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Serological tests for COVID-19 – a primer

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Abstract: Diagnostic testing for COVID-19 plays a critical role in defining the epidemiology of the disease, informing case and contact management, and ultimately in reducing viral transmission. Recently there has been considerable media interest in the use of serological point of care tests (PoCT) as rapid tests to detect prior infection with SARS-CoV-2. To date however, there are limited data available on the performance of these tests, and their specific utility in the overall COVID-19 response is unclear. Here, we provide an update for clinicians on serological testing for COVID-19 and discuss the challenges and opportunities with serological PoCT assays for SARS-CoV-2.

Byline: We discuss the challenges and opportunities with serological point of care assays for SARS-CoV-2.

One of the fundamental pillars in the prevention and control of COVID-19 is timely, scalable and accurate diagnostic testing. Diagnostic testing plays a critical role in defining the epidemiology of the disease, informing case and contact management, and ultimately in reducing viral transmission (1). To date, laboratory testing has comprised detection of SARS-CoV-2 virus using reverse-transcriptase PCR (RT-PCR) assays, predominantly from patients meeting specific epidemiological criteria. However, the unprecedented scale of RT-PCR diagnostic testing has placed extraordinary demands on healthcare and laboratory systems, with both local and global challenges relating to regulatory frameworks, supply chains of reagents, and the human and financial resource required to support population-level testing.

Early laboratory responses included early characterisation and release of the viral whole genome sequence by Chinese investigators in early January 2020, which enabled rapid development of RT-PCR workflows for the detection of SARS-CoV-2 (2). Since then, a range of commercially available diagnostic tests have been developed, including RT-PCR assays and serological tests. The broad array of tests now available vary both in analytical performance and in their particular utility in the overall public health response to COVID-19.

What does serological testing detect?

Serological tests rely on detection of specific anti-viral antibodies (IgM, IgA, IgG or total antibody) in patient serum, plasma or whole blood (3). Determining the optimal antigenic epitopes to maximise sensitivity, but minimise cross-reactivity, particularly against other human coronaviruses, has meant that the development of high-quality serological testing has been slower than molecular-based diagnostics (4, 5). Initial candidate epitopes have largely focused on the immunogenic viral structural proteins nucleocapsid (N), and spike protein (S), particularly the S1 subunit and the receptor binding domain (RBD) (5). To date, a range of serological tests for COVID-19 have been developed, each with particular test characteristics (Table 1). Broadly, these serological tests can be divided into tests that (i) can be performed at the point-of care; (ii) can be performed in routine diagnostic laboratories, and (iii) can only be performed in specialised reference laboratories (Table 1).

Initial studies have reported that most patients with COVID-19 seroconvert by day 10-14 (~80%), with almost 100% seroconversion by day 20 (6, 7). However, comparisons across published studies are challenging due to (i) different antigens used in assays; (ii) differences in the complexity of patient populations, and (iii) variations in the RT-PCR assays used as the 'gold standard' for determining sensitivity of serological assays. Further, it is not clear whether the type and amount of antibody correlate with severity of disease, or more importantly, with immune protection from re-infection. As noted by the World Health Organisation (WHO), the Public Health Laboratory Network of Australia (PHLN) and the Royal College of Pathologists Australasia (RCPA), a negative result using a serological

test does not rule out SARS-CoV-2 infection, particularly in those with strong epidemiological risk factors, and both PHLN and RCPA note that there is no role for serological point of care tests (PoCT) in the acute diagnosis of COVID-19 (8, 9).

At present, the most widely available (and most publicised) serological tests are PoCT, which involve detection of anti-SARS-CoV-2 antibodies through binding to immobilised antigen, generally bound to colloidal gold on a test strip (Figure 1). The relatively cheap and simple nature of lateral flow assays means that production is suited to scaling-up for increased testing capacity. However, there are limited published data on the performance characteristics of serological PoCT, and high-quality data are urgently needed to guide laboratories, public health agencies and governments in the appropriate and responsible deployment of PoCT, and serological assays more broadly (4). Currently WHO recommends the use of PoCT immunodiagnostic assays in research settings only, and not for clinical decision making until further evidence is available (10). Ideally, validation of serological assays, including PoCT, should be performed against a serum panel that includes samples from: (i) patients at acute and convalescent stages of infection (to assess sensitivity), and (ii) patients with other human coronavirus infections (to assess specificity).

What serological assays are available in Australia and how is this regulated?

At the time of writing (6th May, 2020), more than 20 serological PoCT have been approved by the Therapeutic Goods Administration (TGA) for inclusion on the Australian Register of Therapeutic Goods (ARTG), with multiple distributors (available at https://www.tga. gov.au/covid-19-diagnostic-tests-included-artg-legal-supply-australia). An emergency TGA exemption on 22nd March 2020 allows for COVID-19 diagnostic tests to be supplied to accredited pathology laboratories in Australia (11). In addition, unapproved diagnostic tests can also be supplied under this exemption, but again, only to an accredited laboratory. Work is ongoing globally to monitor the clinical performance and safety of new diagnostic tests, particularly in the context of emerging reports of limited sensitivity and specificity to serological PoCT (12).

The National Pathology Accreditation Advisory Council (NPAAC) has existing guidelines on the use of PoCT in Australia (13). These guidelines cover issues such as clinical supervision for performing PoCT; ensuring test quality; staff training and competency for performing PoCT, and appropriate reporting of test results. More recently, this advice has been extended to serological PoCT for COVID-19, with an emphasis on a robust quality framework to support the implementation and deployment of such tests (14). Of note, in Australia, the supply of self-testing (e.g. testing at home) for many infectious diseases, including COVID-19, is prohibited under another TGA regulation, the *Therapeutic Goods (Excluded Purposes) Specification 2010*.

Where might serological assays be used?

Given the time lag from symptom onset to detectable antibody, serological PoCT have no role in the detection of acute COVID-19 infection. However, there are some settings where serological assays, including PoCT, may have potential utility, including defining antibody prevalence in key populations such as frontline workers, and determining the extent of COVID-19 infection within the community. For other applications, such as identifying individuals for further evaluation of therapeutic immunoglobulin donation and vaccine development and evaluation, assays that assess neutralising antibody response are likely to be required, although as mentioned above, there are still limited available data to support the concept of protective immunity following infection with SARS-CoV-2.

Regardless of the type of serological assay used, in order to appropriately deploy serological testing, it is critical to understand the limitations of test performance in the epidemiological context in which tests are used. This is particularly important in a setting such as Australia, which, based on the number of reported cases of COVID-19 (6,849 cases as of 5th May, 2020), has an estimated COVID-19 prevalence of 0.03%. As such, even with serological tests that are highly sensitive and specific, the majority of positive tests are likely to represent false positive results. When considering the use of serology to inform policies relating to relaxing of physical distancing interventions, specificity of the assay becomes critical. If the majority of those considered immune actually represent false positive results, then the threshold to maintain immunity (if this correlates with antibody detection) within the community will not be achieved.

Conclusions

There has been considerable media and government interest in the promise of relatively low-cost, scalable, and easy to use serological assays, particularly in the context of global shortages for reagents for RT-PCR testing. The unprecedented demands on laboratories to rapidly upscale testing for COVID-19 has necessarily led to 'fast-tracking' of normally stringent regulatory requirements for test approval, both globally and in Australia. Peer-reviewed and high-quality validation data are urgently required to guide laboratories, public health agencies and governments in appropriate serological test selection and deployment. Without such data, many countries run the risk of roll-out of sub-optimal tests, which ultimately may cause more harm than good in the COVID-19 response.

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Table 1. Main serological assays used to date for the detection of SARS-CoV-2

Serological assay	Detection method	Advantages	Disadvantages	Implications
Neutralisation	Determines ability of test sera to inhibit live virus replication.	Gold Standard. Highly specific.	Requires PC3 facilities. Technically demanding. Slow turn-around time. Low throughput.	Only undertaken in specialist laboratories. Gold standard for initial validation of other assays and challenging cases. Not suited to routine testing.
IFA (Indirect Fluorescent Antibody)	Whole-virus inactivated and fixed to a slide. Addition of test sera with fluorescent detection of antibody binding.	Can be undertaken at PC2 facilities once slides prepared. Less technically demanding than neutralisation assays.	Preparation of slides requires PC3 facilities. Less specific than neutralisation. Technically demanding. Subjective end point. Low throughput.	Not available in routine laboratories. Not suited to large scale testing.
EIA (Enzyme immunoassay)	Recombinant antigen fixed to solid surface (often 96 well plate), test sera applied and antigen-antibody binding detected by enzyme mediated colour change.	Good sensitivity. Less technically demanding than IFA or neutralisation. Semi-automated. High throughput. Objective end point with machine based optical density reading.	Less specific than neutralisation. Initial expertise and time required to determine, test and manufacture suitable recombinant antigen.	Generally relies on commercial companies to manufacture and distribute test kits. Suitable for routine testing. Good for screening.
Lateral flow EIA	A particular type of EIA. Recombinant antigen present on immunochromatographic paper, test sera applied to test pad, antigen-antibody binding detected visually by colour change on a membrane .	Variable sensitivity. Least technically demanding. Fast turn-around time for individual tests. Test on demand.	May be less sensitive and specific than laboratory-based assays. Limited scalability. Subjective end point. Data capture less robust.	Suited to point-of-care testing. Can be undertaken by non-laboratory staff. Systems for data capture of results need to be implemented.

Figure 1. Schematic of a lateral flow immunoassay for detection of SARS-CoV-2 IgM and IgG antibodies (adapted from reference (15)). The sample is added to the sample pad, and then travels by capillary motion to the conjugation pad. Anti-SARS-CoV-2 IgM and / or IgG antibodies in the patient sample then bind to the specific SARS-CoV-2 antigen. This antigen is bound to colloidal gold, which acts as a colorimetric indicator. The bound antigen-antibody-gold complex then travels to the nitrocellulose membrane and bind to specific anti-human IgM or IgG antibodies, with a resultant colorimetric change. To monitor test validity, excess conjugated colloidal gold binds to antibody on the control line, which allows assessment of whether fluid has successfully migrated across the test strip.