PHLN

Public Health Laboratory Network

PHLN guidance on laboratory testing for SARS-CoV-2 (the virus that causes COVID-19)

Revision History

Version	*Date Endorsed by PHLN*	*Revision note*
2.2	26 January 2022	Updated to align with testing recommendations following the 5 January 2022 National Cabinet.
2.1	29 October 2021	Update to serology guidance.
2.0	7 June 2021	Revision of whole document.
1.16	16 February 2021	Update regarding correlates of immunity and serology testing post vaccination.
1.15	2 November 2020	Update to guidance on point of care testing outside a PC2 facility and include reference to PHLN – CDNA joint statement on antigen testing.
1.14	9 September 2020	Update to advice on serology, inclusion of guidance on saliva, antigen testing, genomics and amend formatting for readability.
1.13	10 August 2020	Update to include guidance on paediatric oropharyngeal and bilateral deep nasal specimen collection.
1.12	26 June 2020	Update to guidance on self-collection.
1.11	16 June 2020	Update guidance on self-collection and Royal College of Pathologists of Australasia Quality Assurance Program (RCPAQAP).
1.10	18 May 2020	Update to guidance on point of care testing outside a PC2 facility.
1.9	11 May 2020	Update Transmission-based Precautions to reflect ICEG guidance.
1.8	4 May 2020	Update guidance on deep nasal collection.
1.7	28 April 2020	Update to include guidance on serology testing and updated advice on point of care testing outside a PC2 facility.

Version	*Date Endorsed by PHLN*	*Revision note*
1.6	15 April 2020	Update to include advice on point of care testing outside a PC2 facility.
1.5	14 April 2020	Update to upper respiratory tract sample collection guidance to also include addition of self-collected combined nasal and throat swab using a single swab as suitable specimen type
1.4	1 April 2020	Updated reference of nasopharyngeal to deep nasal. Deleted reference to dry swabs.
1.3	13 March 2020	Update to upper respiratory tract sample collection guidance to recommend use of a single swab. Inclusion of virus target and quality assurance program information.
1.2	25 February 2020	Update to upper respiratory tract sample collection guidance, virus name and inclusion of reference to Person under Investigation definition.
1.1	6 February 2020	Update to transmission based precautions for sample collection, available PCR assays and biosafety measures for clinical pathology.
1.0	23 January 2020	Initial document

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1. Executive Summary

Testing for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) predominantly involves virus detection using:

- nucleic acid amplification (NAA) tests targeting different viral genes
- rapid antigen tests (RATs) that detect SARS-CoV-2 viral proteins.

High throughput, laboratory-based NAA is usually performed using either reverse transcriptase polymerase chain reaction (RT-PCR) or transcription-mediated amplification (TMA) technologies. Both commercially manufactured devices and assays developed in-house are available (see Section 5.1).

RATs are intended for use at the point-of-care (PoC), or near person care, and can provide a result within 15–30 minutes. In times of high community transmission, RATs can be used to complement NAA testing in order to relieve pressure on the pathology system. Please refer to the *Testing Framework for COVID-19 in Australia* (the Framework) for more information on testing approaches based on the local epidemiological context (see Section 5.3).

Retrospective diagnosis is achieved using pathogen-specific serology (predominantly IgG seroconversion). Serology tests measure antibody responses in a qualitative or quantitative manner (see Section 5.2).

Other testing methods such as viral culture and whole genome sequencing are generally only available in reference public health laboratories (see Section 5.4 and 5.5).

Samples used for diagnosis include nasopharyngeal secretions collected via a swab, combined nasal cavity and oropharyngeal swab collection, and in more severe cases, lower respiratory tract secretions. Saliva is increasingly used for surveillance purposes[1]. Other specimens less frequently tested include faeces, blood and other non-respiratory swabs (conjunctiva)(see Section 2).

National wastewater surveillance testing is also in place as a public health tool to understand SARS-CoV-2 spread in the community (see Section 5.6).

1.1 Transmission-based precautions

Guidance on Personal Protective Equipment (PPE) for specimen collection is available from the Infection Control Expert Group's *Guidance on use of personal protective equipment (PPE) in non-inpatient healthcare settings, during the COVID-19 outbreak*.

2 Approach to testing and specimen selection

The *COVID-19 Communicable Diseases Network Australia (CDNA) Series of National Guidelines for Public Health Units* (the National Guidelines) recommends testing of individuals who meet the suspect case definition and the enhanced testing criteria. Laboratory testing is useful for individual patient diagnosis and therapy, and to guide infection control procedures and public health investigations. Where feasible, perform repeat testing (especially of lower respiratory tract specimens) of clinically compatible cases if initial results are negative and there remains a high suspicion of infection. Investigation for other non-SARS-CoV-2 diagnoses

[1] Please refer to the *PHLN statement on using saliva as a respiratory specimen for SARS-CoV-2 testing* for more information on advantages and disadvantages to this specimen collection method.

of acute respiratory infection and/or sepsis, such as blood and sputum cultures and NAA testing for other respiratory pathogens, should be considered.

This section describes the current specimen types recommended for SARS-CoV-2 testing in Australia. Perform routine tests for acute pneumonia/pneumonitis according to local protocols where indicated. This may include:

- NAA testing for other respiratory pathogens using multiplex methods,
- cultures of blood and sputum,
- acute and convalescent serology, and
- urinary antigen testing for non-SARS-CoV-2 pathogens.

2.1 Upper respiratory tract samples[2]

a. Oropharyngeal and bilateral deep nasal swab:

Oropharyngeal (throat):

- Using either a dacron or rayon (PHLN recommend flocked) swab, sample the tonsillar beds and the back of the throat. Avoid the tongue.

Bilateral deep nasal:

- To conserve swabs, the same swab used to sample the oropharynx may be used for nasal sampling.
- Using a pencil grip, gently rotate the swab, inserting the tip 2–3 cm (or until resistance is met) into the nostril. Keep the swab parallel to the palate to absorb mucoid secretion.
- Rotate the swab several times against the nasal wall.
- Withdraw the swab and repeat the process in the other nostril.
- Place the swab(s) back into the accompanying transport medium.

Sample all three sites, oropharynx and bilateral deep nasal, to optimise the chances of virus detection.

Place the swab(s) directly into the transport medium (TM). This may be viral (VTM) or universal transport medium (UTM), Liquid Amies (LA) or another validated transport medium. While dry swabs are acceptable, the PHLN recommends swabs in transport medium.

b. Self-collected oropharyngeal and bilateral deep nasal swab:

A self-collected oropharyngeal and bilateral deep nasal swab for the purpose of NAA testing may in some circumstances be an appropriate method of specimen collection. However, where possible, the PHLN encourages healthcare workers supervise the collection.

The PHLN has reviewed both domestic and international data that supports a self-administered method of collection. A correctly performed self-collected combined oropharyngeal and bilateral deep nasal swab is equivalent in detecting SARS-CoV-2 to a

[2] For rapid antigen test devices, the user should collect the specimen in accordance with the manufacturer's instructions for use. This applies to self-test devices, home use, and supervised use.

combined nasal and throat swab carried out by a healthcare worker[3][4]. This potentially reduces infection risk to the healthcare worker performing the collection. It also reduces PPE requirements.

It will also need to be part of an agreed procedure with the testing laboratory. For rapid antigen tests, the manufacturer's instructions for use (IFU) must be followed.

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The requesting medical practitioner or the pathology service provider should provide clear written instructions to the patient. It is recommended that an accessible instructional video is provided to support any written instructions. If the requesting clinician provides the swabs directly to the patient, the clinician must make sure that the receiving laboratory is able to process self-collected specimens.

The PHLN recommends laboratories processing these self-collects consider:

- clearly identifying the specimen is a self-collected sample
- validating their ability to obtain equivalent viral loads compared to other collection methods
- monitoring positivity rates using these self-collection kits in comparison to other collecting methods and
- including a human DNA control to the COVID-19 NAA assay to confirm adequacy of sample collection.

Please note, the Therapeutic Goods Administration (TGA) has approved rapid antigen self-tests for supply in Australia from 1 November 2021. Rapid antigen self-tests (home use tests) are tests that can be used unsupervised at home without the involvement of a health practitioner. A person collects their own sample, or a sample from a family member, performs the test and interprets the results by themselves. Some jurisdictions require the registration of positive rapid antigen self-tests results.

c. Paediatric oropharyngeal and bilateral deep nasal swab

Make sure the child is in a comfortable, secure position, preferably on their guardian's lap. The guardian should cross one arm across the child's body, holding their arms and place the other hand on the child's forehead. This will limit the child's ability to roll their head away from the collector. The collector must be calm and confident when collecting the sample.

Swab selection:

- Use a swab with a fine flexible shaft with a nylon flocked or foam tip. Dacron or rayon tips are also suitable.
- If using a nasopharyngeal swab as part of broader respiratory testing, seek appropriate guidance (see section 1d).

Oropharyngeal (throat) collection:

- Using a tongue depressor, hold down the tongue.
- Swab the tonsillar beds and the back of the throat. Avoid the tongue.

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[3] Wehrhahn MC, Robson J, Brown S, Bursle E, Byrne S, New D, Chong S, Newcombe JP, Siversten T, Hadlow N. Self-collection: an appropriate alternative during the SARS-CoV-2 pandemic. Journal of Clinical Virology. 2020 May 4:104417

[4] Tu YP, Jennings R, Hart B, Cangelosi GA, Wood RC, Wehber K, Verma P, Vojta D, Berke EM. Swabs Collected by Patients or Health Care Workers for SARS-CoV-2 Testing. New England Journal of Medicine. 2020 Jun 3.

Bilateral deep nasal collection:

- To conserve swabs, the same swab used to sample the oropharynx may be used for nasal sampling.
- Using a pencil grip, insert the tip of the same swab into the nostril and parallel to the palate. Insert the swab to the following depths, or until resistance is met. As a guide:
    - <2 years, insert 1 cm
    - 2–6 years, insert 1.5 cm
    - 6–12 years, insert 2 cm
    - >12 years, insert 2–3 cm
- Rotate the swab several times against the nasal wall.
- Withdraw the swab and repeat the process in the other nostril.
- Place the swab(s) back into the accompanying transport medium.

This may be VTM, UTM, LA or another validated TM. While dry swabs are acceptable, the PHLN recommends swabs in TM.

d. Nasopharyngeal swab

Only use a swab suitable for nasopharyngeal sampling. If the swab is too large revert to a oropharyngeal bilateral deep nasal sampling technique.

Measure the distance from the nose to the ear to provide an estimate of the distance to insert the swab.

Use a nasopharyngeal swab with a long flexible shaft and fine small tip dacron, rayon or nylon flocked swab. Insert the measured length in a line from the nasal ala to the ear lobe (in line with the nasal floor) until reaching the nasopharynx.

Leave for a few seconds to absorb secretions and rotate on withdrawal.

Directly place the swab in transport medium. While dry swabs are acceptable, the PHLN recommends swabs in TM.

e. Saliva testing

Routine diagnostic saliva testing has been used in some countries with a high prevalence of COVID-19. In Australia, regular saliva testing has been used for quarantine staff and healthcare worker screening.

Collect the saliva:

- directly into a specimen container; or
- using specialised saliva collection devices; or
- using an oral swab[5].

A Low SARS-CoV-2 prevalence alters pre- and post-test probability, and subsequent negative and positive predictive values. The PHLN does not advise routine use of saliva for diagnostic testing except in a low prevalence epidemiological context.

Noting the current evidence, PHLN considers the potential advantages of saliva use for SARS-CoV-2 PCR testing are:

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[5] Please note that the type of swab and amount of viral transport media which the swab is placed into may effect the sensitivity of the test.

- it is minimally invasive and can be reliably self-administered under an approved healthcare professional's supervision
- it reduces the infection risk to healthcare workers and minimises the demand for PPE and
- it is likely, and in some settings proven, to be more acceptable to people, particularly those undergoing repeated sampling.

A 2020 systematic review and meta-analysis found a lower percentage of positive results when testing saliva compared to nasopharyngeal or combined throat and bilateral deep nasal swabs. Test performance was even lower when using saliva specimens collected 7 days or more after symptom onset[5]. Much remains unknown about the impact of saliva collection method variation, processing protocols, and population (i.e., pædiatric vs. adult and late vs. early in disease course) on the sensitivity of testing saliva.

In Australia, the Therapeutic Goods Administration (TGA) must approve the use of in vitro medical devices (IVDs). Currently, few registered SARS-CoV-2 IVDs list saliva as a specimen type in the manufacturers' IFU. Some laboratories have National Association of Testing Authorities (NATA) accreditation for saliva testing by NAA. For further information, including limitations of using saliva, please refer to the *PHLN statement on use of saliva as an alternative specimen for the diagnosis of SARS-COV-2*.

2.2 Lower respiratory tract samples

a. Bronchoalveolar lavage, tracheal aspirate, pleural fluid

Collect 2–3 mL into a sterile, leak-proof, screw-top sputum collection cup or dry sterile container.

b. Sputum

Patient should rinse their mouth with water before collection.

Expectorate deep cough sputum directly into a sterile, leak-proof, screw-top dry sterile container.

The PHLN advises collecting lower respiratory tract specimens for SARS-CoV-2 testing where possible for those with a negative upper respiratory tract specimen with a high clinical suspicion of COVID-19. This is because the lower respiratory tract specimens contain the highest viral loads in lower respiratory disease due to SARS-CoV-2.

2.3 Blood

Collect serum or dried blood spots (DBS) under transmission-based precautions if the patient has suspected COVID-19, or has confirmed COVID-19 infection and is still in isolation.

For serum, collect at least 3 mL in a serum separator tube and transport to the laboratory as soon as possible. Alternatively, dried blood spots (DBS) can be used, and either prepared from capillary blood samples (collected by finger prick using disposable lancets) or whole blood. Place a drop of blood onto the circle on a blood collection card and let dry for at least 30 minutes before transporting or storing.

The self-collection of DBS for the purposes of SARS-CoV-2 antibody testing has not been extensively studied to date. Use standard precautions to collect serum or DBS from proven cases of COVID-19 released from isolation.

3 Specimen handling in the laboratory

3.1 Microbiology laboratory

Laboratory staff should handle specimens under PC2 conditions in line with AS/*NZS 2243.3:2010 Safety in Laboratories Part 3: Microbiological Safety and Containment*. Transport specimens in line with current regulatory requirements as diagnostic samples for testing.

Perform virus culture of SARS-COV-2 under PC3 conditions.

3.2 Point of care testing of respiratory samples outside of a PC2 facility

PHLN members considered the World Health Organization (WHO) guidance *Laboratory biosafety guidance related to coronavirus disease (COVID-19)*[6] and noted the highlighted recommendations which refer to laboratory-based work:

- Perform all procedures based on risk assessment and only by personnel with demonstrated capability. Strictly observe relevant protocols at all times.
- Technicians may use point of care (POC) or near-POC assays on a bench without employing a biosafety cabinet (BSC). This is when dictated by local assessment and proper precautions are in place.

Assess the diagnostic testing steps for specimen processing outside of a PC2 facility, for example, rapid respiratory testing performed at, or near, the POC. Determine if aerosol generation may occur to inform which transmission-based precautions should be applied to provide a barrier between the specimen and personnel during specimen manipulation. If local community transmission is established, consider implementing airborne precautions. When conducting POC tests in non-NATA/RCPA accredited medical pathology facilities, testing should occur within an appropriate quality framework. This includes but is not limited to:

- training and competency systems of staff involved in testing
- management and quality control of reagents
- patient identification
- specimen labelling and documentation of results and
- workflow plans for secondary or confirmatory testing.

Conduct testing in a well ventilated room, preferably with an external exhaust fan. Do not conduct testing without a validated infectious waste process with sufficient capacity for the number of specimens handled[1].

4 Clinical Pathology

Non-respiratory specimens (blood, urine, stool) may be SARS-CoV-2 NAA positive, but culture of SARS-CoV-2 virus from these specimens has rarely been reported. Apply standard precautions for non-microbial pathology testing (such as routine biochemistry and haematology). Where possible, use auto-analysers according to standard practices and/or local protocols. There is evidence that capping and uncapping of samples is not a high risk aerosol generating procedure.

[6] https://www.who.int/publications-detail/laboratory-biosafety-guidance-related-to-coronavirus-disease-(covid-19)

a. Respiratory virus diagnostic testing for laboratories not performing SARS-CoV-2 testing

Perform NAA testing of the upper or lower respiratory tract samples for diagnosing common respiratory viruses. Do this using standard protocols and methods of the hospital or community laboratory.

The PHLN expects this testing to be performed using PC2 laboratory practices, and use of a Class II BSC for aerosol generating procedures, for example, centrifuging without sealed carriers. Only undertake viral culture in an accredited laboratory that has a PC3 facility.

Forward the residue (original swab and remaining eluate) of the respiratory tract sample(s) with any serum, to the reference laboratory for SARS-CoV-2 testing as appropriate.

Clinical liaison with jurisdictional public health officers is essential to coordinate referral and testing.

Apply standard protocols for sample packaging and transport as diagnostic samples for testing (i.e. Category B).

5 SARS-CoV-2 specific testing

5.1 Nucleic acid amplification

NAA testing using real time RT-PCR or TMA are the methods of choice to detect SARS-CoV-2 during the acute illness. The PHLN describes specific diagnostic test approaches for SARS-CoV-2 below in broad terms. There is significant variation in NAA testing employed by different PHLN member laboratories and non-PHLN laboratories. Commercial NAA assays have been available for SARS-CoV-2 testing in Australia since March 2020. Verification to NPAAC standards is required prior to introduction of NAA SARS-CoV-2 assays.

In the early phases of the pandemic, before commercial assays were available, the PHLN member laboratories implemented in-house NAA assays by either designing their own specific RT-PCR primer/probe sets or using published primer/probe sets, for example, those released by the Centers for Disease Control (CDC). Leading international coronavirus reference laboratories recommended these primer sets to the World Health Organization (WHO) to detect SARS-CoV-2. Well pedigreed PCR primer sets, probes and protocols are available from the WHO/ European Virus Archive Global (EVAg).

In Australia, positive control material is available from jurisdictional public health laboratories with cell culture capacity. Synthetic positive control material in the form of nucleic acid templates is also available through WHO/ EVAg.

During these early stages of the pandemic when laboratories were becoming familiar with the performance of these assays, diagnosis of acute infection was often performed as a two-step process with an initial screening NAA testing, followed by a confirmatory NAA test.

Most diagnostic laboratories now use either commercial or in-house developed assays for testing. When community transmission is low, turnaround times are less than 24 hours after the laboratory receives a specimen. Faster turnaround times can be achieved when using rapid, but low throughput NAA assays are used. Specimens may also be pooled to increase the throughput of testing, or when there is a shortage of reagents and consumables.[7]

[7] Chong B, Tran T, Druce J, Ballard S, Simpson J, Catton M, Sample pooling is a viable strategy for SARS-CoV-2 detection in low-prevalence settings, *Pathology*, 52(7): Pages 796-800 2020. https://doi.org/10.1016/j.pathol.2020.09.005.

Laboratories should evaluate the effects of pooling on their assay's limit of detection prior to pooling. Samples within a pool that test positive are then tested individually to determine which one(s) were positive. When the prevalence or pre-test probability of infection is high or when testing previously known positives, pooling is not advised. This is due to the increased likelihood of follow up testing of individual samples negating the workflow efficiencies of pooling.

The RT-PCR Ct (cycle threshold) value reflects the minimum number of amplification cycles required for the fluorescence signal generated to be considered positive. The Ct value is may provide a guide to the level of virus present in the sample. However, one cannot assume the correlation between Ct value and viral load in the specimen to be linear.

SARS-CoV-2 NAA tests are qualitative assays and should be reported as such unless the testing laboratory performs a validated calibration of the NAA test against a recognised standard (such as the WHO International Standard NIBSC 20/146[8]). There can be considerable variability between Ct values due to sampling effects and inter-assay variability. Therefore, it is difficult to directly compare Ct values between different assays, particularly when the target genes are different. There are also assays that do not record Ct values, for example, TMA assays measure in relative light units (RLU). Laboratories using TMA platforms will need to establish criteria for the level of virus in the sample if providing a guide of this.

Most NAA assays detecting SARS-CoV-2 have multiple gene targets to provide optimal analytical sensitivity and specificity. The use of multiple gene targets is recommended as some SARS-CoV-2 lineages or strains have mutations that result in failure to detect particular gene targets in some NAA assays. This is most likely to occur in the genes with the highest diversity, the spike and nucleocapsid protein genes. This genetic variation affecting NAA assay detections can be used for public health purposes. For example, the loss of the spike gene target in some commercial NAA assays was used as a predictive marker of the spread of both B1.1.7 (Alpha) in the UK and more recently the B.1.1.529 (Omicron) lineage globally. . In settings with low SARS-CoV-2 prevalence, there is the potential for false positive NAA test results due to the lower pre-test probability. To minimise these risks, PHLN recommends confirming positive results. Do this by either:

- retesting the original specimen using NAA assay detection of a different target gene (particularly for assays with a single target),
- obtaining a second sample for NAA assay detection, or
- genomic sequencing (see below).

As an example, the pan-sarbecovirus E gene target used in some assays is not specific for SARS-CoV-2. When detected (often called a presumptive positive), test other targets to confirm this result. However, caution should be applied as the other target(s) may not be detected despite the presence of SARS-CoV-2. Also, the specificity of the NAA assay is a function of the size and location of the targeted region of the SARS-CoV-2 genome. Laboratories should also have rigorous controls to prevent contamination within the laboratory environment. At present, it is not possible to differentiate between SARS-CoV-2 reinfection and persistent or intermittent RNA detection in a person where NAA test is positive, unless performing genomic sequencing.

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[8] NIBSC *Coronavirus (COVID-19)-related research reagents available from the NIBSC* https://www.nibsc.org/science_and_research/idd/cfar/covid-19_reagents.aspx

Further PHLN guidance on NAA test result interpretation can be found at *PHLN guidance on nucleic acid test result interpretation for SARS-CoV-2*.

The Biosecurity QAP of the Royal College of Pathologists of Australasia Quality Assurance Program (RCPAQAP) offered three SARS-CoV-2 specific NAA test specimen Proficiency Testing Programs (PTP) in March, May and November 2020. The March 2020 NAAT PTP was the first such national program offered anywhere in the world. This Australian Government-funded Biosecurity program contributed to the COVID-19 pandemic response by providing laboratories with access to a timely PTP for NAA testing, and later for whole genome sequencing (WGS). Laboratories are also encouraged to continuously monitor changes in the SARS-CoV-2 genome that may affect the performance of NAA assays. The WGS PTP for SARS-CoV-2 was offered in March 2021, collaboratively with the Communicable Diseases Genomics Network (CDGN), the first such program internationally. This will be offered again in 2022 to support clinical and public health laboratories in implementing WGS as a diagnostic and epidemiological tool.

5.2 Serology

Serology (antibody) tests for COVID-19 can help identify individuals who have developed detectable antibodies to SARS-CoV-2 as part of an immune response to the virus or vaccine. Serological tests rely on detection of specific anti-SARS-CoV-2 antibody (IgM, IgA, IgG, total or neutralising antibody) in patient serum, plasma or whole blood.

Detection of a specific antibody response to infection with SARS-CoV-2 usually occurs within 1-3 weeks following SARS-CoV-2 infection, with IgG reliably detectable by 21 days post infection. Serological testing within two weeks following the onset of symptoms may result in false negative results. Occasionally the serological response may take longer to develop and approximately 5–10% of individuals may not develop antibodies.[1] In addition, the timing and duration of the antibody response(s) can vary depending on several host factors, including:

- age
- underlying comorbidities
- individual variability and severity of disease and
- assay factors such as the type of antibody being measured and to which viral antigen.

There also remain many gaps in the interpretation of antibody test results, including:

- whether antibody detection from infection or vaccination correlates with protective immunity to reinfection or post-vaccination infection
- whether development of antibody in those infected correlates with cessation of infectiousness to others
- the interchangeability between antibody detected by different test formats or to different epitopes and
- the positive predictive value of serological assays for previous infection in settings with differing prevalence (i.e. identification of true positive results).

Despite these gaps in knowledge, there are several use cases for serological assays.

5.2.1 Uses for serology testing

While serological assays have no role in the detection of acute COVID-19 infection, they can be helpful for the retrospective diagnosis of infection. For example:

- where the result will influence individual or outbreak management, such as testing

patients who have had symptoms consistent with COVID-19 but are:
    - RT-PCR negative
    - were not tested
    - have unexpected positive or inconclusive results on RT-PCR assays or
    - had an epidemiological risk factor for COVID-19 but remained asymptomatic.
- 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 and
- as part of a public health investigation to inform management of a patient.

However to date, there are no serological assays that provide a *definitive* correlation of immunity to SARS-CoV-2.

5.2.2 Testing to confirm immunity status

PHLN does not currently recommend routine diagnostic serological testing pre- or post-vaccination. The detection of anti-spike antibodies cannot distinguish between natural infection and vaccination. Patients with previous SARS-CoV-2 infection may lose specific antibodies at different rates over time.

The antibody response following vaccination will vary depending on the antigens present in the different COVID-19 vaccines, some of which contain spike protein and others consist of whole inactivated virus. Following COVID-19 the antibody profile in terms of anti-spike and anti-nucleocapsid antibody production may also vary. In a minority of infections, antibodies are not produced or are short-lived, especially in asymptomatic infection. Likewise antibodies may not become detectable after vaccination.

Immunocompromised patients may have a reduced immunological response to COVID-19 vaccination, with an ongoing risk of recurrent infection and of onward transmission of SARS-CoV-2. In these specific cases, serology testing may be requested as part of the assessment by a treating medical practitioner to inform a patient's clinical pathway.

On 7 October 2021, the Australian Technical Advisory Group on Immunisation (ATAGI) recommended that individuals who are severely immunocompromised receive a third primary dose of COVID-19 vaccine. In its advice, ATAGI did not recommend antibody testing to assess immunity to SARS-CoV-2 following COVID-19 vaccination, including in immunocompromised individuals after a second or third dose.

On 24 December 2021, ATAGI recommended booster doses of COVID-19 vaccine for all Australians aged 18 years or older, to mitigate against waning immunity to SARS-CoV-2 and emergence of SARS-CoV-2 variants.

Importantly, COVID-19 vaccination does not negate the need for RT-PCR or RAT testing in the context of subsequent COVID-19 compatible clinical symptoms and/or public health investigations.

5.2.3 Test type and performance

PHLN recommends performing antibody detection using a validated laboratory-based immunoassay that meets acceptable and documented performance standards. Similar to NAA tests, the RCPAQAP offers a SARS-CoV-2 specific serology QAP. All laboratories performing SARS-CoV-2 serology are required to participate in a QAP.

Antibody detection can be considered under the following headings:

1. Binding antibody detection – IgG/IgM/IgA/total antibody usually targeting specific antigens (for example, spike or nucleocapsid proteins), although some assays contain multiple antigens. Assay formats include:
    - laboratory based immunoassays – enzyme immunoassays (EIA), chemiluminescent assays (CLIA), chemiluminescent microparticle immunoassays (CMIA), microsphere immunoassays (MIA), immunofluorescence assays (IFA), and
    - point of care lateral flow assays (LFA) IgG IgM/total antibody.
2. Neutralisation antibody detection tests which determine functional ability to prevent infection by virus *in vitro*. Tests include:
    - Virus neutralisation tests (VNT) – reference laboratory viral culture based neutralisation assays. Results can take up to 5 days and require a PC3 laboratory.
    - Competitive neutralisation assays (cVNT) – EIA based competitive neutralisation test for qualitative detection of total neutralising antibodies. Turnaround time is much quicker and can be performed in clinical diagnostic laboratories (PC2).
    - Surrogate virus neutralisation test (sVNT) – such as a microsphere immunoassay-based neutralisation test for qualitative or quantitative detection of total neutralising antibodies. This test also has short turnaround times and does not need to be performed under PC3 requirements.

Neutralisation assays are currently being used as possible surrogates of protection in epidemiological and clinical studies and as confirmatory tests in some settings. The duration of neutralising antibody following infection remains uncertain.

There is also emerging evidence that detecting SARS-CoV-2-specific antibodies using DBS is comparable to that of serum. DBS may be the preferred sample type to determine 'serostatus' in paediatric populations, although further studies are ongoing.

The TGA has approved a number of point-of-care serological tests[9]. These are subject to non-standard conditions of inclusion in the Australian Register of Therapeutic Goods (ARTG), including restrictions on who can use and report them. Their use has generally been discouraged in the Australian setting due to suboptimal analytical sensitivity and specificity.

5.2.4 Interpreting and reporting results

In the absence of seroconversion or a significant rise in antibody level between two specimens, low-prevalence settings will require a combination of testing approaches. The combination of serological assays employed will vary between laboratories but may involve assays with different epitope targets (for example, spike or nucleocapsid) or assays of different formats, including higher specificity assays, such as virus neutralisation assays.

[9] https://www.tga.gov.au/covid-19-test-kits-included-artg-legal-supply-australia

Detection of a positive IgM or IgA antibody without IgG detection is not sufficient evidence of recent COVID-19. PHLN recommends the clinician requests a repeat specimen collection to look for IgG seroconversion.

The combination of a NAA test on a respiratory sample at the same time as acute serum is collected with a follow up blood collection for repeat serology may be indicated to provide the most complete diagnostic picture. Their detection (IgM/IgA) may provide some indication about the timing of infection.

Supplemental or confirmatory serological testing is also recommended when there is low prevalence or pre-test probability (such as the absence of a clinically compatible illness or epidemiological exposure), as the predictive value of a single positive test is low[10].

Such approaches depending on jurisdictional capabilities would include:

1. retesting the initial sample using an alternative commercially available immunoassay or other validated tests with comparable or greater sensitivity/specificity (ideally the retest targets a different antigen to the initial test), or
2. retesting the initial sample using a virus neutralisation assay or other alternative format reference laboratory antibody test, such as IFA or MIA.

Currently, no Western Blot assays are available for confirmatory testing.

Pathologists and medical laboratory scientists should interpret serological test results in association with the patient's clinical and epidemiological information and other laboratory results. When serological results for COVID-19 are reported, it is important to understand:

(i) the analytical sensitivity and specificity of the assay
(ii) the likely prevalence of the patient population being tested
(iii) timing of specimen collection relative to symptoms and
(iv) the vaccination status of the patient.

PHLN recognises that individual laboratories will tailor comments according to their local situation.

Most serological assays using enzyme-linked immunosorbent or chemiluminescent processes are not quantifiable. While serological assays can be reported as units/mL or semi-quantitatively as titres (which equate roughly to the amount of antibody detected), the results of these assays cannot be directly compared across assays. Apart from aiding the interpretation of significant changes in antibody levels between acute and convalescent samples, PHLN does not recommend reporting titres or quantitative results at this time.

5.3 Antigen

Antigen tests are immunoassays that detect the presence of a specific viral antigen, usually the NC protein.

There are two types of antigen tests:

- point-of-care, and
- laboratory-based antigen

[10] Public Health Laboratory Network Guidance for serological testing in COVID-19 Sept 2020

From 1 November 2021, antigen point-of-care self-tests were permitted for use at home without supervision in Australia. Approved tests are listed on the ARTG. Prior to this date the TGA prohibited their use without appropriate health care supervision.

Antigen tests are most accurate when used for the diagnosis of a SARS-CoV-2 infection in the early stages of symptomatic infection. They are generally intended for use with nasopharyngeal, throat or nasal swabs and should be performed by following the manufacturer's IFU. A positive direct virus detection assay result most likely implies current viral infection. In areas of low community transmission, it is recommended that a positive RAT result be confirmed by a follow-up RT-PCR test, where feasible. Where NAA testing is unavailable, RAT should be used to confirm a probable case (as defined in the National Guidelines).

The advantages of antigen tests include:

- ease of use
- rapid turnaround times
- accessibility
- affordability and
- potential for wide scale testing in community settings (such as airports).

While RATs can provide a result within 15–30 minutes, they are generally less sensitive and have low throughput compared to NAA tests. NAA tests are currently the gold-standard in active SARS-CoV-2 infection diagnosis.

PHLN and CDNA have published a *joint statement on SARS-CoV-2 rapid antigen tests* outlining the opportunities and risks associated with the use of these devices.

These types of tests need to be continuously evaluated to determine their optimal clinical utility in various setting as the pandemic evolves and testing requirements change. The ARTG website lists the current antigen tests available in Australia. The WHO has published interim guidance *Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays.*

5.4 Genomics

Whole genome sequencing of the approximately 30,000 nucleotides that comprise the SARS-CoV-2 genome has allowed prompt identification of the virus and rapid development of diagnostic tests. This has subsequently facilitated vaccine development. WGS is used to compare the genomic relatedness from multiple COVID-19 cases to facilitate investigation of clusters by public health units. It has also allowed mutational analysis for variants of concern (VoC), and informed studies of SARS-CoV-2 transmission. In urgent situations expedited processing of prioritised samples can rapidly produce whole genome sequences to inform public health interventions in real time.

Other current and potential future roles for SARS-CoV-2 genomics include:

- identifying genetic factors that may impact susceptibility and disease severity
- selecting effective targets for potential therapies
- vaccines and monitoring future vaccine effectiveness
- identifying mutations associated with antiviral resistance to monitor for the emergence of resistant strains and to help guide treatment decisions and

- identifying mutations that may impact NAA test performance.

All states and territories have public WGS sequencing capability. Specimens from surveillance programs such as wastewater may also be suitable for WGS.

a. WGS methodology for SARS-CoV-2

(i) Sequencing

RNA is extracted from SARS-CoV-2-positive samples, reverse transcribed into complementary DNA (cDNA) and the cDNA segments amplified to create a DNA library for sequencing. The DNA is sequenced using one of several methods and the sequence readings, known as FASTQ files, are then analysed using a bioinformatics process.

PCR amplicon-based sequencing methods such as the protocols developed by the ARTIC Network perform WGS directly from SARS-CoV-2 PCR-positive specimens. To note, some SARS-CoV-2 PCR-positive specimens have low viral loads allowing no or only partial coverage of the SARS-CoV-2 genome. This in turn may limit the representativeness of a COVID-19 cluster investigation.

(ii) Bioinformatics

Using multiple software programs the sequence reads undergo quality control checks prior to constructing a whole genome consensus sequence. This is then compared to a reference standard or other genomes to identify any sequence variation.

(iii) Genomic epidemiology

Phylogenetic analyses are performed and visually represented in different formats, most commonly as a phylogenetic tree. Incorporation of both the phylogenetic relationships and the distances between sequences are taken into consideration when interpreting the degree of genomic relationships. This can reveal transmission networks and genomic clustering of sequences.

National and international data repositories have built phylogenetic analysis tools for SARS-CoV-2. The GISAID Initiative, originally known as a Global Initiative on Sharing All Influenza Data, allows application of phylogenetic analysis tools for classification of sequences. The tool classifies the sequences into seven major clades (S, L, V, GH, GR, GV, GRY) with a number of subclades. PANGOLIN (Phylogenetic Assignment of Named Global Outbreak LINeages) assigns lineages denoted numerically that are similar, but not identical, to the GISAID clades. It is important to note that PANGO lineages and sub-lineages are subject to change as new data is received.

Early on in the pandemic, Australia employed a comprehensive sequencing strategy facilitated by low SARS-CoV-2 prevalence. Widespread community transmission of Delta and Omicron across Australia has triggered a change of approach from comprehensive sequencing to selective and targeted sequencing, balancing the utility and cost of real-time SARS-CoV-2 genomic surveillance in the environment of the rapid spread of a dominant strain of the virus, and ability of the genomic data to show variations within clusters given the relative stability of the SARS-CoV-2 genome.

To support the collection of timely, targeted and accurate information from SARS-CoV-2 genomic surveillance in areas of widespread community transmission, including detection of emerging variants please refer to the CDGN *Sampling strategy for SARS-CoV-2 genomic surveillance* (the Strategy).

Importantly, genomics provides information on genetic relatedness, but it does not define the direction or mechanism of transmission between people or locations. Epidemiological data aids the interpretation and significance of findings from the WGS information. Combining the epidemiological data with SARS-CoV-2 genomic data provides the best approach for the analyses of clusters and transmission events. The optimal public health impact is achieved when WGS laboratories are closely integrated with existing diagnostic and epidemiological public health programs.

The *Australian National Disease Surveillance Plan for COVID-19* outlines and monitors the roles of SARS-CoV-2 genomics to enhance surveillance data.

Variants and genetic stability

SARS-CoV-2 was initially a genetically stable virus which varied by less than 25 single nucleotide polymorphisms (SNPs) from the first sequenced strain (Wuhan-Hu-1). More recently, variants with multiple mutations have appeared. For some of these variants, there is epidemiological and/or laboratory evidence that these mutations provide a selective advantage by either enhancing transmissibility or facilitating immune (and vaccine) escape. When the supporting evidence is sufficient the WHO or other authorities denote them as VoC. A major role of SARS-CoV-2 WGS is to identify VoC for surveillance purposes and to guide the management of such cases. The CDGN has a VoC working group that regularly reviews the VoC status of SARS-CoV-2 lineages.

The high resolution and specificity of SARS-CoV-2 genomics means it has the capacity to identify transmission events and detect outbreak clusters. However, there are currently no agreed national or international criteria for defining the relatedness between SARS-CoV-2 viruses. Various groups have applied strict criteria of 0-2 SNPs to define relatedness in cluster investigations. However, it is important to note that viruses with more than 2 SNPs may still be part of a single chain of transmission. This is evidenced by strong epidemiological links showing a chain of transmission indicative of a point source, or there being only one credible source of that strain.

b. Recommendations for SARS-CoV-2 WGS laboratories

For laboratories performing SARS-CoV-2 WGS for public health purposes in Australia, PHLN recommends:

- for each jurisdiction, SARS-CoV-2 genomics should be facilitated and/or directed by the respective nominated genomics public health laboratories
- the laboratory should have or be working towards NATA accreditation for pathogen WGS
- the laboratory should have, or be working towards having, an NPAAC/RCPA accredited medical microbiologist (designated person) for medical supervision of WGS
- the laboratory should implement or work towards implementation of an appropriate sampling strategy for genomic surveillance of SARS-CoV-2, and review and adjust the strategy regularly to reflect current or emerging structure or evolution of the virus population, or epidemiological and public health needs,
- encouraging the laboratory to participate in national processes for the consistency in bioinformatic analyses and reporting approaches facilitated through the CDGN,
- the laboratory responsible for analysis and reporting of SARS-CoV-2 genomic data should make sure they consider the limitations and potential sources of bias and they present the genomic analyses in an appropriate format for their public health units,

- encouraging the laboratory to rapidly share WGS data within the jurisdiction, nationally, and internationally as this enhances jurisdictional, national and international understanding of SARS-CoV-2 emergence and transmission, and
- The laboratory should participate in external quality assurance programs, such as the RCPAQAP SARS-CoV-2 WGS PTP or another recognised international program.

5.5 Virus culture

Virus culture has limited utility for the routine diagnosis of SARS-CoV-2 given their complexity, costs, and turnaround times. Virus culture has been used to inform the release of persons with COVID-19 from isolation. Cultures are useful to provide standardised viral stocks for developing and validating new tests as it provides viral RNA in its original configuration. Cultures of SARS-CoV-2 also allow comparison with NAA test sensitivity when considering infectiousness and duration of shedding studies. They provide a large amount of positive control material for diagnostic NAA testing and are required for traditional neutralising antibody assays. Biobanking SARS-CoV-2 cultures of different lineages will facilitate current and future research, such as studies of pathogenesis, transmissibility, antiviral drug resistance, and immune therapy studies.

Culturing SARS-CoV-2 should be performed in reference laboratories with appropriate experience and access to PC3 facilities, consistent with current WHO, national and international recommendations for SARS-CoV-2.

5.6 Wastewater screening

Wastewater is a mixture of water from household use, such as from showers, toilets, etc. and as such usually contains human faeces. It is also commonly referred to as sewage. Fragments of the virus that causes COVID-19, as well as other SARS coronaviruses (referred here as SARS-CoV-1 for clarity), are detectable in wastewater. Sewage testing has been used in other studies of viruses, to detect noroviruses and classify and study the genomics of detected viruses[11]. In such settings, genomic data can be used to understand the relationship between virus genotypes and the epidemiology of virus spread. These findings can be related to diagnosis, and potentially transmission of the pathogens. Importantly these data inform understanding of the potential for future spread throughout the population due to viral genome variation, when combined with WGS.

Wastewater surveillance was used particularly in the initial SARS outbreaks (caused by SARS-CoV-1) in 2003–2004. This was where spread from faeces between humans was seen as a common event in some populations. SARS-CoV-2 is present in stool, with some recent evidence for faeco-oral spread[12]. Although, this is currently not regarded as a clinically significant means of transmission, compared with respiratory spread.

The utility of wastewater in other settings has led to programs in Australia, known to be undertaken in most jurisdictions either weekly or twice weekly. The public health responses include issuing public health messages, in some cases combined with local enhanced testing. The national project, Collaboration on Sewage Surveillance of SARS-CoV-2 (ColoSSoS) from Water Research Australia, aims to provide community wide wastewater surveillance in the

[11] Lun JH et al (2018) *Emerging recombinant noroviruses identified by clinical and waste water screening article.* Emerging Microbes and Infections 7(1):1

[12] Guo M et al. *Potential intestinal infection and faecal–oral transmission of SARS-CoV-2.* Nat Rev Gastroenterol Hepatol 18, 269–283

longer term.[13]

In these, wastewater surveillance for SARS-CoV-2/COVID-19 has been used to sensitively detect the presence of viral RNA. This is similar to other methods for early detection of diseases, for example those used for polio eradication programs. This detection relies on significant expertise within water programs for detecting virus. The potential for further data on quantitative virus detection, and genomic characterization of detected virus, may inform more sophisticated surveillance and prevention programs for COVID-19. Such programs are well advanced in Australia, compared with other countries such as the United States. As an example, *the Centre for Disease Control and Prevention National Wastewater Surveillance System*).

The issues around wastewater testing include:

- lack of sensitivity, due to the large volumes and small amounts of virus. Some jurisdictions indicate detection of 10 cases in an area of 100,000 people (NZ), although the data informing sensitivity in real situations are imprecise,
- infectivity of the RNA detected is not measured, and is unknown, and
- confirmatory testing can be difficult due to the nature of the analyte.

Research to address these issues and further increase potential utility of wastewater surveillance for SARS-CoV-2 is ongoing.[14,15]

6 Further information

Access the PHLN publications on COVID-19 here. The Department of Health also produces a series of resources on COVID-19 for health professionals, including pathology providers and healthcare managers available here.

[13] In collaboration with members of enHealth, enHealth's Water Quality Expert Reference Panel, and CDNA—under the auspices of AHPPC—national frameworks for testing and reporting as well as for public health response and surveillance are being developed for incorporation into the national surveillance plan for COVID-19. A link will be provided when this work is complete and will be available in the relevant update to this PHLN guidance document.

[14] Black J. et al (2021) Epidemiological evaluation of sewage surveillance as a tool to detect the presence of COVID-19 cases in a low case load setting. *Sci Total Environ*. 2021 Sep 10; 786 (www.ncbi.nlm.nih.gov/pmc/articles/PMC8087577/)

[15] Schang C. et al (2021) Passive sampling of viruses for wastewater-based epidemiology: a case-study of SARS-CoV-2

www.researchgate.net/publication/347103410_Passive_sampling_of_viruses_for_wastewater-based_epidemiology_a_case-study_of_SARS-CoV-2