Testing for COVID-19: a 2023 update

SUMMARY

Nucleic acid amplification tests (NAATs), including polymerase chain reaction (PCR) assays, are more sensitive for the detection of SARS-CoV-2 than rapid antigen tests (RATS), and are the gold standard for diagnosis of acute COVID-19. However NAATs can remain positive for weeks following infection due to low-level shedding of non-viable viral fragments.

RATs (in particular self-testing) are the mainstay of COVID-19 diagnosis due to their convenience, speed and high specificity. The sensitivity of RATs is highest within seven days of symptom onset. A negative RAT result may warrant a NAAT or repeat RAT for confirmation.

The presence of spike antibodies is consistent with either vaccination or infection. Nucleocapsid antibodies suggest a previous infection. Serological tests measuring neutralising antibodies that infer immunity are not readily available.

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Introduction

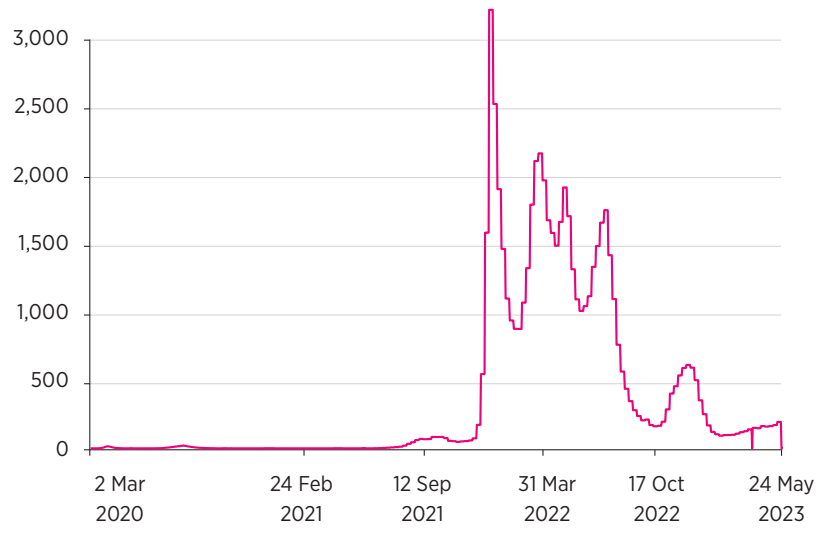
Much has changed since the October 2020 *Australian Prescriber* article on diagnostic tests for SARS-CoV-2.[1] In the first two years of the COVID-19 pandemic, case numbers and mortality were low due to border closures, lockdowns and intensive test, trace, isolate and quarantine policies.[2] Vaccines were developed, tested and manufactured in record time and were rolled out in 2021. With the opening of borders and emergence of new variants of concern, Australia now has widespread community transmission in a relatively well-vaccinated population. Notified cases exceed 11.2 million, with more than 20,300 deaths, the majority of which have occurred since 1 January 2022 (Fig. 1).[2,3] The mortality rates in the highly vaccinated Australian population have remained relatively low in comparison to those in other developed countries.[3]

Variants of concern

Variants of concern are assigned by the World Health Organization when an emerging viral lineage displays increased transmissibility, immune (including vaccine) evasion, resistance to antiviral therapy and/or increased disease severity.[4] Since the emergence of SARS-CoV-2 in December 2019, five viral lineages have been classified as variants of concern: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529).[4]

The Omicron variant emerged in late November 2021, replacing Delta to become the dominant strain globally.[5] Omicron evolved to have increased transmissibility and immune evasion, but does not seem to be more virulent.[5] In 2022 there were several waves associated with the emergence of Omicron sublineages in Australia; initially BA.1, followed by BA.2 and its descendants BA.5 and BA.2.75.[6,7] More recently, recombinant sublineages arising from the exchange of genetic material between strains have emerged and expanded; these include XBB, XBF, XBC, and others.[7,8] The WHO is currently reviewing its classification system for emerging variants to better reflect the current landscape dominated by Omicron descendants.[4]

Fig. 1 **Daily COVID-19 incidence per million population in Australia, March 2020 to May 2023** [3]



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Diagnostic tests

In 2020–21, nucleic acid amplification tests (NAATs) were the main method for diagnosing COVID-19. They remain the most sensitive and specific gold-standard tests.[9] In late 2021, rapid antigen tests (RATs) became available in Australia. Their wide availability, convenience and speed of providing results has led to them becoming the dominant testing method in Australia. Viral culture testing is not available outside specialist reference laboratories and is not routinely performed. There is no readily available test to determine infectivity.

Nucleic acid amplification tests

NAATs detect the genetic material of pathogens in clinical specimens. The most widely used tests are polymerase chain reactions (PCRs). These tests have been authorised for use in laboratories and in point-of-care settings.

Laboratory-based nucleic acid amplification tests

For laboratory-based tests, SARS-CoV-2 nucleic acid (RNA) is extracted and transcribed to DNA, followed by amplification and detection. The recommended specimen is obtained through a single swab of the throat and bilateral deep nasal passages (or nasopharynx). The specimen can either be collected by healthcare workers or can be self-collected. Saliva is a less-sensitive specimen.[10]

There are many assays directed against a variety of different viral gene targets. Most commercial assays are high throughput and include at least two targets. In many jurisdictions, systems have been developed that enable results to be communicated directly to the patient, such as by SMS.

Point-of-care nucleic acid amplification tests

For point-of-care tests, the clinical specimen can be placed directly into a point-of-care NAAT kit. The test kit performs both nucleic acid extraction and amplification and can provide results in approximately one hour. These low-throughput kits are suitable for use outside the main laboratory, such as in emergency departments or remote clinics, but generally require a trained operator. Point-of-care NAATs are performed under the supervision of healthcare professionals according to the clinical governance requirements outlined by the National Pathology Accreditation Advisory Council.[11]

Cycle threshold

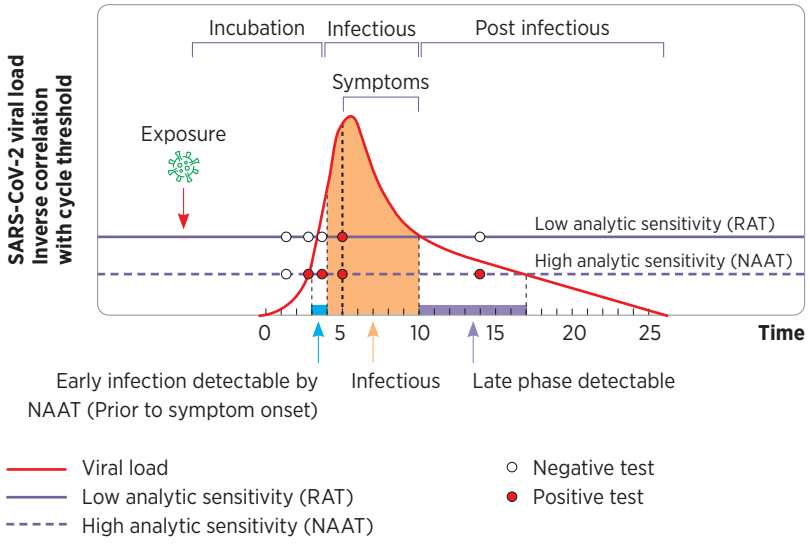
The cycle threshold (Ct) value is the number of amplification cycles before the virus is detected in a PCR. The Ct values from different assays are not directly comparable with each other. A lower Ct value is associated with a larger amount of viral material in a specimen, that is, the Ct value and viral load are inversely related. In acute infection, the viral load usually peaks at around the time of symptom onset, and gradually falls thereafter.[12] A high Ct value may represent very early-stage infection, in which case repeat testing 24–48 hours later would reveal a fall in the Ct value (Fig. 2).[13]

Following SARS-CoV-2 infection, low amounts of SARS-CoV-2 RNA with high Ct values (e.g. more than 35 cycles) can be detected for several months.[14] Studies using viral culture methods have shown that this represents non-infectious shedding of nonviable viral fragments.[15] In some cases, the Ct values can inform decisions about infection control and isolation, alongside testing history and the clinical context. Immunosuppressed individuals may have persistent viral shedding with low Ct values and may have prolonged viral culture positivity and therefore infectivity.[16] Ct values are not included in pathology reports because they vary by assay,[17] although the laboratory may include a comment indicating low-level detection.

Indications

The main indication for a NAAT is to resolve possible false-negative RAT results and, less commonly, suspected false-positive results. For example, in a symptomatic individual with a negative RAT result, a NAAT is indicated to rule out COVID-19. This is particularly important for symptomatic individuals in high-risk settings, including healthcare workers, patients, visitors and carers.

Fig. 2 **Changes in the SARS-CoV-2 viral load over time** [13]



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Rapid antigen tests

Since January 2022, more than half of all notified cases have been detected using a RAT (Fig. 3).[7] These lateral flow immunoassays detect the presence of viral protein (usually the nucleocapsid protein) in a clinical specimen. The sensitivity is greatest during the symptomatic period, and it is recommended that the tests are performed within seven days of symptom onset.[18] RATs available in Australia have high specificity, and have the benefit of providing results within 15–30 minutes. Specimen collection and testing and the interpretation of some RAT results can be done unsupervised at home (self-tests), while others require supervision by a healthcare practitioner or trained staff member (point-of-care tests). RATs are less sensitive than NAATs, so if there are symptoms compatible with COVID-19, a negative RAT result should be followed by a NAAT. If a NAAT is unavailable, serial RATs should be performed. Consistent with their lower sensitivity, prolonged positivity is less of a problem with RATs than for NAATs (Fig. 2).

Postmarketing evaluations are undertaken by or on behalf of the Therapeutic Goods Administration (TGA), and where claimed performance criteria are not met, the TGA registration is withdrawn. To our knowledge, six registrations have been withdrawn at the time of writing.[19] There is currently no evidence to suggest decreased performance associated with emerging variants, but this requires ongoing monitoring.[19]

Rapid antigen self-tests

The TGA approved the use of RAT self-tests for SARS-CoV-2 from November 2021 in Australia, and at the time of writing, there are 73 approved test kits.[20] Each test uses a different methodology (e.g. some use nasal or oral swabs and others use saliva). In June 2022, the first self-administered NAAT was approved.[21] For TGA approval, self-tests must have a minimum clinical sensitivity of 80% for specimens collected within seven days of symptom onset and clinical specificity of at least 98% compared to laboratory NAATs. A summary of tests approved for use in Australia is available on the TGA website, including a rating of the clinical sensitivity of each test:

- 'acceptable' if greater than 80%
- 'high sensitivity' if greater than 90%
- 'very high sensitivity' if greater than 95%.

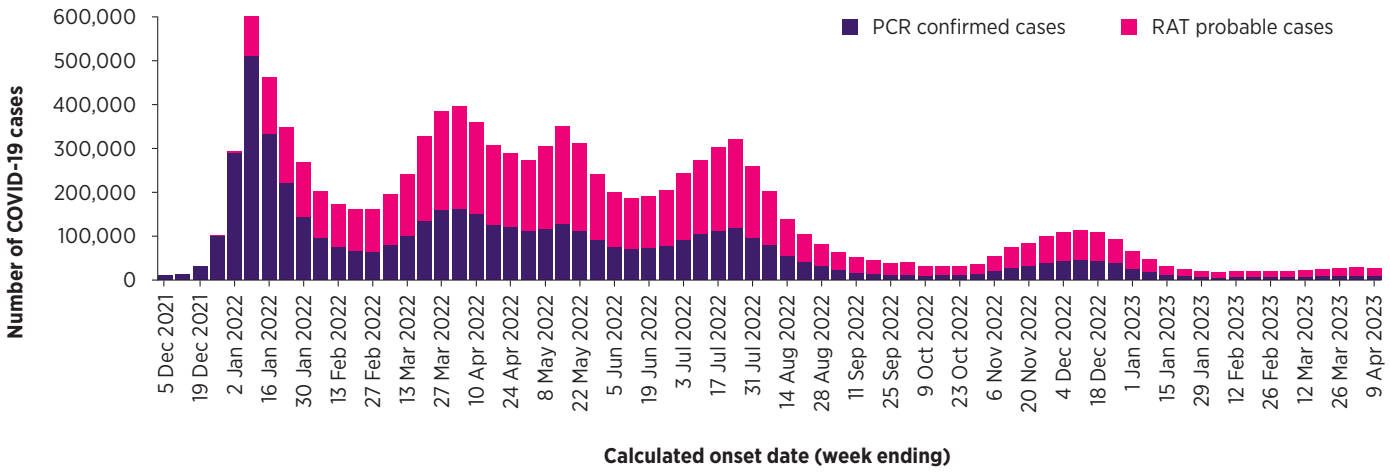
Following a positive RAT self-test result, reporting requirements vary by jurisdiction and can be checked on the relevant state or territory government websites.

If a false self-test result is suspected, the correct methodology for the test including specimen collection, inoculation of the buffer and cartridge, and correct incubation time should be reviewed with the patient, with an interpreter if needed. Specimen collection and testing (e.g. point-of-care RAT or NAAT) by a trained professional should be done if there are concerns.

Point-of-care tests

Similar to point-of-care NAATs, point-of-care RATs are performed under the supervision of healthcare professionals according to the clinical governance requirements outlined by the National Pathology Accreditation Advisory Council.[11] Trained staff are responsible for sample collection and testing, the

Fig. 3 **COVID-19 notifications by rapid antigen tests (probable cases) and nucleic acid amplification (PCR confirmed) tests in Australia** [7]



Source: NNDSS extract from 19 April 2023 for cases with an illness onset from 29 November 2021 to 9 April 2023.
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interpretation of results and the provision of clinical advice if needed. There are currently 51 different point-of-care RATs approved by the TGA.

Serology

The targets for SARS-CoV-2 serological assays include antigens within the spike and nucleocapsid proteins. Antibodies against SARS-CoV-2 can be detected using indirect immunofluorescence assays, enzyme-linked immunosorbent immunoassays and chemiluminescent microparticle immunoassays. IgG appears early and is usually detectable by day 14 after symptom onset, at approximately the same time as IgM.[22] The detection of IgG is more specific and useful than the detection of IgA and IgM.

All vaccines in use in Australia generate antibodies against the spike protein, and the detection of spike antibodies may be due to either vaccination or infection. Nucleocapsid antibodies indicate prior SARS-CoV-2 infection.

Neutralising antibodies confer immunity to SARS-CoV-2, but these are difficult to measure. Testing for these is not readily available outside specialist reference laboratories.[23]

National seroprevalence studies using a convenience sample of blood bank donors indicate that at least 71% of Australian adults were estimated to have had COVID-19 by December 2022, which is twice as high as case notifications would suggest.[24] This nucleocapsid antibody-based snapshot is likely an underestimate, given the assay sensitivity of approximately 84%.[24] Spike antibody prevalence was 99.6%, suggesting that almost the entire Australian population has been vaccinated and/or has had COVID-19 infection.[24]

Uses and limitations of SARS-CoV-2 serology

The detection of spike antibodies can demonstrate a response to vaccination. However, routine post-vaccination testing is not recommended as there are currently no serological correlates of immunity.

Serology is not recommended for the diagnosis of acute infection.[25] The detection of nucleocapsid antibodies can be used to confirm previous infection with COVID-19 (e.g. if a NAAT or RAT was not performed at the time of acute illness or if a false-negative RAT result was suspected). It is important to note that nucleocapsid antibodies wane over time. There is no role for the use of less-sensitive IgG and IgM lateral flow assays, and some jurisdictions have prohibited their use.

Genomics

Genomic sequencing has played a key role in public health management since the beginning of the pandemic. It was through metagenomic sequencing (i.e. sequencing all nucleic acids in a clinical specimen) that a novel coronavirus was identified as the cause of pneumonia in cases with unknown aetiology in Wuhan, China, in December 2019.[26] Rapid sharing of sequence data enabled the development of sensitive and specific NAATs. In 2020–21, a large proportion of notified cases across Australia had a specimen sequenced, with real-time interjurisdictional data sharing, which enabled tracking and tracing of the virus and investigation of the source of cases with no obvious exposure or transmission link.[27,28] As the virus has evolved, genomic surveillance has identified variants of concern and has been used to monitor resistance to pharmacotherapies.[5,8,16] With widespread community transmission, a sampling strategy has been developed to target international arrivals, hospitalised patients with severe illness, outbreaks, immunosuppressed individuals with prolonged infection, and reinfections.[29]

Genomic sequencing can be performed using specimens collected for PCR tests. This is rarely indicated for individual patient management but may assist in differentiating relapse from reinfection, or in the detection of mutations associated with resistance to pharmacotherapies. At the individual patient level, it is most relevant for immunocompromised patients in hospitals.

Other winter viruses

Throughout 2020–21, other respiratory viruses, particularly influenza A and B, disappeared from circulation altogether. Respiratory syncytial virus was still present but had unexpected, unseasonal peaks of activity. The winter of 2022 saw the re-emergence of influenza A with an early and sharp rise in case numbers and deaths.[30]

Twelve combination RATs that include influenza A and B have been registered by the TGA for self-testing. With additional circulating viral respiratory pathogens, multiplex NAATs for common respiratory viruses including influenza A and B, respiratory syncytial virus and SARS-CoV-2 were recommended for the winter of 2022.[31] This is referred to as a 'respiratory virus panel' and is the recommended method for diagnosing these other respiratory viruses. Some panels also include human metapneumovirus, parainfluenza virus, rhinovirus, adenovirus and bacterial pathogens as part of respiratory NAATs. It is not uncommon for multiple respiratory viruses including SARS-CoV-2 to be detected at one time, particularly in infants and small children.

Conclusions

While NAATs remain the gold standard for SARS-CoV-2 detection, RATs and self-testing are currently the main method for diagnosing COVID-19. RATs are most

sensitive when used within seven days of symptom onset. Serial RATs, or NAATs in high-risk settings, are indicated for symptomatic individuals with an initial negative RAT result. There is no readily available serological test that infers immunity. The detection of nucleocapsid antibodies can confirm previous infection, and the presence of spike antibodies can be due to either infection or vaccination. The development, refinement and implementation of diagnostic tests for COVID-19 have been a major achievement and critical to the relatively well-managed pandemic in Australia. The involvement of patients in their own diagnostic testing, public health notification and management (primarily isolation) has represented a paradigm shift. This will form the basis of future development in the management of infectious diseases and outbreaks. <

Conflicts of interest: none declared

REFERENCES

1. Brischetto A, Robson J. Testing for COVID-19. *Aust Prescr* 2020;43:204-8. https://doi.org/10.18773/austprescr.2020.067
2. World Health Organization. Australia: WHO coronavirus disease (COVID-19) dashboard with vaccination data. https://covid19.who.int/region/wpro/country/au [cited 2023 May 15]
3. Mathieu E, Ritchie H, Rodés-Guirao L, Appel C, Giattino C, Hasell J, et al. Coronavirus Pandemic (COVID-19). OurWorldInData. Coronavirus pandemic (COVID-19). https://ourworldindata.org/coronavirus#explore-the-global-situation [cited 2023 May 24]
4. World Health Organization. Tracking SARS-CoV-2 variants. www.who.int/activities/tracking-SARS-CoV-2-variants [cited 2023 May 15]
5. Viana R, Moyo S, Amoako DG, Tegally H, Scheepers C, Althaus CL, et al. Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. Nature 2022;603:679-86. https://doi.org/10.1038/s41586-022-04411-y
6. Duchene S, Porter A. From Centaurus to XBB: your handy guide to the latest COVID subvariants (and why some are more worrying than others). https://www.cdgn.org.au/news/why-are-there-so-many-new-omicron-sub-variants-like-ba4-and-ba5-will-i-be-reinfected-is-the-virus-mutating-faster-7l2lr [cited 2023 May 15]
7. COVID-19 Epidemiology and Surveillance Team. COVID-19 Australia: Epidemiology Report 73 – reporting period ending 9 April 2023. Commun Dis Intell 2023;47. https://doi.org/10.33321/cdi.2023.47.25
8. Akerman A, Milogiannakis V, Jean T, Esneau C, Silva MR, Ison T, et al. Emergence and antibody evasion of BQ, BA.2.75 and SARS-CoV-2 recombinant sub-lineages in the face of maturing antibody breadth at the population level. EBioMedicine 2023;90:104545. https://doi.org/10.1016/j.ebiom.2023.104545
9. Communicable Diseases Network Australia, Public Health Laboratory Network. Revised testing framework for COVID-19 in Australia. March 2022. www.health.gov.au/resources/publications/coronavirus-covid-19-testing-framework-for-covid-19-in-australia?language=en [cited 2023 May 15]
10. Public Health Laboratory Network. PHLN guidance on laboratory testing for SARS-CoV-2 (the virus that causes COVID-19). www.health.gov.au/resources/publications/phln-guidance-on-laboratory-testing-for-sars-cov-2-the-virus-that-causes-covid-19 [cited 2023 May 15]
11. Australian Government Department of Health and Aged Care. Requirements for point of care testing (second edition 2021). 2022 Jan 1. www1.health.gov.au/internet/main/publishing.nsf/Content/Requirements-for-Point-of-Care-Testing-(Second-Edition-2021) [cited 2023 May 15]
12. Jang S, Rhee JY, Wi YM, Jung BK. Viral kinetics of SARS-CoV-2 over the preclinical, clinical, and postclinical period. Int J Infect Dis 2021;102:561-5. https://doi.org/10.1016/j.ijid.2020.10.099
13. Crozier A, Rajan S, Buchan I, McKee M. Put to the test: use of rapid testing technologies for COVID-19. BMJ 2021;372:n208 https://doi.org/10.1136/bmj.n208.
14. Singanayagam A, Patel M, Charlett A, Bernal JL, Saliba V, Ellis J, et al. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. Euro Surveill 2020;25:2001483. https://doi.org/10.2807/1560-7917.ES.2020.25.32.2001483
15. Basile K, McPhie K, Carter I, Alderson S, Rahman H, Donovan L, et al. Cell-based culture informs infectivity and safe de-isolation assessments in patients with coronavirus disease 2019. Clin Infect Dis 2021;73:e2952-9. https://doi.org/10.1093/cid/ciaa1579
16. Rockett R, Basile K, Maddocks S, Fong W, Agius JE, Johnson-Mackinnon J, et al. Resistance mutations in SARS-CoV-2 Delta variant after sotrovimab use. N Engl J Med 2022;386:1477-9. https://doi.org/10.1056/NEJMc2120219
17. Carroll A, McNamara E. Comparison and correlation of commercial SARS-CoV-2 real-time-PCR assays, Ireland, June 2020. Euro Surveill 2021;26:2002079. https://doi.org/10.2807/1560-7917.ES.2021.26.6.2002079
18. Dinnes J, Deeks JJ, Berhane S, Taylor M, Adriano A, Davenport C, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev 2021:CD013705. https://doi.org/10.1002/14651858.cd013705.pub2
19. Therapeutic Goods Administration. Post-market review of antigen and rapid antigen tests. Last updated 12 Apr 2023. https://www.tga.gov.au/products/covid-19/covid-19-tests/post-market-review-antigen-and-rapid-antigen-tests [cited 2023 May 15]
20. Australian Government Department of Health and Aged Care, Therapeutic Goods Administration. COVID-19 rapid antigen self-tests that are approved in Australia. 2022 Dec 7. www.tga.gov.au/products/covid-19/covid-19-tests/covid-19-rapid-antigen-self-tests-home-use/covid-19-rapid-antigen-self-tests-are-approved-australia [cited 2023 May 15]
21. Australian Government Department of Health and Aged Care, Therapeutic Goods Administration. EasyNAT COVID-19 RNA test. www.tga.gov.au/resources/covid-19-test-kits/easynat-covid-19-rna-test [cited 2023 May 15]
22. Wehrhahn MC, Brown SJ, Newcombe JP, Chong S, Evans J, Figtree M, et al. An evaluation of 4 commercial assays for the detection of SARS-CoV-2 antibodies in a predominantly mildly symptomatic low prevalence Australian population. J Clin Virol 2021;138:104797. https://doi.org/10.1016/j.jcv.2021.104797
23. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med 2021;27:1205-11. https://doi.org/10.1038/s41591-021-01377-8
24. Australian COVID-19 Serosurveillance Network. Seroprevalence of SARS-CoV-2-specific antibodies among Australian blood donors: Round 3 update. 3 Nov 2022. Sydney: Kirby Institute. https://kirby.unsw.edu.au/project/serosurveillance-sars-cov-2-infection-inform-public-health-responses [cited 2023 May 15]
25. Royal College of Pathologists of Australasia. Position statement: COVID-19 serology. www.rcpa.edu.au/getattachment/5fb8a015-8e4e-4472-8eb7-46268288a20f/COVID-19-Serology.aspx [cited 2023 May 15]
26. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579:265-9. https://doi.org/10.1038/s41586-020-2008-3
27. Hoang T, da Silva AG, Jennison AV, Williamson DA, Howden BP, Seemann T. AusTrakka: fast-tracking nationalized genomics surveillance in response to the COVID-19 pandemic. Nat Commun 2022;13:865. https://doi.org/10.1038/s41467-022-28529-9
28. Lane CR, Sherry NL, Porter AF, Duchene S, Horan K, Andersson P, et al. Genomics-informed responses in the elimination of COVID-19 in Victoria, Australia: an observational, genomic epidemiological study. Lancet Public Health 2021;6:e547-e56. https://doi.org/10.1016/S2468-2667(21)00133-X
29. Communicable Diseases Genomics Network, Public Health Laboratory Network, Communicable Diseases Network Australia. CDGN, PHLN and CDNA sampling strategy for SARS-CoV-2 genomic surveillance. www.health.gov.au/resources/publications/cdgn-phln-and-cdna-sampling-strategy-for-sars-cov-2-genomic-surveillance [cited 2023 May 15]
30. Australian Government Department of Health and Aged Care. Influenza surveillance program. www.health.gov.au/our-work/influenza-surveillance-program [cited 2023 May 15].
31. Public Health Laboratory Network, Communicable Diseases Network Australia. Joint statement on patient referral and respiratory virus test requesting for winter 2022. www.health.gov.au/resources/publications/phln-and-cdna-joint-statement-on-patient-referral-and-respiratory-virus-test-requesting-for-winter-2022 [cited 2023 May 15]