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

Revision History

Version	*Date Endorsed by PHLN*	*Revision note*
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 must be used when collecting respiratory specimens. These include:

- For most patients in community settings collection of respiratory specimens is a low risk procedure and can be performed using contact and droplet precautions:
    - perform hand hygiene before donning gown, gloves, eye protection (goggles or face shield) and surgical mask;
    - to collect combined deep nasal and oropharyngeal swabs, stand slightly to the side of the patient to avoid exposure to respiratory secretions, should the patient cough or sneeze; and
    - at completion of consultation, remove PPE and perform hand hygiene, wipe any contacted/contaminated surfaces with detergent/disinfectant.
- If the patient has severe symptoms (fever, breathing difficulty, suggestive of pneumonia) or frequent, severe or productive coughing episodes then contact and airborne precautions should be observed:

- if possible, specimens should be collected in a negative pressure room (e.g. in a hospital setting);
- if this is not possible, then collect the specimens in a room with the door closed and leave the room, which should be left vacant for at least 30 minutes after specimen collection (cleaning can be performed during this time by a person wearing PPE);
- perform hand hygiene before donning gown, gloves, eye protection (goggles or face shield) and P2/N95 respirator – which should be fit checked;
- at completion of consultation, remove gown and gloves, perform hand hygiene; remove eye protection and P2/N95 respirator. Do not touch the front of any item of PPE during removal; perform hand hygiene; and
- the room surfaces should be wiped clean with disinfectant wipes by a person wearing gloves, gown and surgical mask.

Routine tests for acute pneumonia/pneumonitis should be performed where indicated, including bacterial cultures, acute and convalescent serology, urinary antigen testing and nucleic acid tests for respiratory pathogens, according to local protocols.

Serology for SARS-CoV-2 is not yet available. Collection of serum for storage by the SARS-CoV-2 testing laboratory is recommended to facilitate retrospective testing, if this is relevant, once serology tests become available.

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 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 (to be stored pending serology availability)

Upper respiratory tract samples

1. Deep 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: swab the right or left nostril by gently inserting the swab along the floor of the nasal cavity parallel to the palate until resistance is encountered, and rotate gently for 10-15 seconds; then withdraw and repeat the process in the other nostril. To conserve swabs the same swab that has been used to sample the oropharynx should be utilised for deep 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 be placed in transport medium, which may be viral transport medium (VTM) or Liquid Amies.

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.

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 should be 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), stored, and when serology testing becomes available tested in parallel with convalescent sera collected 3 or more weeks after acute infection. 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.

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 hematology). 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 aersol 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.

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 (ie Category B).

SARS-CoV-2 specific testing

Nucleic acid testing (NAT) using real time polymerase chain reaction (RT-PCR) is the method of choice for detection of SARS-CoV-2. 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 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 (available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance). 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) (available at: https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2).

Many PCR assays, including those available through WHO will also detect other zoonotic coronaviruses such as SARS-CoV, 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 Royal College of Pathologists of Australasia Quality Assurance Program (RCPAQAP) with Australian Government support, performed the first SARS-CoV-2 specific QAP, which closed on 11th March 2020, and involved 16 public and private laboratories in all Australian states and in New Zealand. This proficiency testing program (PTP) supplemented 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. The second PTP is planned for distribution to a wider range of laboratories, once testing has been extended to more laboratories.

Randox Laboratories and Quality Control for Molecular Diagnostics (QCMD) have announced a pilot EQA scheme for coronaviruses which will include inactivated SARS-CoV-2.

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.