

The utility of SARS-CoV-2-specific serology in COVID-19 diagnosis

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By 31 July 2020, 3,600 confirmed infections of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19), had been recorded in New South Wales (NSW), Australia.¹ Prior to the second wave of COVID-19 in Australia (commencing late June in the state of Victoria), New South Wales had the highest number of cases in Australia (NSW 3,203 cases vs. Victoria 2,231 cases by 1 July 2020).² During the initial wave of infections in New South Wales, the vast majority (62%)³ of cases occurred in returned travellers and their close contacts.⁴ In particular, cruise ship clusters formed a large number of cases (>10%).³ By the end of July 2020, only 10.8% of cases in New South Wales had an unknown source of transmission, indicating a very low rate of community transmission at this time.^{1,3,5}

New South Wales has a network of seventeen Public Health Units (PHU) coordinated by NSW Health and serving a population of 7.5 million. During the early stages of COVID-19, prior to the World Health Organization (WHO) declaration of a pandemic, NSW Health evoked the progressive stages of an existing framework for pandemic management.⁶ This included the coordination of a system for a coherent and structured emergency response, overseen by the Public Health Emergency Operations Centre (PHEOC) and State Health Emergency Operations Centre (SHEOC).⁷ Within the public health measures, novel Coronavirus 2019, as it was then known, was deemed a mandatory notifiable disease on 21 January 2020 (*NSW Public Health Act 2010*).⁸ Every case of COVID-19 in New South

Abstract

Introduction: In May 2020, The Communicable Diseases Network of Australia (CDNA) case definition introduced serological criteria to support the diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We present findings that support the utility of SARS-CoV-2-specific serology for public health investigations.

Methods: From 24 January to 31 July 2020, the following information was collected from individuals with positive SARS-CoV-2-specific immunofluorescence antibody tests: history of contact with COVID-19 cases; recent travel; symptoms consistent with COVID-19; and SARS-CoV-2 nucleic acid testing (NAT) results. Individuals were classified as confirmed or probable by CDNA criteria or additionally as possible (SARS-CoV-2-specific IgG positive with compatible symptoms or epidemiologic risk) or indeterminate (SARS-CoV-2-specific IgA/IgM positive only) cases.

Results: A total of 10,595 individuals were tested in the six-month period. Of these, 9.8% (1,037) individuals had positive SARS-CoV-2-specific serology of which 566 (53.6%) were NAT-confirmed COVID-19 cases and 286 (27.6%) were part of a cruise ship outbreak sero-survey. The remaining 185 individuals (NAT negative) were individually classified as serologically confirmed (4, 0.4%), probable (72, 6.9%) possible (66, 6.4%) and indeterminate (38, 3.7%) cases. Maternal antibody transfer was inferred in one infant and four were unclassified.

Conclusion: SARS-CoV-2-specific serology is a key diagnostic tool for retrospective identification of COVID-19 infection.

Implications for public health: SARS-CoV-2 specific serology can enhance the ability to find cases, link missing cases in clusters of infection and identify the epidemiological extent of SARS-CoV-2 outbreaks. A combination of epidemiological criteria, clinical criteria and a quantitative serological test can be used as an adjunct to classify SARS-CoV-2 cases. Our study confirms the low level of community transmission in NSW during the first year of the COVID-19 pandemic

Key words: covid-19, serology, case definitions

Wales has been formally investigated by local PHUs with robust measures to mitigate further transmission. In May 2020, SARS-CoV-2-specific serology was introduced into the national CDNA guidance for the investigation of cases and contacts.⁹ The comprehensive statewide approach to management and control of COVID-19, which includes various modalities of laboratory diagnostics, provides

a valid population study of the utility of serology for diagnosis.

The aim of this study was to retrospectively assess individual SARS-CoV-2-specific serology results, in conjunction with epidemiological or clinical criteria for COVID-19, to determine the added benefit of SARS-CoV-2-specific IgG, IgM and IgAas – an adjunct in identifying SARS-CoV-2 infections.

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This study was approved by Western Sydney Local Health District Human Research Ethics Committee (2008-10 QA).

Methods

We performed a retrospective cross-sectional study on all serology tests performed by NSW Health Pathology's (NSWHP) Institute of Clinical Pathology and Medical Research (ICPMR), Westmead, Sydney, Australia between 24 January and the 31 July, 2020. NSWHP-ICPMR Westmead was the first laboratory in Australia to offer routine and quantitative SARS-CoV-2-specific serology testing, and so received sera from individuals with clinical suspicion of recent or past COVID-19 throughout New South Wales as well as other states and territories (Western Australia, South Australia, Tasmania, Victoria and the Australian Capital Territory) and countries (New Zealand).

Sera were tested using a validated in-house quantitative indirect immunofluorescence assay (IFA) for SARS-CoV-2-specific immunoglobulin (Ig) M, IgA and IgG with a sensitivity of 91.3% and specificity of 98.9% (10). Although the assay was developed in February 2020, retrospective serology tests were performed on samples collected in late January. A positive SARS-CoV-2-specific serology test was defined by detection of either IgM or IgA or IgG at a titre of 10 or higher. In a subset of individuals, SARS-CoV-2 neutralising antibodies were also tested as a confirmatory assay (10).

For each positive SARS-CoV-2 serology result, the following information was collated: history of contact with confirmed or probable COVID-19 cases; history of overseas travel since January 2020; if the individual was a healthcare worker; presence and timing of COVID-19 symptoms (fever, cough, shortness of breath, sore throat, loss of smell or taste); and results of SARS-CoV-2 nucleic acid testing (NAT).

A systematic and comprehensive collation of data was obtained from pathology request forms, Laboratory Information Systems, electronic medical records, and the NSW Health Notifiable Conditions Information Management System (NCIMS), the mandatory surveillance database for all COVID-19 cases and close contacts in New South Wales. Where required, further information was obtained by telephone from the requesting clinician, and local or interstate public health authorities.

Serology assists in the retrospective diagnosis of missed cases, for example, for those who present late after symptoms onset, thus broadening the understanding of the extent of SARS-CoV-2 community transmission. As such, each positive SARS-CoV-2 result was classified into a category, using the Communicable Diseases Network of Australia (CDNA) Case Definition for COVID-19 that was available at the end of July 2020 (Box 1).⁹ Individuals who did not meet the definition of confirmed or probable cases were categorised into one of the following groups:

1. Possible cases – positive SARS-CoV-2-specific IgG without a positive NAT and either:
 - With a compatible clinical illness BUT NOT meeting epidemiologic criteria
 - Meeting epidemiologic criteria BUT WITHOUT a compatible clinical illness
2. Indeterminate: those who do not meet criteria for confirmed, probable or possible cases after review of clinical and epidemiological information.
3. Unclassifiable due to insufficient clinical or epidemiological information.

Results

Between 24 January 2020 and 31 July 2020, 11,874 samples were tested for SARS-CoV-2-specific serology from 10,549 individuals, of which 421 were from interstate. Serology was performed for the following reasons: a) high-risk populations such as healthcare workers (HCW); b) close contacts of confirmed cases; c) international travel history; d) illness without a clear alternate diagnosis or false-negative NAT suspected; e) public health investigations to identify source of infection; f) identification of historical or asymptomatic cases; g) provide an estimate for the timing of infection in confirmed cases. Of the serology tests performed, 1,395 samples were positive in 1,037 individuals (9.8%) (Figure 1).

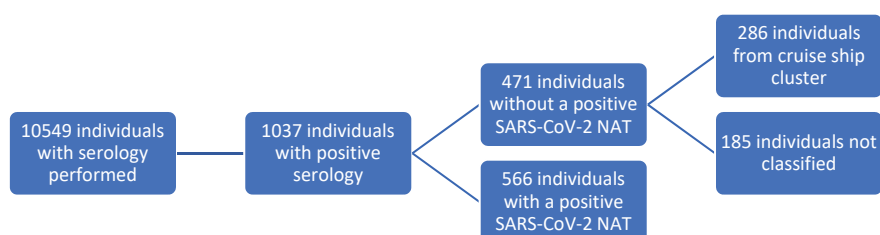
Serology was performed in 625 individuals who were confirmed cases with positive

Box 1: Communicable Diseases Network of Australia Case Definition for COVID-19 as at 31 July 2020 (version 3.6).

Confirmed case
A person who:
i. tests positive to a validated specific SARS-CoV-2 nucleic acid test;
OR
ii. has the virus isolated in cell culture, with NAT confirmation using a validated method;
OR
iii. undergoes a seroconversion to or has a significant rise in SARS-CoV-2 neutralising or IgG antibody level (e.g. four-fold or greater rise in titre).
Probable case
A person who has detection of SARS-CoV-2 neutralising or IgG antibody AND has had a compatible clinical illness AND meets one or more of the epidemiological criteria:
Clinical criteria:
Fever ($\geq 37.5^{\circ}\text{C}$) or history of fever (e.g. night sweats, chills) OR acute respiratory infection (e.g. cough, shortness of breath, sore throat) OR loss of smell or loss of taste.
Epidemiological criteria:
In the 14 days prior to illness onset:
• Close contact with a confirmed or probable case
• International travel
• Passengers or crew who have travelled on a cruise ship
• Healthcare, aged or residential care workers and staff with direct patient contact
• People who have lived in or travelled through a geographically localised area with elevated risk of community transmission, as defined by public health authorities.

SARS-CoV-2 NAT tests. Of these, 89% (556/625) were seropositive with SARS-CoV-2-specific IgG titres ranging from 10 to 2,560. Within this seropositive group, SARS-CoV-2-specific IgG was undetectable in four individuals: three had an IgA titre of 10 and one had an IgM titre of 20 (serology taken six to 26 days after symptoms onset). SARS-CoV-2-specific antibodies were not detected in 59 individuals (9.6%); in two-thirds (39/59) only a single serology sample was collected (median time from date of symptom onset or date of positive NAT to date of serology specimen seven days; IQR 3.25 to 27.5). There were 17 (2.7%) individuals who remained seronegative, despite at least 14 days between symptom onset and serological testing.

Figure 1: Breakdown of positive serology performed by NSW Health Pathology January to July 2020.



Four hundred and seventy-one who were not identified as confirmed cases (negative NAT or not tested) were seropositive. Two hundred and eighty-six of these were part of a single cruise ship outbreak. While most of these individuals would be classified as possible or probable given the strong epidemiological risk, data regarding symptoms of these individuals were unavailable. The remaining 185 individuals were individually assessed and classified by the specified criteria. Twenty-seven individuals were referred specimens from the states of Victoria, Tasmania, South Australia and Western Australia. Of the 185 individuals, 102 (55.1%) were female with a median age of 44.5 (IQR 30.0-63.3 years). Twelve (6.5%) were under 18 years of age (Table 1).

The classification of the 185 individuals is presented in Figure 2. Of these, 76 had a NAT performed, all of which were negative. Four of 1,037 (0.4%) individuals were classified as confirmed SARS-CoV-2 cases as they had a greater than fourfold increase in SARS-CoV-2-specific IgG titres (only one of these had a NAT performed which was negative). A further 72 (6.9%) met the criteria of probable SARS-CoV-2 cases. Twenty-two (2.1%) individuals without epidemiologic risk factors and 44 (4.2%) without a clinically compatible illness (asymptomatic) were classified as possible cases. Interestingly, one neonate was noted to have a SARS-CoV-2-specific IgG titre of 20, presumed to be on the basis of placental transmission during pregnancy. Although the mother was known to be NAT positive, at an unspecified time prior to delivery, she did not have serology performed.

Of the remaining sero-positive individuals, 38 (3.7%) were classified as indeterminate by serology due to a lack of epidemiological or clinical risk factors (nine individuals) or in those with a detectable SARS-CoV-2-

specific IgM or IgA but a negative IgG result (29 individuals). Four could not be classified as further clinical and epidemiological information could not be obtained. Three examples of the utility of the SARS-CoV-2 serology test are presented in Box 2.

The median highest measured antibody titres for individuals classified as NAT-confirmed, confirmed by serology, probable, possible with no epidemiologic risk factors and possible with no symptoms were 320 (80 to 1280), 320 (320 to 320), 40 (10 to 1280), 20 (10 to 320) and 40 (10 to 320), respectively. This demonstrates a gradient in titres, with higher levels found in those classified as confirmed cases, through to lower levels in indeterminate cases. It is likely that this is due to increasing numbers of false-positive results across the categories and supports our previous finding that false-positive SARS-CoV-2-specific IgG results are associated with low antibody titres.¹⁰

Neutralising antibodies

Neutralising antibody (NAb) titres were performed in a subset of individuals with probable and possible infection. Of those with detectable NAb, 12/72 (16.7%) and 2/66 (3.0%) of cases classified as probable and possible had neutralising antibodies detected.

Cases confirmed by serology

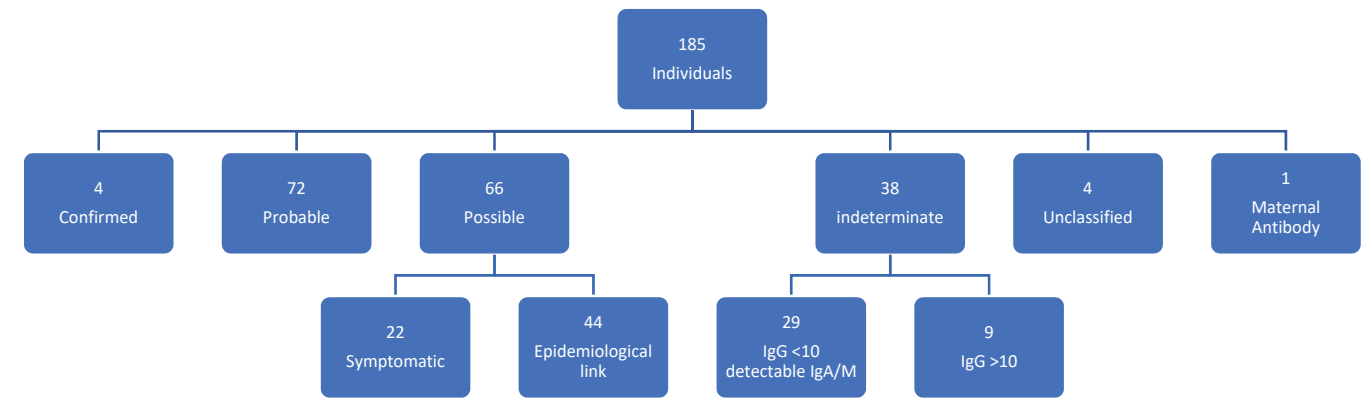
There were four serologically confirmed cases with a four-fold increase in titre or by sero-conversion of SARS-CoV-2-specific IgG. Although not part of the current public health definitions, an additional individual had a four-fold fall in IgG titre and another had a four-fold fall in Nab titre. Follow-up serology was performed between 24 and 59 days (average 46.5 days) after initial serology

Table 1: Demographic features of 185 unclassified sero-positive individuals.

	Number (% of total, n=185)
Age (years)	
0-<18	12 (6.5)
18-<35	49 (26.5)
35-<55	61 (33.0)
55-<75	44 (23.8)
75 and over	19 (10.3)
Sex	
Male	83 (44.9)
Female	102 (55.1)
Hospitalised	
Yes	18 (9.7)
No	162 (87.6)
Unknown	5 (2.7)
Clinical symptoms	
Yes	120 (64.9)
No	61 (33.0)
Unknown	4 (2.2)
Epidemiological risk factors	
Health care worker	31 (16.8)
Overseas travel	44 (23.8)
Close contact of case	39 (21.1)
Casual contact of case	24 (13.0)
No risk factors	42 (22.7)
Unknown	5 (2.7)
Timing of serology specimen in relation to date of symptom onset	
<=10 days	23 (12.4)
10-14 days	2 (1.1)
>14 days	46 (24.9)
Asymptomatic	60 (32.4)
Unknown	54 (29.2)
Total serology performed by ICPMR to July 31st 2020	11874
Total NAT tests in NSW to July 31st 2020	1,552,627

Note:
ICPMR Institute for Clinical Pathology and Medical Research, NAT nucleic acid test, NSW New South Wales

Figure 2: Classification of 185 SARS-CoV-2 sero-positive individuals.



testing. Two individuals were returned travellers, one had household contacts with confirmed COVID-19 and one was an HCW without known exposure to COVID-19 at the time of the serology test. This HCW had a four-fold decrease in IgG titre. Two had no known epidemiological risks for COVID-19 infection. All but one were symptomatic, with the asymptomatic individual having the lowest IgG titres, with a rise in titre from 10 to 80 demonstrated.

Results from HCWs

In the current study, 1,969 (18.7% of total tested) individuals who had serology performed were known to be HCWs. Of these, 74 had positive serology, with 44 having NAT-confirmed COVID-19. Of the remaining 30 HCW, six were classified as probable, 12 possible and 12 indeterminate. All indeterminate cases had an IgG titre of <10 with a positive IgA or IgM. Of the possible cases, seven had IgG titres less than 40. Two of the probable HCW cases had non-occupational exposure to known COVID-19 cases; none of the probable cases had known occupational exposure to COVID-19 cases.

Discussion

The low prevalence of SARS-CoV-2 infection in New South Wales of 29.5 per 100,000³ compared to other global settings provided an ideal opportunity to explore the utility of serological studies as a tool to identify the extent of community transmission of SARS-

CoV-2 in a sero-naïve population. While sero-surveys in targeted populations in China, the US and Europe had suggested infection rates of 3.2% – 10.9%¹¹⁻¹⁵ by July 2020, these studies were unable to fully elucidate the significance of positive results due to the volume of cases and lack of clinical and epidemiological evidence for each patient. We are not aware of other studies which have individually assessed those with detectable and quantifiable SARS-CoV-2 antibodies with other indicators of infection.

This study evaluated the use of the SARS-CoV-2-specific serology in an Australian population. A total of 9.8% of the individuals tested had measurable SARS-CoV-2-specific IFA antibodies by the end of July 2020, of whom almost half were not identified by NAT. If all serologically confirmed, probable and possible cases were included in surveillance data, an additional 122 infected individuals (3.3% of infections up to 31 July) would be added to the New South Wales tally. Serology is a useful tool as an adjunct in understanding the extent of community spread of COVID-19.

Serology can be used in public health investigations of epidemiological clusters by identifying missing links within the transmission network (see Box 2). Targeted serological investigations may assist in establishing the extent of community infection as has been demonstrated in the school setting in New South Wales.¹⁵ A quantitative SARS-CoV-2-specific assay can also be invaluable to retrospectively diagnose cases where NAT results are negative (such

as in circumstances of source finding for acute cases where source cases may already return negative NAT post-acute infection). False-negative NAT can occur where testing is performed too early or too late in the course of infection, or due to sampling or laboratory error.¹⁶

Most sero-studies use qualitative tests that do not measure SARS-CoV-2-specific antibody titres.¹¹⁻¹³ A quantitative assay provides additional depth by the study of antibody kinetics and can be interpreted with individual clinical and epidemiological information to indicate the recency of infection.¹⁰ In addition, if sampled over time, titres are a measure of the host immunological response, which is critical to aid planning for implementation of vaccine programs. In this study, only a single individual in the confirmed case category sero-converted. In effect, a qualitative assay would have missed the remaining confirmed cases who had a four-fold increase in IgG titre. A quantitative titre provides an indicator of the immunological response¹⁰ and higher titres were seen in those who were in the probable category overall; however, even in asymptomatic individuals, titres up to 320 were found. This adds to the evidence that asymptomatic individuals frequently mount an immune response and provides additional information for surveillance and investigation of transmission networks.^{17,18} A more robust SARS-CoV-2-specific IgG response has been demonstrated in more severe COVID-19,¹⁹ with lower levels in asymptomatic individuals²⁰ and overall data available point to a dynamic immune response that warrants further exploration.

Working in a healthcare setting is a risk factor for SARS-CoV-2 infection.^{3,21} By July 2020, the globally reported seroprevalence of SARS-CoV-2 in HCW ranged from 2.7% in low prevalence locations to 45% in high prevalence locations.²¹⁻²³ These numbers are not directly comparable due to differences in antibody detection methodology. This is the first study in Australia reporting HCW infection data in Australia. This study identified an additional 30 HCW with measurable SARS-CoV-2 antibodies of whom 18 were probable or possible cases. Fifteen were asymptomatic (13 possible, 2 indeterminate) and in this group, the median SARS-CoV-2-specific IgG was 20 (IQR 10-60). In these cases, baseline serology (unavailable), may have assisted in classifying cases further. In a low COVID-19 prevalence setting, the

Box 2: Brief examples of the use of SARS-CoV-2 serology.

Case 1: Diagnosis of resolved infection

A 54-year-old woman presented to the fever clinic with mild upper respiratory tract symptoms in April 2020. Her husband was a SARS-CoV-2 NAT confirmed case in March, with her last exposure to him six weeks prior to NAT testing (he had been hospitalised thereafter). NAT testing was negative in April; however serology demonstrated a SARS-CoV-2-specific IgG titre of 320, IgA of 10 and IgM of 80. She was classified as a possible probable case under the CDNA case definition. Serology therefore allowed the retrospective diagnosis of an asymptomatic episode of infection prior to NAT testing. Her symptoms in April were thought likely to be unrelated.

Case 2: Timing of infection onset

A 17-year-old school boy who lives with his parents and four siblings had a weak positive SARS-CoV-2 NAT. Both parents were NAT confirmed COVID-19 cases, along with a 13-year-old sibling. The parents and the 13-year-old sibling were symptomatic while the 17-year-old was asymptomatic. SARS-CoV-2-specific serology was performed on all family members four days after the symptom onset in parents: all were negative. There was no known epidemiological link to a known cluster at the time of the initial NAT and serology. As his NAT was weak positive, initial concern was that he may have been at the end of his illness and therefore possibly the source of infection for his family members. However, as antibodies can take 10-14 days to develop after symptom onset, negative serology as well as a follow-up NAT test which demonstrated significant drop in Ct value, was able to inform the public health department that the boy was unlikely to be the index source of infection for this family.

Case 3: Retrospective diagnosis of a case

A 72-year-old male travelled on a cruise ship in March 2020. His wife was a NAT confirmed COVID-19 case. Initially asymptomatic and by the time symptoms developed, he no longer met criteria for NAT testing according to guidelines in place at that time. He subsequently had progressive dyspnea with radiology demonstrating ground glass changes suggestive of COVID-19. Serology performed three months after disembarking from the cruise ship demonstrated a SARS-CoV-2-specific IgG titre of 10, with negative IgA and IgM. This was classified as a probable case, allowing the retrospective diagnosis of COVID-19. The low IgG titre may be due to the timing of sample collection.

exact risk to HCW working outside of high-risk areas is uncertain, and the interpretation of low detectable SARS-CoV-2-specific IgG or isolated IgA/M in these individuals needs further exploration.

Only 3.7% of serology results were indeterminate, with the majority having detectable SARS-CoV-2-specific IgA or IgM with undetectable IgG (20/36). Some of these results may represent false-positive serology, especially in the setting of lower overall SARS-CoV-2 prevalence.²⁴ It is also possible that individuals classified as probable or possible may represent a false-positive result, however, repeat serology results, in particular for those with low IgG titres, would have been beneficial to further elucidate this issue. We also report a case of maternal SARS-CoV-2-specific antibody transfer, which has been previously described.²⁵

Although not analysed in the present study, serological testing is also valuable in assessing suspected false-positive NAT results.²⁶ In New South Wales, such cases are considered by an expert panel, taking into account epidemiological, clinical and laboratory data including serological testing. Cases determined by the panel to be false-positive NAT are removed from the official case tally. Although to date this has constituted a very small portion of overall cases, it is important to note that false-positive NAT results are a particular concern in settings of low prevalence²⁷ with high levels of testing, as has been the case in Australia.

Some limitations of this study related to the evolving nature of the Australian public health response to the COVID-19 pandemic and the corresponding changes to case definitions in the first half of 2020. For example, serology was added into the national case definition for the diagnosis of COVID-19 in May 2020,⁹ so in the first few months of the pandemic, serology diagnostics were targeted towards NAT-confirmed cases. Subsequently, the use of serology diversified including for retrospective diagnosis. Within the timescale of this study, the variability in the timing of serology sampling (relative to the date of symptom onset) has limited the ability to interpret serology. Whilst highly specific NAb testing may have aided the interpretation of these results, testing was limited due to laboratory constraints. It has also been demonstrated the NAb immunological response fades over time,^{28,29} which may be

a contributing factor to discordant serology and NAb results.

In the Australian setting of low SARS-CoV-2 prevalence and high rates of testing, the positive predictive value of laboratory tests, including SARS-CoV-2-specific serology, is reduced. It is possible some of the low titre antibody results, particularly for indeterminate cases, may represent false positives. Ideally, the collection of baseline serology, tested in parallel with convalescent samples, would have aided the interpretation of these results. Finally, this study does not represent a sero-survey; the majority of individuals who were tested had epidemiological or clinical indications for testing. However, our results suggest that seroprevalence will be low in the Australian context; this was later confirmed with the Sydney sero-survey.⁵

With the rollout of COVID-19 vaccinations, it will be important to use serological tests that detect and distinguish antibodies induced by vaccination from those induced by infection. Assays based on antigens that are not present in the vaccine (e.g. nucleocapsid) should be useful for this purpose. This will be more difficult for jurisdictions implementing vaccines based on whole inactivated virions.

Currently, international guidelines for COVID-19 case definitions preclude serological diagnosis of asymptomatic cases with epidemiological links, e.g. healthcare worker or close/casual contacts. In this study, serology: a) provides additional information for classification of COVID-19 cases including previously undetected cases; b) enhances surveillance by comprehensive identification of all cases; c) facilitates public health investigations of transmission networks; and d) provides an indicator for the period of infectivity of individual cases via the study of antibody kinetics in the context of clinical features. Since the study was conducted, the Australian SARS-CoV-2 infection case definitions have been updated to include a category of 'historic' infection, which would capture many of those classified as possible cases in our study.

To optimise the interpretation of any serology result, the public health response should include testing in parallel with baseline serology (if available) or follow-up repeat serology. In line with international recommendations, serology should be requested ideally between two to five weeks after symptom onset as SARS-CoV-2-specific IgG response may wane over time.³⁰

Conclusion

The current study demonstrates the utility of serology as an adjunct in aiding the diagnosis of COVID-19 and understanding the epidemiological link between clusters. When serology is combined with clinical and epidemiological information, it provides a much more dynamic interpretation and understanding of the COVID-19 pandemic.

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