affected by the quality of sample

collection. Furthermore, while RT-PCR can diagnose current infection, it cannot indicate whether someone has been previously infected with SARS-CoV-2.

Unlike RT-PCR, which detects viral nucleic acid, serological tests measure the host antibody response to the virus. In general, individuals will mount an antibody response to pathogens after infection; thus, serology can be used to assess previous exposure to pathogens, including SARS-CoV-2. Antibodies are produced through the B cell or humoral immune response and for some infectious diseases, can be used as a correlate of immunity. It is currently not clear whether this is true for COVID-19. Another arm of the adaptive immune response to infection is mediated through T cell or cellular immunity (i.e. not antibody-mediated). Serological assays do not measure cell-mediated immunity. Serological tests use an antigen to bind and detect antibodies specific to that antigen in a biological specimen. The amount of antibody in a sample is then quantified, either by reporting an antibody concentration or by reporting qualitative results (i.e. reactive, non-reactive) ([Theel et al.](#)).

The purpose of this document is to outline what we know about serological testing for COVID-19, including the types of assays currently available; determinants of sensitivity and specificity; and what we know about the implications of positive test results, as they relate to immunity to COVID-19.

COVID-19 Serology Tests

- For COVID-19, there are approximately 200 commercial serological tests available ([Foundation for Innovative New Diagnostics](#)). These include immunoassays used in the laboratory, which are either manual or automated, as well as rapid diagnostic tests administered at the point of care on an individual basis. Specific assays available include enzyme-linked immunosorbent assays (EIA), chemiluminescent immunoassays (CLIA), gold immunochromatographic assays (GICA), and lateral flow immunoassays (LFIA). However, the test characteristics of most of these assays have not been evaluated yet ([Johns Hopkins University](#)). In addition, neutralization assays, which measure functional neutralizing antibodies, can also be performed. However, these assays are not used routinely for clinical testing.
- COVID-19 serology tests have been designed to detect a variety of antibody isotypes, which are present at different times during infection ([Foundation for Innovative New Diagnostics](#)):
 - IgM – Generally, a marker of early infection. Estimates vary, but IgM is detectable in the first week, is thought to peak 12 days post-symptom onset, and persist for approximately 32 days, after which levels decline ([Tan et al.](#)).
 - IgA – Another marker of early infection. IgA is generally produced at mucosal surfaces and can be used for diagnosis of infections that affect the respiratory mucosa. Similar to IgM, IgA is detectable within the first week of illness and peaks in the second week ([Guo et al.](#)).
 - IgG – Develops later in infection compared to IgM or IgA, but continues to circulate in blood after IgM or IgA levels drop ([Bryan et al.](#), [Chen et al.](#), [Cassaniti et al.](#)). IgG can be detected in early infection in some patients but it is more often detected in samples of individuals infected with COVID-19 by the third week after symptom onset. Although it is not known how long IgG persists, in Severe Acute Respiratory Syndrome (SARS) patients, it has been found to persist for several years ([Johns Hopkins University](#), [Lin et al.](#), [Tang et al.](#)).
- COVID-19 serology tests use one or more viral antigens to detect SARS-CoV-2-specific antibodies. Most commonly the spike (S) protein or the nucleocapsid (N) protein is used:
 - S protein – This viral surface protein is involved in binding and fusion to host cells during infection. COVID-19 serological assays use either the whole S protein, or only a part of it (i.e. – the S1 region, S2 region, or the receptor binding domain) ([CDC](#)). The S protein is the

most divergent protein, potentially allowing for better differentiation from other coronaviruses and greater specificity ([Petherick et al.](#)). It is thought that, since it protrudes from the viral membrane, it is easily recognizable by the host immune system and induces the most neutralizing antibodies ([Mousavizadeh et al.](#)).

- N protein - This structural protein binds and packages viral RNA, and is the most abundant protein in SARS-CoV-2 ([Petherick et al.](#)).

Sensitivity of COVID-19 Serological Assays

The overall sensitivity of COVID-19 serological assays has been shown to vary dramatically, ranging from 0% - 100% for both IgM ([Wang et al.](#), [Xiao et al.](#), [Perera et al.](#)) and IgG ([Chen et al.](#), [Okba et al.](#), [Zhang et al.](#)). There are various determinants for serology test sensitivity, including test factors and patient factors.

- Test-related factors:
 - Generally, laboratory-based tests have higher sensitivity than point-of-care tests ([World Health Organization](#)).
 - Some studies comparing test sensitivity using the N protein and S protein (whole or part) have shown that the sensitivity varies depending on which antigen is used ([Okba et al.](#), [Zhong et al.](#), [To et al.](#)).
 - Test sensitivity may be dependent on where the laboratory cut-off is set. A lower cut-off may increase sensitivity, but potentially at the expense of specificity. For commercial kits, cut-offs are usually set by the manufacturer.
- Patient factors:
 - Since individuals are not likely to have measurable antibody immediately when they are infected, test sensitivity increases with increasing time between symptom onset and sample collection ([Gao et al.](#), [Guo et al.](#)) for all antibody isotypes. Test sensitivity peaks earlier for IgM and IgA than IgG, but declines faster too. That said, there are patients who do not develop an antibody response above the laboratory cut-off reported in the study ([Perera et al.](#), [Okba et al.](#)).
 - Some studies have reported that individuals with mild infection generate less antibodies than those with severe infection ([Okba et al.](#), [Wu et al.](#), [Zhao et al.](#)).
 - There is a paucity of data on the relationship between age and antibody response. However, one study, which has not yet been peer-reviewed ([Wu et al.](#)), has noted that middle-aged and older individuals had higher COVID-19 antibody titres than younger individuals.
 - A study investigating the relationship between COVID-19 antibody levels and co-morbidities found no statistically significant difference in the antibody concentration of COVID-19 patients with comorbidities compared to those without comorbidities ([To et al.](#)). Furthermore, the effect of immune system-compromising conditions and medications on antibody production is unknown ([World Health Organization](#)).

Specificity of COVID-19 Serological Assays

The overall specificity of the COVID-19 serological assays has also been shown to vary, ranging from 6.9% - 100% for IgM ([Guo et al.](#), [Cassaniti et al.](#), [Li et al.](#)) and 0% - 100% for IgG ([Okba et al.](#), [Cassaniti et al.](#), [Perera et al.](#)). However, several commercial assays exhibit excellent specificity. Like sensitivity, there are various determinants for serology test specificity, including test factors and patient factors.

- Test-related factors:
 - The antigen used in the serological test may affect specificity. For example, SARS-CoV-2 N protein exhibits 90% amino acid homology to SARS-CoV nucleocapsid protein ([Okba et al.](#)); the S2 region of the COVID-19 S protein also exhibits 90% amino acid homology to the SARS-CoV protein ([Okba et al.](#)). This may affect test specificity in individuals previously infected with SARS-CoV, resulting in SARS-specific antibodies binding to COVID-19 antigens.
 - There are data suggesting that the specificity of IgM- and IgA-based tests for COVID-19 may be lower than those of IgG tests ([Ma et al.](#), [Government of Canada](#)).
 - Test specificity may be dependent on where the assay cut-off is set, which for commercial tests is set by the manufacturer. A higher cut-off may increase specificity, but potentially at the expense of sensitivity.
 - Previous coronavirus infection, including with SARS-CoV, Middle East Respiratory Syndrome (MERS) and the four seasonal human coronaviruses NL63, OC43, 229E and HKU1 (in particular with OC43 and HKU1, which are betacoronaviruses) may result in cross-reactivity when testing using COVID-19 serology assays. Various studies evaluating serological assays have reported antibody cross-reaction in samples from patients who were previously infected with SARS, MERS and seasonal coronavirus infections ([Guo et al.](#), [Perera et al.](#), [Okba et al.](#)). It has been shown that over 90% of adults over 50 years of age have antibodies to seasonal coronaviruses ([Gorse et al.](#)), which may result in cross-reactivity.
- Patient factors:
 - Studies have shown that test specificity is lower in samples from patients that tested negative for COVID-19 using molecular methods compared to sera from patients before the COVID-19 pandemic. This may reflect the fact that individuals who have tested negative using molecular methods are not currently infected but it does not rule out a previous exposure ([Pan et al.](#), [Guo et al.](#)).
 - Other host factors, including rheumatoid factor and heterophile antibodies, can potentially cross-react and generate false positive SARS-CoV-2 serology results ([Zhong et al.](#), [Wang et al.](#)).

Correlation of Serology with Neutralization Assays

In contrast to other serology assays, which detect the presence of antibodies, neutralization assays (e.g. plaque reduction neutralization tests (PRNT) or microneutralization assays) are a gold-standard method to measure functional humoral antibody, and can provide a relative measure of the quantity of antibody present. This is important because not every antibody produced during an immune response can neutralize virus. To date, studies comparing serological assays to neutralization assays have been limited. COVID-19 neutralization assays require expertise, are laborious, and must be conducted in a Biosafety Level 3 facility.

- For IgG, IgM and IgA, the correlation between serological test results and neutralization assay results varies ([Perera et al.](#), [Okba et al.](#), [Wölfel et al.](#)), based on limited studies so far. Variation in results may be due to different serological test platforms and antigens used; what cut-offs were used for both the serological assays and the neutralization assays; and when the samples were collected following symptom onset.
- It is proposed that, in general, IgG and IgA contribute more to neutralizing immunity than IgM ([Bryan et al.](#), [Casadevall et al.](#)).

Correlation of Serology Tests with Immunity

Although IgG antibody levels often correlate with immunity against other pathogens, it is currently not known whether the IgG response correlates with immunity in COVID-19, and if it does, at what threshold is immunity determined and what the duration of protection may be ([Centers for Disease Control and Prevention \(CDC\)](#), [World Health Organization \(WHO\)](#)). Furthermore, it is not yet known whether currently available serological assays are appropriate tools to determine and measure immunity since not all IgG antibodies are neutralizing.

- Current data suggests that humoral (antibody) immunity does not, on its own, determine immunity to COVID-19.
 - It has been shown that COVID-19 patients can generate an antibody response while still exhibiting substantial viral loads, and while still symptomatic ([Wölfel et al.](#)).
 - Since some individuals do not generate a measurable antibody response to COVID-19 infection, but still recover, it is possible that other immune functions, such as cell-mediated immunity, are also important ([Thevarajan et al.](#)).
- Studies of patients infected with other betacoronaviruses may shed light on COVID-19 immunity. There are several reports of detectable neutralizing antibodies in individuals previously infected with SARS-CoV lasting for 2-3 years post-infection ([Wu et al.](#)). However, antibody levels decreased over time ([Lin et al.](#), [Tang et al.](#)). Neutralizing antibody following infection with MERS-CoV have been shown to persist for more than 2.5 years in recovered patients ([Payne et al.](#)).
- A study in rhesus monkeys showed that the immunological response from previous COVID-19 infection provided some protection against re-infection; however, they were not able to differentiate between the roles of antibody-mediated and T-cell mediated protection ([Chandrashekar et al.](#)).

Interpretation of COVID-19 Serological Results

It is important to underscore that a positive serology test result does not necessarily indicate immunity to COVID-19. Given the variability in test performance and the limited knowledge about the immunological response in COVID-19, serological test results must be interpreted with caution.

- A positive serology test result may indicate previous exposure to COVID-19. This is; however, dependent on the test characteristics of the assay used, and on the prevalence of infection in the population. If infection prevalence is low, the positive predictive value of the test will also be low. For example, if the population antibody prevalence is 5%, the positive predictive value is only 49%, meaning that less than half of those with positive tests will be true positives ([CDC](#)).
- A negative serology test result does not necessarily mean that an individual has not been exposed to COVID-19. This depends on the test characteristics and the time between exposure and testing (since it takes some time to generate an antibody response). In addition, it is important to note that not every individual may mount a measurable antibody response to COVID-19.

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