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FOCUS ON SARS-COV-2

Laboratory diagnosis of severe acute respiratory syndrome coronavirus 2

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Summary

The first laboratory confirmed case of Coronavirus disease 2019 (COVID-19) in Australia was in Victoria on 25 January 2020 in a man returning from Wuhan city, Hubei province, the People's Republic of China. This was followed by three cases in New South Wales the following day. The Australian Government activated the Australian Health Sector Emergency Response Plan for Novel Coronavirus on 27 February 2020 in anticipation of a pandemic. Subsequently, the World Health Organization declared COVID-19 to be a Public Health Emergency of International Concern followed by a pandemic on 30 January 2020 and 11 March 2020, respectively. Laboratory testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for COVID-19, is key in identifying infected persons to guide timely public health actions of contact tracing and patient isolation to limit transmission of infection. This article aims to provide a comprehensive overview of current laboratory diagnostic methods for SARS-CoV-2, including nucleic acid testing, serology, rapid antigen detection and antibody tests, virus isolation and whole genome sequencing. The relative advantages and disadvantages of the different diagnostic tests are presented, as well as their value in different clinical, infection control and public health contexts. We also describe the challenges in the provision of SARS-CoV-2 diagnostics in Australia, a country with a relatively low COVID-19 incidence in the first pandemic wave but in which prevalence could rapidly change.

Key words: SARS-CoV-2; COVID-19; laboratory diagnosis.

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INTRODUCTION

On 31 December 2019, the Chinese Center for Disease Control and Prevention reported to the World Health Organization (WHO) a series of patients with pneumonia of uncertain aetiology in Wuhan city, Hubei province, the People's Republic of China (PRC).[1] The pathogen responsible for this outbreak was identified on 7 January 2020 as a novel group 2B *Betacoronavirus,* designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease arising from SARS-CoV-2 infection, Coronavirus disease 2019 (COVID-19), subsequently spread rapidly worldwide. The Australian Government activated the Australian Health Sector Emergency Response Plan for Novel Coronavirus on 27 February 2020 and the WHO declared COVID-19 as a pandemic on 11 March 2020. At the latter time, there were 118,319 cases and 4,292 deaths reported across 113 countries and territories from COVID-19 around the world.[2] Border screening of arrivals from Wuhan commenced in Sydney on 23 January 2020. The first confirmed case in Australia was diagnosed in Victoria on 25 January 2020 in a man returning from Wuhan; three other cases were confirmed in New South Wales (NSW) the following day. On 20 March 2020, Australian borders were closed to non-residents and several 'non-essential' services were shut down. As of 27 September 2020, there have been 27,061 cases and 870 deaths in Australia. A total of 7,517,520 tests has been performed with a positivity rate of 0.4%.[3]

Laboratory confirmation of SARS-CoV-2 is key in identifying infected persons to guide appropriate public health interventions of contact tracing and patient isolation to prevent further transmission of infection, which is essential in the absence of specific antivirals or vaccines.[4] The rapid spread of the COVID-19 pandemic worldwide meant that there was a critical need for urgent development and implementation of *in vitro* diagnostic (IVD) tests to diagnose SARS-CoV-2. In Australia, the rigorous requirements to validate IVDs to satisfy regulatory standards prior to approval for use would not have been met by diagnostic laboratories in the early stages of the pandemic. On 31 January 2020, the Therapeutic Goods Administration (TGA) Australia issued an emergency

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exemption allowing supply of COVID-19 IVDs to laboratories within the Public Health Laboratory Network (PHLN). This was later expanded to include all accredited pathology laboratories in Australia. A disclaimer was included in early reports indicating that the nucleic acid tests (NATs) used were not validated for testing per National Pathology Accreditation Advisory Council (NPAAC) requirements.[5]

This manuscript presents a comprehensive overview of currently available laboratory methods for diagnosis of SARS-CoV-2 including NAT assays, serology, rapid antigen detection and antibody tests, virus isolation and whole genome sequencing (WGS) (Table 1). These tests have different applications, and vary in analytical performance, availability, costs, turnaround times and throughput. A summary of the relative advantages and disadvantages of the different diagnostics tests are discussed, as well as their applications in different clinical and infection control situations. We also discuss issues related to reagent shortages, quality control and assurance, and future challenges including the sustainability of ever-increasing demands for testing.

BASIC VIROLOGY

The International Committee on Taxonomy of Viruses (ICTV) recognises five hierarchical ranks that define viral taxonomy; order, family, subfamily, genus and species.[6] SARS-CoV-2 belongs to the *Coronaviridae* family, one of four families within the order *Nidovirales*, which can be further subdivided into two subfamilies *Coronavirinae* and *Torovirinae*. There are four genera, *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus* within *Coronavirinae*.[6] Species within the *Betacoronavirus* genus are further differentiated on the basis of genome sequences into lineages A, B, C and D, although these lineages do not have formal taxonomic status. SARS-CoV-2 belongs to the subgenus Sarbecovirus and is a group 2B *Betacoronavirus*. SARS- CoV-2 shares 80% homology with SARS-CoV which is also in the Sarbecovirus subgenus.[6]

Coronaviruses (CoVs) are named due to their striking crown surface projections, reminiscent of the solar corona, which can be seen by electron microscopy (EM) (Fig. 1). CoVs are enveloped linear, positive sense, single-stranded RNA viruses. The genome is approximately 30 kb in size, capped with a polyadenylated 3′ end, containing seven known genes in the arrangement 5′-ORF1a-ORF1b-S-ORF3-E-M-N-3′.[7] There are non-structural proteins in two overlapping open reading frames, ORF1a and ORF1b, representing two-thirds of the genome, with the remaining third encoding structural proteins.[8] These are translated from a nested set of subgenomic mRNAs and contain additional accessory genes specific to CoVs. The enveloped virions are spherical or pleomorphic and contain a helical nucleocapsid of nucleoprotein (N) associated with the RNA genome. Embedded in the envelope are 20 nm long trimers of spike (S) glycoprotein, also called peplomers, which have a club-shaped morphology and facilitate attachment to cells. The envelope also contains integral membrane (M) and envelope (E) proteins. The genes encoding proteins N, S, M and E are the targets of many of the NAT assays and the S protein is one of the proteins being investigated as a vaccine target for SARS-CoV-2.[9]

SAMPLE COLLECTION AND TRANSPORT

Suitable samples from persons with suspected COVID-19 include those collected from the upper (nasal and oropharyngeal swabs, nasopharyngeal swabs and nasal wash/aspirates) and lower respiratory tract [bronchoalveolar lavage (BAL), tracheal aspirates, pleural fluid and sputum].[10] Healthcare worker (HCW) collected oro- and nasopharyngeal swabs have superior sensitivity, but other sample collections methods may be appropriate in some circumstances.[11] Testing of self-collected oro-pharyngeal and/or nasal swab specimens have shown comparable performance to HCW collected swabs in some studies but lower sensitivity in others,[11–13] whilst reducing the risk of HCW infection and obviating the need for personal protective equipment (PPE). Self-collected saliva specimens also offer the same benefits in addition to reducing the need for swabs and discomfort associated with repeated sampling of the oro- or nasopharynx.[14,15] Overall, the detection of SARS-CoV-2 in saliva specimens is comparable to that of oro- or nasopharyngeal swabs, although there are different methods in the collection and laboratory processing of saliva in published studies.[14,16–19] SARS-CoV-2 RNA is generally detectable in respiratory samples from 48 hours prior to the onset of symptoms, although it has been detected by NAT and isolated in samples collected 6 days prior to the onset of symptoms.[20] Serum or plasma samples are suitable for serology testing, and acute and convalescent samples should be collected. Other samples where SARS-CoV-2 has been detected include stool, cerebrospinal fluid, conjunctival fluid and placental tissue.[21–24] Following collection, samples should be transported to the laboratory as soon as possible or refrigerated at 4°C.

LABORATORY SAFETY

SARS-CoV-2 is classified as a Risk Group 3 organism and therefore appropriate safety precautions when handling specimens in the laboratory are required. NAT testing can be performed safely in a Physical Containment (PC) Level 2 laboratory using biosafety level 2 (BSL-2) cabinets once extraction reagent has been added to the sample, however viral cultures and serological assays that require preparation of immunofluorescence slides and microneutralisation assays need to be performed in a PC3 laboratory given live virus manipulation. To date, there have been no reports of laboratory-acquired SARS-CoV-2 infection, in contrast to SARS-CoV.[25]

SARS-CoV-2 NUCLEIC ACID TESTING

NAT using real-time reverse transcriptase polymerase chain reaction (RT-PCR) has become the predominant method to detect SARS-CoV-2. Prior to the availability of the SARS-CoV-2 genome, initial detection of SARS-CoV-2 RNA relied on the use of generic primers targeting CoVs that may not be specific for SARS-CoV-2, and gel-electrophoresis techniques, which are costly, labour intensive and low throughput. The publication of the SARS-CoV-2 genome on 10 January 2020 (subsequently Wuhan-1, GenBank accession number MN908947.3)[26] enabled the rapid development and roll out of RT-PCR assays. Several diagnostic pathology providers developed RT-PCR assays based on in-house primer sets, or using primer sets and probes published by

Table 1 Summary of SARS-CoV-2 diagnostics available in Australia for routine and reference use

Methods	Sample type	Comments	Advantages	Disadvantages	TAT/approximate reagent cost[a]	Availability in Australia
Nucleic acid testing (NAT) or nucleic acid amplification test (NAAT)	Upper and Lower respiratory tract samples	In-house initially; available commercially since February 2020	Acute diagnosis	Low viral titres can mean lack of reproducibility Reduced predictive values in low incidence settings False positives, contamination	1–6 hours (once sample in lab) ~AU$10–50	Widespread in both public and private laboratories across Australia Assays in use include: In-house tests targeting various combinations of (E, M, N, ORF1a/b, ORF1b, RdRp and S) Commercial assays (see Table 2)
Serology	Serum	In-house/ commercial POCT IgM, IgA, IgG MN IFA ELISA	Useful for diagnosis of past cases (i.e. follow up of suspected cases who either did not undergo NAT during the acute illness or were NAT negative) Broad-based population serological surveillance, vaccine efficacy and research activities	Not useful for acute diagnosis. Not clear if development of neutralising antibodies is indicative of immunity Not all PCR-positive cases have positive serology Falling titres over time (2–3 months)	Usually <12 hours ~AU$10	Limited availability, generally state public health reference laboratories Commercial kits (see Table 3)
Virus culture	Upper and lower respiratory tract samples		Infectivity demonstrated	Need PC3 laboratory. Need equipment and skilled scientist	4–7 days ~AU$100	PC3 laboratory facilities State public health reference laboratories
Sequencing	RNA extracts	Generally needs higher viral loads, represented by a Ct value of <30 on most commercial assays	Linking transmission Cluster analysis Mutation development informing diagnostic targets, later vaccine studies	Needs to be PCR positive with high enough viral load/ low Ct to produce adequate sequencing results	1–7 days ~AU$100 Usually batched which increases TAT High costs compared with RT-PCR	State public health reference laboratories Research institutes
Electron microscopy (EM)	EM specific preparation of respiratory tract samples	Requires highly trained staff; available in few centres	Virus agnostic (that is not dependent upon genomic sequence)	Labour intensive	Several days	State public health reference laboratories, some only

ELISA, enzyme linked immunosorbent assay; IFA, immunofluorescent assay; MN, microneutralisation; NAT, nucleic acid test; PC3, Physical Containment level 3; POCT, point of care test; TAT, turnaround time.
[a] Cost is very approximate in Australian dollars for reagents and does not include labour costs.

the WHO,[27] US Centers for Disease Control (CDC)[28] or Hong Kong University.[29]

The gene targets used in these RT-PCRs were initially the RNA dependent RNA polymerase (RdRp) gene of the ORF1ab sequence, E gene, N gene and S gene.[30] In Australia, the PHLN recommended early on that positive screening results should be confirmed by a secondary NAT targeting another gene or by WGS. There is also variability in the analytical performance of these targets in the detection of SARS-CoV-2, with several studies showing that the E gene has the highest sensitivity.[31] However, the E gene is not specific for SARS-CoV-2 as it is a pan-Sarbecovirus target and there may be cross-reactivity with other CoVs including SARS-CoV. There is no cross reactivity with the commonly circulating human corona-viruses, and since SARS-CoV is not thought to be currently circulating, detection of the E gene generally indicates the presence of SARS-CoV-2. Formal viral quantitation is not routinely available in many diagnostic laboratories, though RT-PCR cycle threshold (Ct) values may provide some indication of viral load.

As the pandemic unfolded, commercial assays targeting different genes became available to meet the exponential increase in global testing demands. These assays have been generally developed for existing platforms, some of which incorporate automated nucleic acid extraction. The targets of these assays are also varied, and as with in-house or laboratory-developed assays, there is variable performance in the detection of SARS-CoV-2 (Table 2). The unprecedented rapid increase in testing volumes worldwide has resulted in shortages of reagents and consumables required for NAT. This includes swabs, transport media for swabs, plasticware required for sample processing and storage (such as pipettes and cryovials tubes), reagents for automated nucleic acid extraction platforms and testing kits.

To overcome some of these limitations, laboratories have adapted and developed innovative methods for testing, such as the pooling of two or more samples either prior to, or after, nucleic acid extraction,[32–35] which has been done previously for the mass screening of samples for influenza virus.[36] If SARS-CoV-2 RNA is subsequently detected, repeat testing of the individual samples from the original pool is performed

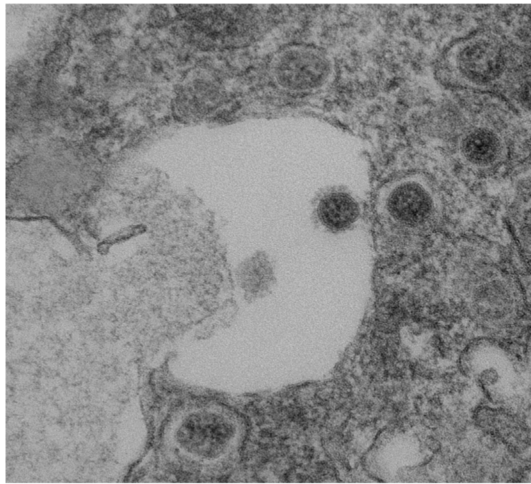

Fig. 1 Electron microscopy image of SARS-CoV-2 (100 nm, HV=80 kV, direct magnification 80,000x).

to determine which sample(s) have returned the positive result. Although testing by pooling increases throughput, it is important for laboratories to validate their pooling method to determine how pooling affects the limit of detection of their assays. More complex testing algorithms have also been developed mathematically such that repeat testing of individual samples may not be required if the initial sample is tested in multiple pools.[37] However, the advantages of pooling are negated when the prevalence of COVID-19 in the population tested is high, as more pooled samples will require individual testing. Other laboratories have also incorporated SARS-CoV-2 targets in existing respiratory multiplex panels to determine alternate viral aetiologies for respiratory infection and to exclude co-infections.[38,39]

Similar to the detection of other respiratory viruses such as influenza and respiratory syncytial virus, turnaround times for NATs may be reduced by using rapid RT-PCR platforms or loop isothermal amplification (LAMP) methods.[40] Although the RT-PCR assays show comparable performance to the standard non-rapid assays,[41,42] the throughput of these assays is generally low, but the reduced turnaround times of less than one hour make these assays useful for certain clinical scenarios where an urgent result is required. LAMP assays are being developed for commercialisation, particularly for point-of-care applications in remote areas or communities with limited laboratory access, given the lack of specialised equipment needed, rapid turnaround times required and with previous data showing comparable performance to RT-PCR.[43–45]

Rapid antigen testing

Similar to rapid influenza diagnostic tests,[46] rapid antigen detection tests (RADTs) have also been developed to detect SARS-CoV-2. RADTs are available in several formats including lateral immunochromatography and fluorescent immunoassays. Typically used in a point-of-care format, RADTs offer the benefits of rapid turnaround times (10–20 minutes) to guide individual patient management and infection control measures but without the requirement of testing to be performed by skilled technical laboratory staff. However, RADTs are less sensitive than NATs, have less throughput and are generally more expensive. Modelling studies have suggested that the reduced analytical sensitivity of RADTs may in part be overcome by more regular testing of the same person (such as daily screening of asymptomatic workers in high-risk settings).[47] In addition, the sensitivity of RADTs in detecting SARS-CoV-2 was 100% in samples where the corresponding Ct of the NAT was <25.1.[48] RADTs are also more sensitive when performed on samples collected from persons within one week of symptom onset.[48] This suggests that RADTs may be used to reliably detect virus in patients shedding high levels of virus, and who therefore have the highest risk of transmission.

SEROLOGY

Serology is generally not utilised for the diagnosis of acute SARS-CoV-2 infection, but may be used: (1) for retrospective diagnosis of persons with symptoms consistent with acute COVID-19 who have not undergone NAT, or those

Table 2 Nucleic acid test (NAT) assays in routine use in Australia

Assay	Target	Approved in Australia	LOD NDU/mL[83,a]	Performance evaluation[b]
Abbott (USA) RealTime SARS-CoV-2	RdRp, N	17 April 2020	5400	Degli-Angeli *et al.*[84] Mostafa *et al.*[85]
AusDiagnostics (Australia) respiratory virus panel (incl SARS-CoV-2)	ORF1a, ORF8	19 March 2020	NT	Attwood *et al.*[86] Rahman *et al.*[31]
Becton Dickinson (USA) BD SARS-CoV-2 for BD Max System	N1, N2	17 April 2020	1800	Mostafa *et al.*[85]
Cepheid (USA) Xpert Xpress SARS-CoV-2	E, N2	22 March 2020	5400	Loeffelholz *et al.*[87] Mostafa *et al.*[85]
CerTest Biotc SL (Spain) VIASURE SARS-CoV-2 Real Time PCR Detection Kit	ORF1ab, N	31 March 2020	NT	
Genetic Signatures (Australia) EasyScreen SARS-CoV-2 Detection Kit	N, E	13 April 2020	NT	Public Health England[88]
Hologic (USA) Panther Fusion SARS-CoV-2 Assay	ORF1ab (Region 1 and 2)	20 May 2020	600	Hogan *et al.*[89]
Roche (Switzerland) Cobas SARS-CoV-2	ORF1ab, E	20 March 2020	1800	Poljak *et al.*[90] Mostafa *et al.*[85]
Seegene (Korea) Allplex 2019-nCoV Assay	E, N, RdRp	27 March 2020	DNR	Hur *et al.*[91]

DNR, data not returned; NT, not offered testing by FDA SARS-CoV-2 reference panel.
[a] NAAT detectable units/mL: data from FDA SARS-CoV-2 reference panel.
[b] Independent performance evaluation: some assays have been evaluated by two different groups and therefore have two sets of performance data.

with negative or inconclusive NAT results; (2) to estimate the timing of infection to help define the infectious period; and (3) for serosurveys to examine rates of exposure and infection in a given population. Confirmation of infection requires the demonstration of seroconversion, or a four-fold or greater rise in antibody titres between acute and convalescent sera. Other uses of serology tests include the screening of persons that have recovered from COVID-19 for convalescent plasma harvesting and monitoring immune responses including the assessment of SARS-CoV-2 vaccine effectiveness.

In-house developed and commercial serology tests using different methods such as lateral flow immunochromatography, enzyme linked immunosorbent assays (ELISA), immunofluorescent antibody (IFA) and viral neutralisation assays are available for detection of SARS-CoV-2 antibodies. Viral neutralisation represents the 'gold standard' method but is labour intensive (and therefore has low throughput) and requires technical expertise and testing to be done in a PC3 laboratory. By contrast, ELISAs that detect the binding of antibodies to antigens such as the S protein, the S1 and/or receptor-binding subdomains of the S protein or nucleoprotein can be adapted to high throughput platforms. The moderate throughput IFA assay detects the presence of antibodies that bind to viral antigens expressed on fixed cells, but like ELISA, do not measure functional antibodies compared to viral neutralisation. The performance of various serology assays is highlighted in Table 3.

When assessing the performance of different serology tests, it is important to observe when the samples were collected relative to symptom onset, as false negatives may occur if samples are collected too early during the window period, which may be up to 14–20 days.[49–51] Of relevance is also what the serology test was compared against, which is typically NAT rather than the 'gold standard' serological test of viral neutralisation. It is estimated that 5–10% of infected persons do not generate antibody responses following infection.[52] There are inconsistent data on the correlation between antibody development and disease severity following CoV infections, with some investigators reporting early robust responses in association with mild

Table 3 Immunoassays approved in Australia

Assay	Target	Sensitivity	Approved in Australia	Performance evaluation[a]
Abbott (Ireland) SARS-CoV-2 IgG kit[b]	N protein	<7 days 8.3% 7–14 days 61.9% >14 days 100%	28 July 2020	Meschi *et al.*[92]
Beckman Coulter (USA) Access SARS-CoV-2 IgG Antibody Test	S1 RBD	<7 days 65% 7–14 days 94% >14 days 100%	24 July 2020	Hogan *et al.*[93]
BioMerieux (France) VIDAS SARS-CoV-2 IgM	S protein	<7 days 40% 7–14 days 80.7% >15 days 73.3%	3 August 2020	Wolff *et al.*[94]
BioMerieux (France) VIDAS SARS-CoV-2 IgG	S protein	<7 days 57.1% 7–14 days 71% >15 days 86.7%	3 August 2020	Wolff *et al.*[94]
Bio-Rad (France) Platelia SARS-CoV-2 Total Ab	N protein		23 June 2020	
DiaSorin SpA (Italy) LIAISON SARS-CoV-2 S1/S2 IgG and IgM[b]	S1/S2 protein	<7 days 59% 7–14 days 88% >14 days 100% <7 days 51.4% 7–14 days 67.7% >15 days 87.7%	31 July 2020	Hogan *et al.*[93] Wolff *et al.*[94]
EUROIMMUN Medizinische Labordiagnostika (Germany) Anti-SARS-CoV-2 ELISA (IgG)	S1 protein	<7 days 60% 7–14 days 71% >15 days 91.1% >4 days 100%	18 May 2020	Wolff *et al.*[94] Beavis *et al.*[95]
EUROIMMUN Medizinische Labordiagnostika (Germany) Anti-SARS-CoV-2 ELISA (IgA)	S1 protein	<7 days 71.4% 7–14 days 80.7% >15 days 93.3% >4 days 90.5%	18 May 2020	Wolff *et al.*[94] Moran *et al.*[41]
Ortho-Clinical Diagnostics (United Kingdom) VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total	S1 protein	<11 days 45.5% >14 days 91.8%	19 June 2020	Public Health England[96]
Roche (Switzerland) Elecsys Anti-SARS-CoV-2[b]	N protein	<7 days 59% 7–14 days 94% >14 days 100% <7 days 68.6% 7–14 days 83.9% >15 days 88.9%	20 May 2020	Hogan *et al.*[93] Wolff *et al.*[94]
Siemens (USA) ADVIA Centaur SARS-CoV-2 Total (COV2T) assay	S1 RBD		5 June 2020	
Siemens (USA) Atellica IM SARS-CoV-2 Total (COV2T) assay	S1 RBD	>14 days 89.4% >21 days 92.4%	5 June 2020	Public Health England[97]
Siemens (USA) Dimensions EXL SARS-CoV-2 Total antibody assay	S1 RBD		5 June 2020	
Shenzhen YHLO Biotech (China) iFlash-SARS-CoV-2 IgG and IgM	N and S proteins	Median 16 days: IgM 48.1% IgG 88.9%	31 July 2020	Jin *et al.*[98]

RBD receptor binding domain.
[a] Independent performance evaluation: some assays have been evaluated by two different groups and therefore have two sets of performance data.
[b] Currently in routine use in Australia.

infection,[53,54] whilst others have observed higher antibody titres with severe disease.[55,56] Of note, SARS-CoV-2-specific IgA is detected more often and at higher titres and is at least as specific compared to IgM following acute infection.[51,57]

Following infection, antibodies against SARS-CoV-2 may decline with time,[58] although B- and T-cell responses may provide longer term protection than is indicated by circulating antibody levels. Further data are awaited on the robustness and duration of humoral antibody responses and the cellular immunity responses after SARS-CoV-2 infection.

Rapid antibody testing

Lateral flow immunoassays (LFAs) detecting SARS-CoV-2 antibodies are typically used in a point-of-care setting. Although LFAs are rapid and easy to perform (as they require capillary blood only and do not need specialised equipment), they are insensitive compared to standard serology tests, particularly in the early phases of infection. For example, IgM was only detected in 25.9–42.9% of persons with COVID-19 during the first 5 days following symptom onset. On the other hand, IgG was detected in 66.7–90.9% of sera from persons infected with SARS-CoV-2 collected more than 20 days after symptom onset.[59] Specificity ranged from 84.3% to 100%, with false positive LFA results occurring due to cross-reactivity with other antibodies from circulating seasonal non-SARS-CoV-2 CoVs.

SARS-CoV-2 WHOLE GENOME SEQUENCING

Following the first release of the SARS-CoV-2 genome on 10 January 2020,[60] public health and research laboratories worldwide have rapidly shared sequences on public data repositories such as GISAID, with over 93,000 genomes published as of 31 August 2020.[61] This has provided near real-time snapshots of global diversity through public analytic and visualisation tools.[62–65] At a local level, WGS enhances disease surveillance and control by confirming transmission events and clusters, which are critical to timely intervention to limit spread.[66] The recognition of previously unidentified outbreaks through linkage of genomic and epidemiological data in real-time or near real-time improves public health investigations, particularly in cases of unknown community transmissions.

In Australia, the initial high diversity of sequenced SARS-CoV-2 genomes was attributable to the concurrent and independent importations of the virus by overseas travellers from different countries and regions.[63–65] By contrast, most of the cases in the second wave of the pandemic after the closure of our international borders showed limited sequence diversity, suggesting local community transmission of SARS-CoV-2 originating from more limited sources. WGS can be used to identify transmission pathways where there is more than one plausible epidemiological exposure and also allows determination of nosocomial acquisition of infection.[67,68] Other applications of WGS for SARS-CoV-2 include the identification of mutations that may confer increased virulence, transmissibility or antiviral resistance.

Various protocols have been developed to sequence SARS-CoV-2 using Illumina, Ion Torrent, PacBio and Oxford Nanopore platforms. High quality consensus sequences are often difficult to obtain from samples with low virus burden (as evidenced by RT-PCR Ct values >30 for example) using amplicon-based Illumina sequencing methods.[63,65] Early studies suggest that amplicon-based or hybrid capture-based target enrichment methods may potentially overcome this limitation. Metagenomic sequencing is relatively slow (Illumina) or insensitive (Nanopore) for the purposes of cost-effective near- to real-time SARS-CoV-2 WGS.[69] Partial sequences do not necessarily provide adequate granularity to identify relatedness and transmission links in general.

WGS is not widely available in many countries given the substantial infrastructure and relatively high costs required, and sequences on databases such as GISAID are overwhelmingly represented from resource-rich countries.[63] Data analysis requires sufficient technical and bioinformatic expertise, and where resources are limited, there may be a need for prioritisation of samples for WGS to inform efficient and effective public health responses.

VIRUS CULTURE

Cell culture is not widely available for the routine diagnosis of SARS-CoV-2, but is useful for: (1) providing adequate positive control material for the development, validation, evaluation and quality assurance of SARS-CoV-2 diagnostic assays; (2) supporting the development of vaccines and therapeutic agents; and (3) enabling research into viral virulence and transmission. At a clinical level, cell culture has also been used to guide de-isolation of persons with persistent or intermittent shedding of SARS-CoV-2 RNA from both the respiratory and gastrointestinal tracts,[70–72] with Lan *et al.* reporting cases out to 50 days following symptom onset.[73]

Cell culture has long turnaround times, is labour intensive and the WHO recommends that SARS-CoV-2 cultures be performed in laboratories under PC3 conditions. Suitable cell lines include Vero E6, Vero CCL81, Calu3, Caco2, Huh7 and 293T. Cytopathic effects (CPE) are observed in the majority of cultures 48–72 hours post-inoculation,[72,74] and may be confirmed by SARS-CoV-2 NAT of cell culture supernatant. A negligible reduction in Ct values of the terminal cell culture supernatant compared to the original sample suggests a lack of growth.[72] Of note, the yield of cell cultures from samples with low levels of virus (inferred from Ct values of >32–34 for E and N genes) is low,[71,72] but the threshold for successful culture needs to be established in individual laboratories given the potential variability in culture methods. Similar to SARS-CoV and MERS-CoV, the addition of exogenous trypsin may improve the recovery of SARS-CoV-2 in culture by inducing the proteolytic cleavage of the S protein and promoting virus-cell membrane fusion.[75–77]

ELECTRON MICROSCOPY

On transmission EM, CoVs including SARS-CoV can be identified by their characteristic projections forming a ‘corona’ or crown-like structure around a spherical virion measuring 80 nm in diameter. EM is useful as a complementary method for detection of novel viruses including CoVs; however, this method is not routinely available given the need for specialised equipment and technical expertise. It is also time consuming and labour intensive.

QUALITY ASSURANCE PROGRAMS

The participation in Quality Assurance Programs (QAPs) is a requirement not only for laboratory accreditation, but crucial for benchmarking with the relative rapid development and deployment of diagnostic tests for SARS-CoV-2. The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) Biosecurity has been offering proficiency testing programs for SARS-CoV-2 NAT. The first survey was circulated to 16 public and private laboratories in early March 2020 prior to the WHO declaring COVID-19 a pandemic. As some of the items included in the survey contained synthetic SARS-CoV-2 gene fragments only, these were not detected by laboratories using NAT assays that do not target these genes. Reassuringly, all 16 laboratories detected total RNA that was extracted from cell culture isolated SARS-CoV-2. Randox Laboratories (England) and Quality Control for Molecular Diagnostics (QCMD; Scotland) also provide external QAP for SARS-CoV-2 NAT assays which include inactivated preparations of SARS-CoV-2.[10] At the time of writing, the RCPAQAP for serology has been developed and results from the first survey are expected to be available in the second half of 2020.

SARS-CoV-2 TESTING IN AUSTRALIA: PAST, PRESENT AND FUTURE

The case definitions for COVID-19 and persons recommended for testing in Australia have continued to evolve since the beginning of the pandemic. Initially, testing was confined only to symptomatic travellers returning from Wuhan City, followed by Hubei Province, before widening to all of PRC. Other high-risk countries including South Korea, Iran, Italy, and Cambodia were subsequently added prior to the closure of Australia's international borders. Since January 2020, testing has further broadened to include at-risk groups [such as HCWs, residents in aged care facilities, Aboriginal and Torres Strait Islanders (ATSI), cruise ship passengers and meat processing facility workers] with regular revision of the national guidelines. The United Nations (UN) has advised that COVID-19 poses a grave threat to the health of indigenous communities worldwide.[78] The Australian Government has issued warnings that ATSI and people in remote communities are at a greater risk of COVID-19 due to the increased incidence of comorbidities, household crowding and lack of access to healthcare.[79] By the end of June 2020, 60 (representing 0.8% of all cases) persons infected with COVID-19 in Australia were of ATSI background. Half of these infections were acquired overseas and the rest were acquired locally from major Australian cities.

Australian laboratories increased their testing capacity quickly and within weeks tested thousands of samples per day with short turnaround times. In some jurisdictions, such as NSW, innovative approaches for result delivery, such as the direct text messaging of negative results to patients, have helped reduce turnaround times between specimen collection and result to within 24 hours in general. Some laboratories have several platforms operating simultaneously so that testing can be flexible in view of shortages of reagents and consumables. Extraction-free NAT assays have also been developed to circumvent the worldwide shortage of RNA extraction reagents.[80,81] Workforce fatigue is a further issue that was encountered with the sudden and unexpected surges in demands for laboratory testing.

Australia has one of the highest testing rates per capita in the world with over 6.4% of the population tested.[3] Widespread testing, together with Australia's geographic advantage, border control, social distancing and public health messaging measures have all contributed to limit the number of infections of COVID-19. A low prevalence of COVID-19 in Australia (<1%) means that the pre-test probability will be low, and that even with highly specific assays, there will be false positive results. For example, the positive predictive value (PPV) of SARS-CoV-2 NATs with a specificity of 99% is only 50% when the prevalence of infection is 1%. By late March 2020, the prevalence of COVID-19 was over 3%[82] in some Australian jurisdictions, and NAT assays with the same analytical performance had a PPV of approximately 75%. When prevalence of infection is low, it is recommended that positive results be confirmed by testing the same sample using another assay, preferably one with different gene targets. If there is doubt about a positive result (such as only a single target positive or collected from a person with low pretest probability), a repeat collection of another respiratory tract sample for supplementary testing and serum for the detection of SARS-CoV-2-specific antibodies can be performed. Implications of false positive results, which could be avoided, include unnecessary quarantining, contact tracing, delays in the recognition and treatment of the true illness, patient anxiety, potential nosocomial exposure to infection from the cohorting of confirmed COVID-19 patients, wastage of PPE and costs of confirmatory testing.

In the absence of specific antiviral therapies and vaccines, it is likely that SARS-CoV-2 testing will remain key to limiting spread of infection. Outside the laboratory setting, the role of soon to be available RADTs in a point-of-care format for asymptomatic screening remains to be defined. The 'opening of society', particularly with fatigue and complacency in maintaining effective physical distancing measures, may rely on the use of such assays (for example, prior to international travel) even if the sensitivity of these tests is substantially lower than NATs. Similarly, rapid antibody tests have been promoted to determine 'immunity passports', but such strategies are not without flaws. Performing these tests outside the laboratory setting may reduce the accuracy of captured data such as testing volumes and analytical performance of testing, and the number of ancillary tests such as WGS or culture.

CONCLUSIONS

Laboratory diagnosis of SARS-CoV-2 remains the cornerstone for controlling COVID-19, and the strong laboratory network in Australia has contributed substantially to our early and continued success in tackling the pandemic. The rapid scaling up and strategic expansion of testing has limited the spread of COVID-19 by the rapid identification of cases, clusters and transmission events. Continued investment in infrastructure, equipment and personnel are key factors in ensuring Australia remains at the forefront in dealing with pathogens of pandemic potential.

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