PHLN
Public Health Laboratory Network

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Public Health Laboratory Network Guidance for serological testing in COVID-19

High-quality serological assays are now available in Australia for detection of SARS-CoV-2 (the virus that causes COVID-19) antibodies. Before widespread deployment of these assays, appropriate frameworks are required to inform:

(i) which patients to test
(ii) which assays and confirmatory protocols to use, and
(iii) how to report test results.

Background

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. To date, laboratory testing has largely comprised detection of SARS-CoV-2 using reverse-transcriptase PCR (RT-PCR) assays. Recently however, a range of serological tests have been developed. Situations where serological testing may be of possible use include:

- Testing patients who have had symptoms consistent with COVID-19 but are
  - PCR negative OR
  - were not tested OR
  - have unexpected positive or inconclusive PCR results
- Seroepidemiological studies to define the degree of population infection
- Surveillance of frontline healthcare workers to define potential occupational infection
- Convalescent patients, for plasma donation
- Patients who may have been, or are, part of an outbreak investigation
- Estimating timing of infection to help define the likely infectious period where this is not evident from clinical symptoms or exposure history.

Serological tests rely on detection of specific anti-SARS-CoV-2 antibodies (IgM, IgA, IgG or total antibody) in patient serum, plasma or whole blood. Determining the optimal antigenic epitopes to maximise sensitivity, but minimise cross-reactivity, particularly against other human coronaviruses, has meant the development of high-quality serological testing has been slower than molecular-based diagnostics. Candidate epitopes have largely focused on the immunogenic viral structural proteins nucleocapsid (N), and spike protein (S), particularly the S1 and S2 subunits and the receptor binding domain (RBD), in addition to whole virus antigen. [1]

Broadly, serological tests for COVID-19 can be divided into tests that

(i) can be performed at the point-of care
(ii) can be performed in routine diagnostic laboratories
(iii) can only be performed in specialised reference laboratories.

Despite the increase in availability of serological assays, many have undergone only limited validation, making selection of assays difficult. In addition, there remain fundamental knowledge gaps in the interpretation of serological test results, including:

- Whether antibody detection correlates with protective immunity to reinfection
- Whether antibody detection correlates with lack of infectiousness to others
- The amount and duration of antibody response, particularly in mild or asymptomatic infection
- The interpretation of serological assays in low prevalence settings (i.e. identification of true positive results).

Who and when to test for clinical purposes?

In general, pathology testing should only be performed if it is likely to contribute to the management of the individual and/or public health. Given the time lag from symptom onset to detectable antibody, serological assays have no role in the detection of acute COVID-19 infection. Serology should not be requested for acute diagnosis if a patient has acute symptoms of COVID-19, but may be collected and stored for later testing. In the first instance, Reverse Transcription Polymerase Chain Reaction (RT-PCR) tests should be requested for acute diagnosis of COVID-19 as it is the gold-standard.[2] However, serology may be used to support diagnosis in cases where PCR results are inconclusive.

As a guide, serology for COVID-19 diagnosis may be considered for a person who meets the following:

- at least two weeks have passed since the onset of symptoms (fever (37.5^0C) OR
- they have a history of fever OR
- they have acute respiratory symptoms or other symptoms outlined in the current national case definition and where PCR results are inconclusive. [3]

AND/OR the person has one of the following risk factors:

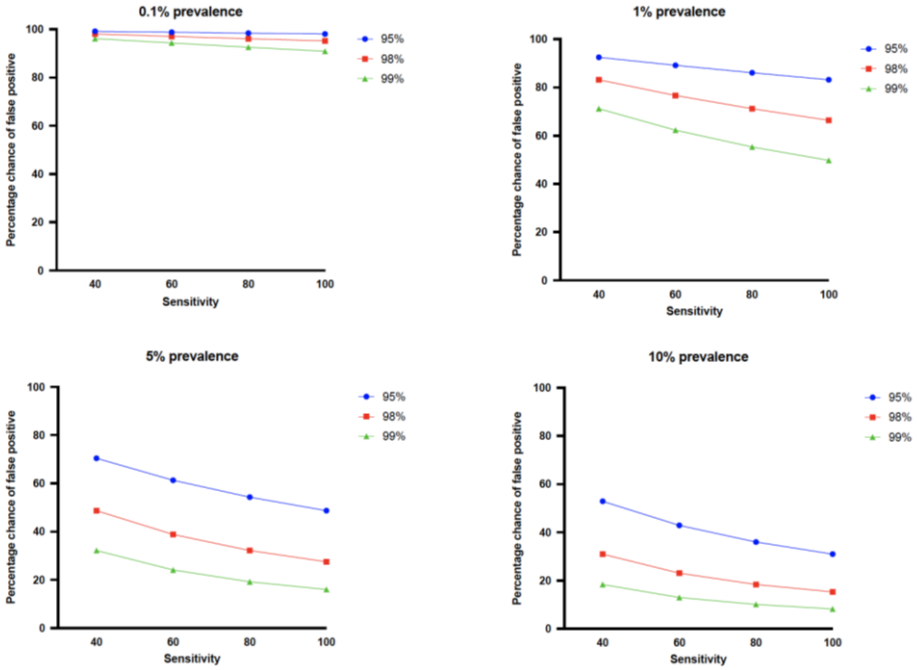
- is a close contact of a confirmed case OR
- is identified as related to an outbreak setting or public health investigation OR
- has travelled (internationally and/or interstate) since 1/1/20.

If serology is requested, the requesting clinician should provide the date of onset of symptoms (where possible) to enable accurate interpretation of serological result. Serological testing for COVID-19 should be undertaken with guidance from the local laboratory. Serological testing before two weeks from the onset of symptoms may result in false negative results. Occasionally the serological response may take longer to develop. Approximately 5-10% of individuals may not develop antibodies.[4] If the clinical suspicion is high, testing out to 28 days post-presumed exposure or symptom onset (if available) is recommended. As more data become available regarding immune responses to SARS-CoV-2, the clinical indications for serological testing may become more evident and broader.

How to test?

As for all laboratory tests, the positive predictive value of serology testing is inversely proportionate to disease prevalence in the tested population (Figure 1). In Australia where the prevalence of COVID-19 is very low (<1%), testing of those without risk factors (see above) will lead to a high proportion of false positive results. Robust validation of serological assays for COVID-19 is essential to assess their accuracy in the Australian setting, as is the identification of optimal approaches to confirming true positive tests.

Figure 1. False positive rates of serological assays stratified by population prevalence of disease and assay specificity and sensitivity. Coloured lines denote specificity.



Current CDNA guidelines state that a seroconversion, or significant rise (e.g. four-fold or greater titre rise) in either neutralising or IgG antibody level is definitive laboratory evidence of SARS-CoV-2 infection, whereas detection of neutralising or IgG antibody in a single specimen from a person with suspected COVID- 19 is only suggestive evidence of SARS-CoV-2 infection and should be considered in conjunction with clinical and epidemiological evidence.[3] This distinction is to convey the inherent uncertainty associated with single reactive results in the context of low population prevalence, and limits in the specificity of currently available serological tests.

At present, based on published sensitivity and specificity data of commercially available serological assays, there is no single serological assay that:

(i) has sufficient sensitivity to detect all cases – i.e. minimise false negatives, and
(ii) can correctly identify all true positive cases – i.e. minimise non-specific reactivity or false positives.

Accordingly, it is likely that a combination of testing approaches will be required in a low-prevalence setting such as Australia. Specific approaches will vary according to jurisdictional access to high specificity assays, particularly virus neutralisation assays. At present,

neutralisation assays are only available in three jurisdictions (Victoria, NSW and QLD). Currently, no Western Blot assays are available for confirmatory testing.

Detection of a positive IgM or IgA antibody without IgG detection is not sufficient evidence of recent COVID-19 (see suggested comments below) and a repeat specimen should be requested to look for IgG seroconversion.

In the event of a single IgG positive specimen (with or without IgM and/or IgA antibody) from a suspected case of COVID-19 with supportive clinical and epidemiological evidence, a follow-up sample should be requested to look for a significant rise in antibody.

In the event of a single IgG positive specimen without supportive clinical and epidemiological evidence, the following are two suggested approaches to help confirm the presence of anti-SARS-CoV-2 antibodies, depending on jurisdictional capabilities:

Approach 1: Initial testing using a commercially available enzyme immunoassay (or other validated test). If positive (based on manufacturer's guidelines), request a follow-up sample (see below), and consider retesting initial sample using an alternative commercially-available enzyme immunoassay (or other validated test) with comparable or greater sensitivity/specificity, ideally targeting a different antigen to the initial test.

Approach 2: Initial testing using a commercially available enzyme immunoassay (or other validated test). If positive (based on manufacturer's guidelines), request a follow-up sample (see below), and retest initial sample using a virus neutralisation assay or other alternate format reference laboratory antibody test, such as immunofluorescent assay or microsphere immunoassay.

How to report?

Serological test results should be interpreted in association with all patient information (clinical, epidemiological and laboratory). When serological results for COVID-19 are reported, it is important to understand (i) the analytical sensitivity and specificity of the assay; and (ii) the likely prevalence of the patient population being tested.

The following comments are suggested as a framework for reporting serological tests for COVID-19.

SARS-CoV-2 IgG	SARS-CoV-2 IgM/ IgA	Interpretive comment
Non-reactive	Non-reactive	No serological evidence of infection with SARS-CoV-2. Please submit a further sample in 10-14 days if recent infection is suspected to assist in confirming or excluding infection.
Non-reactive	Equivocal	Possible recent infection with SARS-CoV-2 or nonspecific reactivity. Please submit a further sample in 10-14 days if recent infection is suspected to assist in confirming or excluding infection.
Non-reactive	REACTIVE	Possible recent infection with SARS-CoV-2 or nonspecific reactivity. Please submit a further sample in 10-14 days if recent infection is suspected to assist in confirming or excluding infection.
Equivocal	Non-reactive	Possible recent infection with SARS-CoV-2 or nonspecific reactivity. Please submit a further sample in 10-14 days to assist in confirming or excluding infection

Equivocal	Equivocal	Possible recent infection with SARS-CoV-2 or nonspecific reactivity. Please submit a further sample in 10-14 days to assist in confirming or excluding infection.
Equivocal	REACTIVE	Possible recent infection with SARS-CoV-2. Please submit a further sample in 10-14 days
REACTIVE	Non-reactive	Consistent with recent or past infection with SARS-CoV-2. This result should be considered with clinical and other information. Please submit a further sample in 10-14 days if recent infection is suspected.
REACTIVE	Equivocal	Consistent with recent infection with SARS-CoV-2. This result should be considered with clinical and other information. Please submit a further sample in 10-14 days to monitor antibody response.
REACTIVE	REACTIVE	Consistent with recent infection with SARS-CoV-2. This result should be considered with clinical and other information. Please submit a further sample in 10-14 days to monitor antibody response.

In addition, the following comments are suggested (depending on local context) when reporting all serological test results for COVID-19.

- If the patient has an acute infection, please submit a respiratory sample for PCR, and liaise with local public health authorities
- Serological testing is not appropriate for the acute diagnosis of COVID-19
- Testing is for antibodies to SARS-CoV-2. SARS-CoV-2 is the causative agent of COVID-19
- Results should be interpreted in association with all other information (clinical, epidemiological and laboratory) on the patient
- Reports based on this assay are not currently NATA accredited.

Laboratories offering SARS-CoV-2 serology should be NATA accredited following submission of validation or verification data and participate in a recognised Quality Assurance Programme as offered by the RCPAQAP.

References

1. Krammer F, Simon V. Serology assays to manage COVID-19. *Science*. 2020;368:1060-1.
2. Public Health Laboratory Network Australia. Statement on point-of-care serology testing for SARS-CoV-2. Available at: https://www.health.gov.au/resources/publications/phln-statement-on-point-of-care-serology-testing-for-SARS-CoV-2-the-virus-that-causes-COVID-19 (2020).
3. Communicable Diseases Network of Australia. Coronavirus Disease 2019 (COVID-19) CDNA National Guidelines for Public Health Units. Available at: https://www1.health.gov.au/internet/main/publishing.nsf/Content/7A8654A8CB144F5FCA2584F8001F91E2/$File/COVID-19-SoNG-v3.2.pdf
4. Tang MS, Hock KG, Logsdon NM, et al. Clinical Performance of Two SARS-CoV-2 Serologic Assays. *Clin Chem* 2020.