



PHLN

Public Health Laboratory Network

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

Revision History

<i>Version</i>	<i>Date Endorsed by PHLN</i>	<i>Revision note</i>
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.
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.

<i>Version</i>	<i>Date Endorsed by PHLN</i>	<i>Revision note</i>
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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The [COVID-19 CDNA National Guidelines for Public Health Units](#) defines people required to be considered for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing as 'suspect cases'. Where applicable, consult with your state/territory communicable diseases agency to seek advice on:

- which laboratories can provide SARS-CoV-2 testing;
- appropriate specimen type, collection and transport; and
- how to facilitate contact management, if indicated.

Transmission-based Precautions

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

Approach to testing and specimen selection

Laboratory testing is useful for individual patient diagnosis and to guide infection control procedures and public health investigations. Suitable samples for testing are below. The main sample types submitted for testing include respiratory tract samples (upper and lower tract) and sera. Perform routine tests for acute pneumonia/pneumonitis where indicated and according to local protocols. This may include bacterial cultures, acute and convalescent serology, urinary antigen testing and nucleic acid tests for other respiratory pathogens. The rate of viral coinfection in SARS-CoV-2 has been negligible in Australia to date. However, if SARS-CoV-2 is not detected, testing for other common respiratory viruses in a person with an acute respiratory tract infection may be clinically appropriate.

Sections 1-4 describe the current specimen collection types that are available in Australia:

1. Upper respiratory tract samples

a. Oropharyngeal and bilateral deep nasal swab:

- Oropharyngeal (throat): Using either a dacron or rayon (PHLN recommend flocked) swab, swab 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 should be utilised 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 both sites, oropharynx and deep nasal, to optimise the chances of virus detection.

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

If undertaking testing of SARS-CoV-2 in a different laboratory testing for other respiratory viruses, the original swab and remaining eluate should be forwarded for SARS-CoV-2 testing.

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

A self-collected oropharyngeal and bilateral deep nasal swab 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 this method of collection. It is equivalent to a medical practitioner conducting a combined nasal and throat swab in detecting coronavirus¹²
- This potentially reduces infection risk to the health care worker providing the collection. It also reduces PPE requirements.
- Only offer this method of collection at the request of a medical practitioner, or under public health direction. It will also need to be part of an agreed procedure with the testing laboratory.
 - Clearly document the specimen collection method as ‘SELF COLLECTION’ on the request form, by the requesting clinician or Approved Collection Centre personnel. Instruct the patient to document this on the swab collection tube, once collected.
- The requesting medical practitioner or the pathology service provider should provide clear written instructions to the patient.
 - An accessible instructional video could support the written instructions
 - If the requesting clinician provides the swabs directly to the patient, the clinician must ensure that the receiving laboratory is able to process self-collect specimens.
- It is at the patient’s discretion to decide on the location where their self-collection is conducted.
- The self-collect consists of a combined oropharyngeal and bilateral deep nasal swab that accesses the throat and then the nasal cavity. Patients should conduct the swab, following the instructions above. There may be variations in types of swabs available in different jurisdictions and from different pathology providers
- 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

¹ 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

² 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.

- Adding a human DNA control to the COVID-19 PCR assay to confirm adequacy of sample collection.

c. Paediatric oropharyngeal and bilateral deep nasal swab

Ensure 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 and bilateral deep nasal collections

- Oropharyngeal (throat)
 - Using a tongue depressor, hold down the tongue
 - Swab 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 should be utilised 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.

Directly place the swab in transport medium. This may include VTM, UTM, Liquid Amies or another validated transport medium. Whilst dry swabs are acceptable, the PHLN recommends swabs in transport medium.

If undertaking SARS-CoV-2 testing in a different laboratory to testing for other respiratory viruses, forward the original swab and remaining eluate for SARS-CoV-2 testing.

d. Nasopharyngeal swab

- Only use a swab suitable for nasopharyngeal sampling. If the swab is too large revert to a combined 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 flocculated 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. This may include VTM, UTM, Liquid Amies or another validated transport medium. Whilst dry swabs are acceptable, the PHLN recommends swabs in transport medium.

If undertaking SARS-CoV-2 testing in a different laboratory to testing for other respiratory viruses, forward the original swab and remaining eluate for SARS-CoV-2 testing.

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. This is because the lower respiratory tract specimens contain the highest viral loads in SARS-CoV and MERS-CoV. Initial experience in testing for SARS-CoV-2 seems to be consistent with this prior experience. 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.

3. Saliva

The PHLN continues to monitor the emerging literature with saliva as alternative specimen for use in PCR testing. Some early validation studies have been conducted both internationally and in Australia. Results are promising. However, consider the limitations of using saliva at:

[PHLN Statement on use of saliva as an alternative specimen for the diagnosis of SARS-CoV-2.](#)

4. Blood serum

Collect at least 3 mL in a serum separator tube and transport to the laboratory as soon as possible. Collect serum under transmission-based precautions if the patient is a suspected COVID-19 case, or has confirmed COVID-19 infection and is still in isolation. Collect serum from proven cases of COVID-19 released from isolation, using standard precautions.

Specimen handling in the laboratory

Microbiology Laboratory

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

Point of care testing outside a PC2 facility

PHLN members considered the WHO guidance [Laboratory biosafety guidance related to coronavirus disease \(COVID-19\)](#)³ 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.
- Point of care (POC) or near-POC assays may be used on a bench without employing a BSC, when dictated by local assessment and proper precautions are in place.

Assess diagnostic testing steps, for specimens outside of a PC2 facility (such as rapid respiratory testing performed at, or near, the point of care). Determine if aerosol generation may occur. This will inform which Transmission-based Precautions to apply, 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, including excess specimens in place¹.

Clinical Pathology

Non respiratory specimens (blood, urine, stool) are known to contain the virus. 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.

³ [https://www.who.int/publications-detail/laboratory-biosafety-guidance-related-to-coronavirus-disease-\(covid-19\)](https://www.who.int/publications-detail/laboratory-biosafety-guidance-related-to-coronavirus-disease-(covid-19))

Respiratory Virus Diagnostic Testing for Laboratories not Performing SARS-CoV-2 Testing

Perform Nucleic Acid Testing (NAT) of the upper or lower respiratory tract samples for diagnosing common respiratory viruses. Perform these tests using standard protocols and methods of the hospital or community laboratory.

Use standard protocols of the testing laboratory for respiratory sample processing. The PHLN expects this to consist of PC2 laboratory practices, and use of a Class II Biosafety cabinet for aerosol generating procedures. For example centrifuging without sealed carriers and overtaking. Viral culture can only be undertaken in an accredited laboratory that has a PC3 facility.

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

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).

SARS-CoV-2 specific testing

Nucleic acid testing (NAT)

NAT using real time polymerase chain reaction (RT-PCR) is the method 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 PCR assays employed by different PHLN member laboratories and non-PHLN laboratories. Commercial NAT assays have been available for SARS-CoV-2 testing in Australia since March 2020. Perform an evaluation of these prior to introduction.

In the early phases of the pandemic, before commercial assays were available, the PHLN member laboratories designed their own specific RT-PCR primer sets or implemented primer sets. 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 Viral Archive \(Eva\)](#). During this stage, NAT was generally done as a two step process with an initial screening, followed by a confirmatory test.

Complementary DNA (cDNA) synthesized from the VIDRL SARS-CoV-2 is now available to all PHLN member laboratories as a test positive control. Synthetic positive control material in the form of nucleic acid templates is also available through WHO/ EVAg.

The majority of diagnostic laboratories now employ either commercial or in-house developed assays for testing. The 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, RT-assays.

Many PCR assays, including those available through WHO, will also detect other zoonotic coronaviruses such as SARS-CoV. This is sometimes with a recognisable shift in the cycle

threshold value (Ct). This compares to the SARS-CoV-2 target, but not the commonly circulating coronaviruses usually detected by commercial assays (e.g. NL63, 229E strains).

To minimise the risks of false positive results in low prevalence settings, confirming positive results is done with either:

- RT-PCR assays detecting a different target gene (particularly for assays with a single target); or
- 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, test other targets to confirm the results.

Find further PHLN guidance on NAT result interpretation at [PHLN guidance on nucleic acid test result interpretation for SARS-CoV-2](#).

The RCPAQAP, with Australian Government support, offers a SARS-CoV-2 specific NAT QAP. This proficiency testing program (PTP) supplements previous SARS-CoV, MERS-CoV and other coronaviruses PTP.

Serology

Serology does not have a defined role in the diagnosis of COVID-19 during the acute illness but can be helpful for the diagnosis of past cases, and in going forward possibly for evaluation of vaccination programs. For example, it can be useful for public health follow up of suspected cases who either did not undergo NAT during the acute illness, or where NAT is negative. Serial serological testing to assess changes in antibody levels may be of use in public health management. More specific information on serology testing for SARS-CoV-2 is available in the [Public Health Laboratory Network Guidance for serological testing in COVID-19](#).

PHLN recommends performing antibody detection using a validated assay, meeting acceptable and documented performance standards. Laboratory-based antibody assays available or in development include enzyme immuno assays (EIA), microsphere immunoassays, immunofluorescence assays, and as reference neutralization assays. The Therapeutic Goods Administration have approved a number of point-of-care serological tests, subject to conditions, including restrictions on who can obtain them. See [Public Health Laboratory Network Statement on Point-of-Care Serology Testing for SARS-CoV-2 \(the virus that causes COVID-19\)](#) for more information. No SARS-CoV-2 western blot assay tests are currently available.

The RCPAQAP, with Australian Government support, offers a SARS-CoV-2 specific serology QAP.

To date, there are no serological assays that provide a definitive correlation of immunity to SARS-CoV-2. The ability of the serological assays to detect anti-spike antibody following vaccination is unknown. The detection of anti-spike antibody cannot distinguish between natural infection and vaccination. Therefore, serological testing may be used in a research setting following vaccination, however, PHLN **does not currently recommend** routine

diagnostic serological testing post-vaccination. Importantly, COVID-19 vaccination does not negate the need for RT-PCR testing in the context of:

- subsequent COVID-19 compatible clinical symptoms; and / or
- public health investigations.

Antigen

On 8 October 2020, PHLN and CDNA published a [joint statement on SARS-CoV-2 rapid antigen tests](#) on the opportunities and risks associated with the use of these devices. The statement advises that rapid antigen tests should only be used in certain situations under the direction of the relevant public health authority, with medical supervision.⁴

Rapid antigen tests intended for use at the point-of-care detect the presence of viral protein from SARS-CoV-2. They may be used in the diagnosis of a SARS-CoV-2 infection in a symptomatic patient. Health professionals should perform COVID-19 antigen tests in accordance with the manufacturer's instructions for use. They are generally intended for use with nasopharyngeal, throat or nasal swabs.

While rapid antigen tests can provide a result within 15-30 minutes, they are generally less sensitive than a RT-PCR test. RT-PCR tests are currently the gold-standard in SARS-CoV-2 diagnosis.

A number of protein based antigen tests for acute diagnosis of SARS-CoV-2 infections are emerging domestically and internationally. The advantages of use include:

- ease of use,
- rapid turnaround time,
- accessibility, and
- potential for wide scale testing.

These types of tests are currently being evaluated to determine clinical utility in various settings. Current antigen tests available in Australia are listed on the [Australian Register of Therapeutic Goods](#).

Genomics

Genomics is the study of the structure, function, evolution, and mapping of genomes. Pathogen genomics enables infectious disease control by permitting precise and accurate national and international pathogen characterisation and comparisons. Increasingly, the PHLN are using pathogen genomics to enhance surveillance and investigate COVID-19 clusters and transmission of SARS-CoV-2 across Australia.

Whole genome sequencing (WGS) of the approximately 30,000 RNA nucleotides that comprise the SARS-CoV-2 genome can be used to reveal the genetic makeup of the virus. Laboratories can also use WGS to discriminate between mutation patterns of the SARS-CoV-2

⁴ https://www.health.gov.au/sites/default/files/documents/2020/10/phln-and-cdna-joint-statement-on-sars-cov-2-rapid-antigen-tests_0.pdf

virus from different samples. By comparison of the SARS-CoV-2 genomes from multiple COVID-19 cases, clusters of COVID-19 and transmission of SARS-CoV-2 can be investigated. This facilitates identifying the source of infection and routes of transmission by monitoring the emergence of variants over time and through communities. It can also be used to determine whether the patient acquired the infection overseas, or locally from a known or unknown contact. Information regarding the source and spread of the infection is then used by public health units in consultation with laboratories to plan and deliver public health measures to minimise the impact of the disease.

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

- identifying genetic factors that may impact susceptibility and disease severity. For example, mutations that increase virus entry into host cells, increased transmissibility, or production of an abnormal inflammatory response;
- selecting effective targets for potential vaccines and monitoring future vaccine effectiveness. For example, receptor-binding domain studies to monitor for escape from host neutralising antibodies; and
- identifying mutations associated with drug resistance to monitor for the emergence of resistant strains and to help guide treatment decisions.

WGS for SARS-CoV-2 involves three steps:

(i) The sequencing steps

The laboratory extracts the RNA from the samples found to be positive for SARS-CoV-2 using a TGA approved diagnostic test. It is then converted to copy DNA (cDNA) and segments of the cDNA are amplified. This creates a DNA library. Different next generation sequencers then use several methods to sequence the DNA. For one manufacturer the DNA library is checked and prepared by adding adapters to the DNA ends which bind to a flow-cell for sequencing. Millions of single stranded DNA copies are produced, and the sequences read. The sequence readings, known as FASTQ files, are then analysed using a bioinformatics process.

Despite having smaller genomes compared to bacteria, virus sequencing can be more difficult. This is due to the small amount of virus genetic material compared to host nucleic acid in the specimens. To overcome this problem metagenomic sequencing and target enrichment approaches, for example PCR amplicon sequencing, have been used.

Metagenomic sequencing, where all host, bacterial and virus genetic material is sequenced, does not require reference sequences. However, it requires more complex sample preparation. This includes a very high sequencing depth, powerful data processing applications and specialised bioinformatics skills. PCR amplicon sequencing is a well-established method, is especially useful for samples with low viral loads. It is applicable to large numbers of clinical samples. However, it is less suitable for viruses with significant genetic variability, and involves multiple PCR reactions. The number of distinct amplicons is dependent on the size of the virus genome. The PCR process can be multiplexed to improve efficiency and reduce reagent and equipment use, as well as laboratory staff time.

PCR amplicon-based sequencing methods can achieve high quality results for WGS of samples with lower viral loads of SARS-CoV-2. Such methods as the protocols developed by the [ARTIC Network](#). Sequencing using a number of commercial long read next generation sequencing platforms can be performed from virus cultures or from SARS-CoV-2 PCR-positive specimens. However, some SARS-CoV-2 PCR-positive specimens have viral loads too low to enable WGS. Moreover, it is not always possible to obtain 100% coverage of the SARS-CoV-2 genome from low viral load SARS-CoV-2 PCR-positive specimens. This, in turn, may limit the representativeness of a COVID-19 cluster investigation.

(ii) The bioinformatics step

Using multiple software programs the sequence reads undergo quality control (QC) checks. The whole genome consensus sequence is then constructed (assembled from the individual sequence reads). The genome is then characterised to identify any sequence changes compared to a reference standard or other genomes. This work requires specialised bioinformatics expertise. Virologists and bioinformaticians often need to collaborate with respect to the application and adaptation of analytical computational tools. Phylogenetic analysis is often performed after trimming off the 3' and 5' non-coding regions, but these regions may be important for other analyses, such as pathogenicity studies. Currently in development are QC metrics, including % genome coverage, sequencing depth, and SNP distance from Wuhan-Hu-1 sequence.

(iii) The genomic epidemiological step

Once the SARS-CoV-2 sequences have passed QC, bioinformaticians and genomic epidemiologists include the sequences to conduct phylogenetic analyses. This is to accurately interpret and analyse the genomic data. Genomics sequences are used to generate a phylogenetic tree. The phylogenetic tree is a visual representation of the most likely evolutionary relationships between the sequences given the data. 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.

The use of SARS-CoV-2 genomics in public health responses

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, applies a phylogenetics analysis tool. This tool classifies sequences into four major clades (S, L, V and G) with a number of subclades. Alternatively, other sequence analysis tools, such as PANGOLIN (Phylogenetic Assignment of Named Global Outbreak LINEages) assigns lineages that are similar, but not identical, to the GISAID clades.⁵ There are other potential software programs becoming available for phylogenetic analysis. The AusTrakka platform, developed by the Communicable Diseases Genomics Network (CDGN), is Australia's central and secure platform. It provides the tools and bioinformatics pipelines for national phylogenetic analysis of SARS-CoV-2 sequences across Australia and New Zealand. AusTrakka uses the international best practice bioinformatics protocols based on analysis pipelines used

⁵ <https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.32.2001410#figuresntables>

for the Ebola and Zika outbreak response. The bioinformatics pipeline uses iVar to generate consensus sequences. It does this by mapping to the Wuhan-Hu-1 reference sequence, then MAFFT to generate multisequence alignments, and finally IQTree to generate the maximum likelihood tree.

To date, SARS-CoV-2 genomic studies indicate it is a genetically stable virus with minimal evidence of evolution since emerging within human populations in late 2019. Currently, the sequenced genomes have varied by less than 25 single nucleotide polymorphisms (SNPs) from the first sequenced strain (Wuhan-Hu-1). There are currently no agreed national or international criteria for defining the relatedness of the SARS-CoV-2 viruses between cases. Due to the conserved nature of this virus to date, various groups have strict criteria of 0-2 SNPs to define relatedness in cluster investigations. Viruses with more than 2 SNPs may still be part of a single chain of transmission. This is evidenced by other supporting factors. These include strong epidemiological links showing a chain of transmission indicative of a point source, or only one credible source of that strain.

The high resolution and specificity of SARS-CoV-2 genomics means it has the capacity to identify transmission events and detect outbreak clusters. This is demonstrated by the diversification of the virus that traditional epidemiological investigations may not have revealed. However, genomic links do not, of themselves, define the direction or mechanism of transmission between different people or different locations. Combining the epidemiological data with SARS-CoV-2 genomic data provides the best approach to the interpretation and significance of findings from the WGS information. Public health action is more accurately informed through:

- integrating traditional epidemiological investigation and SARS-CoV-2;
- investigating SARS-CoV-2 transmission; and
- clustering events.

State and territory public health laboratories have established pathogen genomics capacity and capability at varying levels. Some jurisdictions have demonstrated major benefits of using SARS-CoV-2 genomics to inform their public health response. The Australian National Disease Surveillance Plan for COVID-19 outlines and monitors the roles of SARS-CoV-2 genomics to enhance surveillance data. In silico digital data sharing currently occurs between jurisdictions to help inform the national picture of SARS-CoV-2 transmission. There has been national agreement to share data and undertake national SARS-CoV-2 genomic surveillance using the AusTrakka platform (AusTrakka.net.au). Facilitated through the CDGN, there are arrangements for states and territories without SARS-CoV-2 WGS capability or capacity to refer samples to the jurisdictions with established genomics programs. This assists with the sequencing and/or bioinformatic analysis of their samples. Implementing this technology more broadly provides an opportunity to expand this capacity to public health laboratories in each state and territory. This provides local access to high-quality genomic analysis, enabling precise public health responses for all population groups and regions in Australia.

Consider the following recommendations for laboratories performing SARS-CoV-2 WGS for public health purposes in Australia:

- For each jurisdiction, SARS-CoV-2 genomics should be facilitated and/or directed by the respective nominated lead genomics public health laboratory. This is for the jurisdiction responsible for uploading of all SARS-CoV-2 genomic sequences to AusTrakka under the *Agreement for SARS-CoV-2 data sharing and analysis using AusTrakka* (endorsed by PHLN on XX August 2020)
- The laboratory should have or be working towards NATA accreditation for high-throughput parallel pathogen sequencing
- The laboratory should have or be working towards hiring an NPAAC/RCPA accredited medical microbiologist (designated person) for medical supervision of genomics
- At the jurisdictional level, analysis and reporting of SARS-CoV-2 genomic data is the responsibility of the accredited PHL. It is facilitated/enhanced through inclusion of “genomic epidemiologists” embedded in the laboratory. Their role is to facilitate genomic and epidemiological data integration.
- The PHLN strongly encourages rapid WGS data sharing within the jurisdiction (if there are multiple laboratories conducting SARS-CoV-2 WGS within the jurisdiction), nationally, and internationally. This enhances jurisdictional, national and international understanding of SARS-CoV-2 emergence and transmission.
- The PHLN encourages laboratories to participate in national processes for the consistency in bioinformatic analysis and reporting approaches facilitated through the CDGN.
- The laboratory should participate in external quality assurance programs. For example, the program developed by the RCPA QAP, or other recognised international programs.

Prioritisation of samples for WGS

The majority of public health laboratories across all states and territories are aiming to sequence all SARS-CoV-2 samples within their capacity. However, sample prioritising for WGS is being implemented where possible for vulnerable populations such as:

- Aboriginal and Torres Strait Islander peoples
- people 70 years of age and older
- people with weakened immune systems
- groups working in high-risk settings. These include healthcare, aged care, abattoirs and detention facilities.

Samples from identified cases of public health interest, notified by the public health unit or through National or International Focal Points, may also be prioritised. Combined with sample prioritisation, WGS is required of a representative sample i.e. a subset of the population, to provide context. It is also needed to accurately reflect the characteristics of the population. For example, in the case of an infected household, WGS of one of the household members would provide the representative sample for the household.

Prioritising samples for WGS will reduce turnaround times required to inform rapid implementation of public health interventions. This is to prevent further transmission and spread between cases of greater risk of severe illness. And, in settings that are at a higher SARS-COV-2 transmission risk. In order for public health laboratories to prioritise samples for WGS, the relevant information must be provided on the referred samples for rapid identification. This may differ between jurisdictions but may be provided on the referral slips to the public health laboratory or direct communication between public health units to the public health laboratory.

Viral Culture

Avoid performing viral culture for routine diagnosis, and only attempt culturing in reference laboratories with appropriate experience and containment facilities. Currently, where attempted, this is being done at Physical Containment Level 3 (PC3). This is consistent with current recommendations for SARS-CoV, pending specific SARS-CoV-2 international recommendations.

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](#).