

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>
1.12	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.
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

Patients to be considered for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (the virus that causes coronavirus disease 2019 (COVID-19)) testing are described under the 'suspect case' definition in the COVID-19 CDNA National Guidelines for Public Health Units.

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 also to facilitate contact management if indicated.

Transmission-based Precautions

Guidance on Personal Protective Equipment (PPE) required 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.

Routine tests for acute pneumonia/pneumonitis should be performed where indicated, according to local protocols. This includes:

- bacterial cultures,
- acute and convalescent serology, and
- urinary antigen testing and nucleic acid tests for respiratory pathogens

Serology for SARS-CoV-2 can help identify individuals who have developed detectable antibodies as part of an immune response to the virus. Collection of serum must be performed under transmission-based precautions if they are suspected COVID-19 cases or have proven COVID-19 infection and have not been released from isolation. Collection of serum from proven cases of COVID-19 who have been released from isolation can be performed using standard precautions.

Laboratory testing for SARS-CoV-2 continues to evolve rapidly with the accumulation of clinical data, and as reagents and protocols are refined.

The aim of testing is to, if clinically appropriate, exclude common respiratory viruses. This is done by using local hospital and community nucleic acid testing capacity, and to simultaneously refer onward to a laboratory with capacity to test for SARS-CoV-2. As co-infection is possible, initial testing protocols should include testing for SARS-CoV-2 in patients with epidemiological risk, even where another infection is shown to be present.

Samples for testing:

- (i) upper respiratory tract samples
- (ii) lower respiratory tract sample if the lower tract is involved
- (iii) serum

Upper respiratory tract samples

1. a. Nasal and oropharyngeal swab: may be Dacron or rayon, although flocked preferred
 - Oropharyngeal (throat): swab the tonsillar beds and the back of the throat, avoiding the tongue.
 - Deep Nasal:
 - Using a pencil grip and while gently rotating the swab, insert the tip 2–3 cm (or until resistance is met) into the nostril, 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. **To conserve swabs**, the same swab used to sample the oropharynx should be utilised for nasal sampling
- Place the swab(s) back into the accompanying transport medium

Sampling both sites, deep nasal and oropharynx, is recommended to optimise the chances of virus detection.

The swab(s) should directly be placed in transport medium. This may include viral (VTM) or universal transport medium (UTM), Liquid Amies or another validated transport medium. Whilst dry swabs are acceptable, swabs in transport medium are preferred.

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

b. A self-collected oropharyngeal and bilateral deep nasal swab may in some circumstances be an appropriate method of specimen collection. However, where possible, PHLN encourage health care worker supervision of the collection.

- PHLN has reviewed both domestic and international data that supports that this method of collection. It is equivalent to a medical practitioner conducting a combined nasal and throat swab in detecting coronavirus.¹²
- This has the potential to reduce infection risk to the health care worker providing the collection and also reduces the requirements for PPE.
- This method of collection should only be offered at the request of a medical practitioner or under public health direction as part of an agreed procedure with the testing laboratory.
 - The specimen collection method 'SELF COLLECTION' should be clearly documented on the request form by the requesting clinician or Approved Collection Centre personnel. The patient should be instructed to document this also on the swab, following collection.
- Clear written instructions should be provided to the patient by the requesting medical practitioner or the pathology service providing the testing.
 - Provision of an accessible instructional video could support the written instructions.
 - If the requesting clinician is providing the swabs directly to the patient (rather than referring the patient to a specific pathology collection centre) then the clinician is responsible for ensuring that the receiving laboratory is able to process self-collect specimens.
- The location at which the self-collect is conducted is at the discretion of the patient.
- The self-collect consists of a combined oropharyngeal and bilateral deep nasal, that accesses the throat and then the nasal cavity. It should be conducted by

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.

following the instructions above, noting there may be variations in types of swabs available in different jurisdictions and from different pathology providers.

- It is recommended that 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 compared to other methods of collection; and
 - Adding a human DNA control to the COVID-19 PCR assay to confirm adequacy of sample collection.

2. Nasal wash/aspirates

- Collect 2–3 mL into a sterile, leak-proof, screw-top dry sterile container

A nasal wash or aspirate if available, may be substituted for the deep nasal swab sample described above.

Lower respiratory tract samples

1. Bronchoalveolar lavage, tracheal aspirate, pleural fluid

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

2. Sputum

- Patient should rinse his/her mouth with water before collection
- Expectorate deep cough sputum directly into a sterile, leak-proof, screw-top dry sterile container

As lower respiratory tract specimens contain the highest viral loads in SARS-CoV and MERS-CoV, it is advised that lower respiratory tract specimens are collected for SARS-CoV-2 testing where possible. Initial experience in testing for SARS-CoV-2 seems to be consistent with this prior experience. Repeat testing (especially of lower respiratory tract specimens) in clinically compatible cases should be performed if initial results are negative and there remains a high index of suspicion of infection.

Serology

Serum should be collected during the acute phase of the illness (preferably within the first 7 days of symptom onset). It should be stored and tested in parallel with convalescent sera collected 2 or more weeks after the onset of illness. If no acute sample was collected, sera collected 14 or more days after symptom onset may be tested.

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.

Specimens should be transported 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:

- All procedures must be performed based on risk assessment and only by personnel with demonstrated capability, in strict observance of any relevant protocols at all times; and
- Point of care (POC) or near-POC assays can be performed on a bench without employing a BSC, when the local risk assessment so dictates and proper precautions are in place.

Diagnostic testing steps for specimens conducted outside of a PC2 facility (such as rapid respiratory testing performed at, or near, the point of care) should be assessed to determine if aerosol generation may occur. This will 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, consideration should be given to implementing airborne precautions. Staff undertaking POC or near-POC testing should be adequately trained and assessed in the appropriate use of PPE. They should not be put under increased pressure for test turnaround time. Testing should be done in a well ventilated room, preferably with an external exhaust fan. Testing in non NATA/RCPA accredited medical pathology facilities should also adhere to the current NPAAC regulatory framework with respect to point of care tests. Testing should not be conducted without a validated infectious waste process, including excess specimens in place¹.

Clinical Pathology

Non respiratory specimens (blood, urine, stool) are known to contain virus. Standard precautions should be used for non-microbial pathology testing (such as routine biochemistry and haematology). Where possible auto-analysers should be used 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.

Respiratory Virus Diagnostic Testing

Nucleic acid testing of the upper or lower respiratory tract samples is performed for influenza and other common respiratory viruses using standard protocols and methods of the hospital or community laboratory.

Standard protocols of the testing laboratory for respiratory sample processing should be used. This is expected to consist of PC2 laboratory practices, and use of a Class II Biosafety cabinet for aerosol generating procedures (such as centrifuging without sealed carriers, vortexing). Viral culture can only be undertaken in an accredited laboratory that has a PC3 facility.

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))

The residue (original swab and remaining eluate) of the upper tract sample is forwarded together with the lower tract sample and 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.

Standard protocols should be used for sample packaging and transport as diagnostic samples for testing (i.e. Category B).

SARS-CoV-2 specific testing

Nucleic acid testing

Nucleic acid testing (NAT) using real time polymerase chain reaction (RT-PCR) is the method of choice for detection of SARS-CoV-2 during the acute illness. Specific diagnostic test approaches for SARS-CoV-2 will be described here only in broad terms. There is significant variation in PCR assays employed by different PHLN member laboratories and testing non PHLN laboratories. Test algorithms are likely to be further refined over time. Commercial assays are becoming available from March 2020 and evaluation of these, or reference to another laboratory evaluation, needs to be performed prior to introduction.

Specific Real Time PCR primer sets to detect SARS-CoV-2 are available. Some PHLN member laboratories have designed their own, and some have implemented primer sets [recommended to the World Health Organization \(WHO\)](#) by leading international coronavirus reference laboratories. The majority of PHLN testing capacity now employs relatively swift RT-PCR assays for screening, with a laboratory turnaround time of several hours. Confirmation of positives is being done either with RT-PCR assays detecting a different target gene, or broadly reactive PCR tests with sequencing of amplicons (see below).

Well pedigreed PCR primer sets, probes and protocols are available from the [WHO/ European Viral Archive \(EVAg\)](#).

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) compared to the SARS-CoV-2 target, but not commonly circulating coronaviruses usually detected by commercial assays (eg NL63, 229E strains).

Several Australian PHLN reference laboratories began diagnostic testing for the current outbreak using PCR assays capable of detecting a wide range of coronaviruses, including zoonotic and novel pathogens. A number of these were mapped against the promulgated nucleic acid sequence of SARS-CoV-2 from Wuhan, China (GenBank accession MN908947, December 2019) early in the course of the outbreak. Nucleic acid sequencing of amplicons from positive tests is used to identify the coronavirus in this approach. These assays have relatively long turnaround times and have largely been replaced by RT-PCR other than as a confirmatory test in some laboratories.

Complementary DNA (cDNA) synthesized from the VIDRL SARS-CoV-2 has now been made 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/ European Viral Archive (EVAg).

There is variable use of one or two viral targets for SARS-CoV-2 testing. Confirmatory testing using an alternative target, at least in the early stages, for positive samples, is recommended if using a single target.

Testing algorithms are likely to be revised pending further information about the virus, and the number of specimens received in the laboratory for testing.

Viral culture should not be performed for routine diagnosis, and should only be attempted in reference laboratories with appropriate experience and containment facilities. Currently, where attempted, this is being done at Physical Containment Level 3 (PC3), consistent with current recommendations for SARS-CoV, pending specific SARS-CoV-2 international recommendations.

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. A second, more detailed PTP is planned to be performed by the RCPA QAP in mid 2020. An RCPAQAP sponsored serology proficiency programme is in development with two programs to be offered by the end of 2020.

Serology

Serology does not currently have a role in the diagnosis of COVID-19 during the acute illness but can be helpful for the diagnosis of past cases. 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 were NAT negative. Serology will also be important for broad-based surveillance, vaccine efficacy and research activities.

Serology for the determination of past COVID-19 is performed using either in-house methodology or commercially manufactured kits. The SARS-CoV-2 antigens are the four structural proteins: the spike, membrane, envelope and nucleocapsid proteins. Antibody tests to date have used either whole virion or the complete or certain domains of the spike protein and the nucleocapsid protein. The nucleoprotein is the most abundant viral protein whereas the spike protein is the most diverse across the coronaviruses, and therefore the most specific for SARS-CoV-2. The temporal pattern of antibody expression has not been fully elucidated and the persistence of the antibody isotypes is currently unknown. Early evidence suggests that IgM, IgA, IgG and neutralising antibodies become detectable between one and two weeks after illness onset in the majority of cases but some NAT-confirmed COVID-19 cases have not shown seroconversion.

It is recommended that antibody detection be performed using a validated assay meeting acceptable and documented performance standards. Laboratory-based antibody assays available or in development include neutralization assays, enzyme-linked immunosorbent assays, microsphere immunoassays and immunofluorescence assays. A number of point-of-care serological tests have been approved by the Therapeutic Goods Administration 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 or SARS-CoV-2 antigen test is currently available.

A seroconversion, or significant rise (e.g. four-fold or greater titre rise) in either neutralising or IgG antibody level is definitive laboratory evidence of SARS-CoV-2 infection whereas detection of neutralising or IgG antibody in a single specimen from a person meeting clinical criteria for COVID-19 is suggestive evidence of SARS-CoV-2 infection. The role of serology for the diagnosis of COVID-19 will be reviewed as more information on the serological response to SARS-CoV-2 becomes available.

The role of serology in determining immunity to SARS-CoV-2 is currently unclear. The development of neutralising antibodies to the spike protein, the virus receptor responsible for host cell entry, has been shown but the correlation of antibodies detected using different methods with virus neutralisation is not known. Further, more studies are needed to determine whether the development of neutralising antibodies to SARS-CoV-2 is indicative of protection from reinfection.

Further Information

The Department of Health has produced a series of resources on COVID-19 for health professionals, including pathology providers and healthcare managers, these may be accessed [here](#).