



CLINICAL UPDATE

The role of serological testing in the SARS-CoV-2 outbreak

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Antibody tests for the novel coronavirus, SARS-CoV2, have been developed both as rapid diagnostic assays and for high-throughput formal serology platforms. Although these tests may be a useful adjunct to a diagnostic strategy, they have a number of limitations. Because of the antibody and viral dynamics of the coronavirus, their sensitivity can be variable, especially at early time points after symptom onset. Additional data are required on the performance of the tests in the South African population, especially with regard to development and persistence of antibody responses and whether antibodies are protective against reinfection. These tests may, however, be useful in guiding the public health response, providing data for research (including seroprevalence surveys and vaccine initiatives) and development of therapeutic strategies.

S Afr Med J. Published online 17 July 2020. <https://doi.org/10.7196/SAMJ.2020.v110i9.15098>

SARS-CoV-2 is a novel coronavirus that originated in Wuhan Province, China, in late 2019. Phylogenetic analysis suggests that this virus shows homology with other Betacoronaviridae, including the viruses responsible for the severe acute respiratory syndrome (SARS) outbreak in South-East Asia and the Middle East respiratory syndrome (MERS) outbreak in the Middle East.^[1] A zoonotic reservoir was identified in both these cases, and it is thought that SARS-CoV-2 is also zoonotic, with possible intermediate hosts including bats and pangolins (although these have not been conclusively identified).^[1]

South Africa (SA) has the highest number of infections with SARS-CoV-2 in Africa, with >100 000 cases and >2 000 deaths reported as of the middle of June 2020. In March, a National State of Disaster was declared, followed shortly afterwards by declaration of a national lockdown that was one of the most stringent in the world.^[2] The lockdown was a mitigation strategy to build diagnostic and health capacity and infrastructure and to protect at-risk populations (including the immunocompromised, the elderly, and patients with underlying chronic conditions).^[3-5] A critical component in this strategy is the rapid identification and isolation of individuals who are infected and can transmit infection and the quarantine of their contacts, even if they are asymptomatic or presymptomatic.^[6-12] Challenges have arisen with the molecular reverse transcription polymerase chain reaction (RT-PCR) testing, which have resulted in backlogs and prolonged

turnaround times in both high- and low- to middle-income countries around the world. There is, therefore, an increasing global demand for additional testing kits and to determine strategies and the role of serological assays.^[13-15] Difficulty with development of these strategies is compounded by the high numbers of testing platforms and assays that are entering the market and require oversight and validation in the context of COVID-19 disease control.

Diagnostic and screening modalities for SARS-CoV-2 include:

Molecular testing (RT-PCR, reverse transcription loop-mediated isothermal amplification (PT-LAMP), and clustered regularly interspaced short palindromic repeats (CRISPR)-based diagnostics), recommended for diagnosis of cases in the acute stage of infection. Antigen tests are in development and serve the same purpose as PCR; they are likely to have lower sensitivity but greater ease of use and access at point of care.

Serological testing, including lateral-flow antibody assays, bead-based assays (Luminex technology), enzyme-linked immunosorbent assays (ELISAs) and automated serology platforms.

Clinical stratification based on symptoms and signs including pyrexia, upper respiratory tract symptoms including odynophagia and cough, nonspecific gastrointestinal symptoms including nausea and diarrhoea, and atypical symptoms such as anosmia, with clinical investigations including chest radiographs.^[16-18]

Serological testing

Serological testing is commonly utilised for infectious disease diagnosis for both viruses and bacteria. Serological testing detects antibodies in blood that tend to be specific to the infection and can be used to assess both acute infection (typically immunoglobulin M (IgM)), ongoing infection (typically immunoglobulin G (IgG) and IgM with some agents) and previous exposure (typically IgG). The presence of these antibodies can also be used to assess immunity to pathogens and to evaluate the immunogenicity of, and response to, some vaccines. An important factor in developing serological tests is determining the correct antigen or antigens to include in the test. Antigens are components of the pathogen against which antibodies are formed. The antigenic structure of some organisms is highly variable, which can complicate the development of sensitive and specific assays. Both point-of-care and laboratory-based serological assays for automated and manual platforms are currently available on the market. The South African Health Products Regulatory Authority (SAHPRA) is currently evaluating several commercial antibody assays.

The antibody response to COVID-19

The antibody response to COVID-19 develops after a period of weeks after symptom onset. Samples taken prior to 5 - 7 days after symptom onset are often antibody-negative. From extensive studies, it appears that the antibody response begins to form around day 5 after symptom onset for IgM and around days 10 - 14 for IgG,^[19] although the maximal detectable response may be even later. In ~20% of infected individuals (with localised disease), IgG may not be detected at all. Immunoglobulin A (IgA), an antibody subclass associated with mucosal immunity, may be produced earlier and by more individuals than IgM or IgG. Detectable antibodies (IgM, IgG or IgA) appear to be more commonly produced in individuals with severe disease (defined as disease requiring intensive care admission or mechanical ventilation).^[20-24]

The novel coronavirus has four major protein components against which antibodies are formed following infection. These are the outer nucleocapsid (NC), the spike (S), which includes the S1 and S2 subunits and the receptor binding domain (RBD), the membrane (M) and the envelope (E).^[25] The majority of current serological assays are designed to detect antibodies against the NC or S proteins (which are the most immunogenic).^[25] Not all patients, however, produce antibodies against all of these proteins.^[26]

The protective value of antibodies is not clearly understood in COVID-19. Antibodies directed against certain antigens (specifically S or RBD) are more consistently produced and may provide protection from reinfection.^[27-30] However, seroconversion does not always correspond to a reduced viral load;^[28] indeed, antibody levels are frequently higher in patients with severe disease. In some patients, continuous viral shedding with detectable RNA for up to 50 days has been reported, despite a robust IgG antibody response.^[31-35] Prolonged viral shedding has been associated with more severe disease and requirements for ventilation.^[32] Early data from some studies, including immunisation in a non-human primate model, suggest that antibodies may protect against reinfection, but this is not yet conclusive.^[36]

Types of serological tests for COVID-19

The two basic categories of serological tests currently available for COVID-19 are rapid diagnostic tests (also known as point-of-care, bedside or near-patient tests) and formal laboratory serological tests.

Rapid diagnostic tests often use the lateral flow design, which produces a colour change on a test strip. Over 250 of these rapid kits have been produced (<https://www.finddx.org/covid-19/sarscov2-eval-immuno>). Many but not all of these tests include both IgM and IgG detection. Some of these tests have been evaluated in settings across the world, but a number of studies have been on smaller sample sizes (ranging from 5 to 25 infected individuals), and the sensitivity and specificity have been extremely variable, especially at earlier time points in the course of COVID-19.

The formal assays are either based on the ELISA or the chemiluminescent detection principle. At least three of these assays, which have become available in SA for validation, produced by Euroimmun, Roche Diagnostics and Abbott Diagnostics, have been evaluated in a number of studies and have been authorised for emergency use by the US Food and Drug Administration. The sensitivity of these assays in large validation studies has been variable, especially if time points prior to 14 - 21 days are considered or if sampled populations include asymptomatic patients.^[37] Package inserts from the formal testing quote sensitivities ranging from 33.3% (Euroimmun IgG) prior to 10 days after symptom onset, to 50% prior to day 7 (Abbott IgG assay) to 65.5% (Roche assay) (Table 1).

Limitations of serological testing and possible use cases

There is a growing call for antibody testing to be available for various purposes, including diagnosis. It is important, however, for clinicians to be aware that interpretation of results is not straightforward. The use of serology for diagnosis is limited by the relatively late onset of an IgG response and the fact that some individuals may not produce antibodies in the blood at all. For selective screening, it may be necessary to use a combination of testing strategies which may incorporate antibody testing, especially in individuals at later time points in infection. Some countries are pondering the idea of 'immunity passports', but this approach is limited by our lack of understanding related to the protective role of antibodies and the possibility of false-positive or false-negative tests that are associated with different tests.

Specific challenges are:

- **Sensitivity.** This is determined by the antigen selected for the test, the antibody class measured, and the time point during the disease course at which the test is conducted. Asymptomatic individuals may produce only localised antibody responses, or not have detectable antibody responses at all.^[28]
- **Specificity.** This is a concern because most individuals have undergone previous infection with other human coronaviruses. Antibodies to these viruses may cross-react with SARS-CoV-2 antigens, causing false-positive results. In addition, exposure to common human coronaviruses is likely to increase during the winter season.
- **Uncertainty regarding the protection provided by antibodies.** This limits the utility of serology to determine any conferred immunity to the infection. The role of antibodies may become clearer with additional studies, particularly in the SA population.
- **Durability of the antibody response.** Recent literature suggests that antibody responses wane rapidly after SARS-CoV-2 infection.^[26]

There are, however, some specific roles that antibody tests could play. Unlike PCR testing, serological testing is able to detect past infection, increasing its scope to include outbreak surveillance where individuals

Table 1. Reported sensitivities of high-throughput serological assays

Assay name	Company	Antibody class and antigen used	Sensitivity, %	Specificity, %
Elecsys Total	Roche	Total antibody (NC)	83.87 - 100 ^[37]	100 ^[37]
Anti-SARS-CoV-2 ELISA (IgG)	Euroimmun	IgG (S)	67 - 86.4 ^[38-40]	96.2 - 100 ^[38-41]
SARS-CoV-2 immunoassay	Abbott	IgG (NC)	87.5 - 100 ^[37,42]	99.63 - 100 ^[37,42]

NC = nucleocapsid; S = spike; ELISA = enzyme-linked immunosorbent assay; IgG = immunoglobulin G.

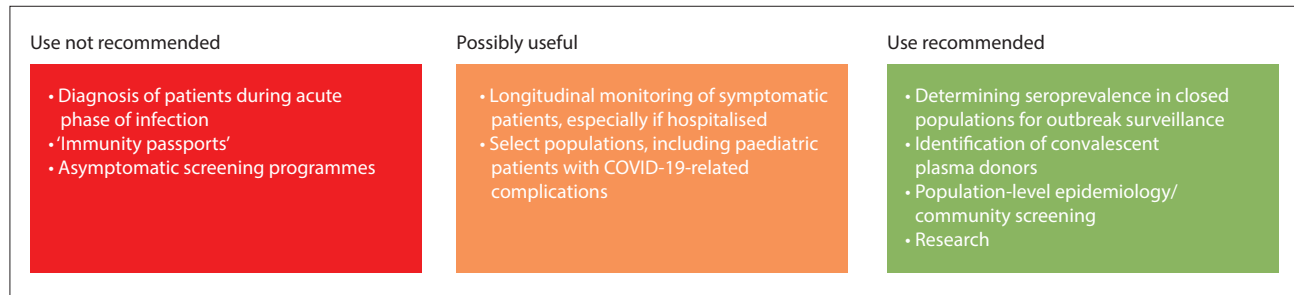


Fig. 1. Possible use cases for serology testing applications.

have been exposed, but have remained asymptomatic. Serological data can potentially provide information on true numbers of infections and enable more accurate determination of case fatality or case infection rates. Serological data can also be used for seroprevalence surveys, which will help to determine how many people in SA were previously infected; however, it should be noted, as per the Africa Centres for Disease Control and Prevention, that a negative antibody test does not exclude past or current infection with SARS-CoV-2 ([africacdc.org/download/interim-guidance-on-the-use-of-rapid-antibody-tests-for-covid-19-response/](https://www.africacdc.org/download/interim-guidance-on-the-use-of-rapid-antibody-tests-for-covid-19-response/)), as antibody responses may wane, leading to false-negative results in people who have had previous infection. However, antibody assays may also be able to assess immune responses to potential vaccines. Molecular testing is still recommended for diagnosis during acute infection, especially in the first 7 days after symptom onset. Because of specificity issues, it is possible that a two-test serological strategy may have some clinical utility (www.who.int/publications-detail/laboratory-testing-strategy-recommendations-for-covid-19-interim-guidance).

A list of potential applications for serological assays, including laboratory-based and rapid tests, could include (Fig. 1):^[43-46]

- **To identify the presence of antibodies directed against COVID-19 in the following cases (as an ancillary diagnostic test):**
 - To identify past exposure to SARS-CoV-2 in individuals at 21 days post infection
 - To identify antibodies in children with COVID-19-associated inflammatory syndrome in children.
- **To investigate outbreaks** in 'hotspots' to identify evidence of subclinical infections.
- **Targeted cohort surveillance**, e.g. for staff, patients, visitors and residents of facilities such as healthcare institutions, prisons and workplaces. Repeat antibody testing over time can allow more comprehensive epidemiological assessments.
- **Population-level epidemiological studies and community surveillance programmes.**
- **Identification of convalescent plasma donors.** For this indication, tests that quantify neutralising antibodies or that correlate with neutralising antibody titres will be required.
- As part of **scientific research studies**, e.g. antibody reactivity as a prognostic marker, for SARS-CoV-2 vaccine trials, additional validation, etc.

Conclusions

It is clear that serological testing, both at point of care and formal, can have high utility. This is especially true in research settings. Interpretation of a negative assay should be performed with caution, especially in asymptomatic individuals or individuals with recent onset of symptoms. Despite potential concerns regarding cross-reactivity with other viruses, the high specificity of these assays can provide reassurance that individuals who test positive have probably been exposed to the infection. It must be noted, however, that this does not necessarily indicate that the patients are no longer infectious.

Funding. EQUIP grant AID-OAA-A-15-00070 – Antiretroviral Therapy Simplification-Optimization of Programs and Services (ART-OPS) COVID supplement, and through iLEAD BMGF (i-LEAD) grant ID OPP1171455.

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Accepted 6 July 2020.