Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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REVIEW

Use of emerging testing technologies and approaches for SARS-CoV-2: review of literature and global experience in an Australian context

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Summary

Emerging testing technologies for detection of SARS-CoV-2 include those that are rapid and can be used at point-of-care (POC), and those facilitating high throughput laboratory-based testing. Tests designed to be performed at POC (such as antigen tests and molecular assays) have the potential to expedite isolation of infectious patients and their contacts, but most are less sensitive than standard-of-care reverse transcription polymerase chain reaction (RT-PCR). Data on clinical performance of the majority of emerging assays are limited with most evaluations performed on contrived or stored laboratory samples. Further evaluations of these assays are required, particularly when performed at POC on symptomatic and asymptomatic patients and at various time-points after symptom onset.

A few studies have so far shown several of these assays have high specificity. However, large prospective evaluations are needed to confirm specificity, particularly before the assays are implemented in low prevalence settings or asymptomatic populations. High throughput laboratory-based testing includes the use of new sample types (e.g., saliva to increase acceptability) or innovative uses of existing technology (e.g., sample pooling). Information detailing population-wide testing strategies for SARS-COV-2 is largely missing from peer-reviewed literature. Logistics and supply chains are key considerations in any plan to 'scale up' testing in the Australian context.

The strategic use of novel assays will help strike the balance between achieving adequate test numbers without overwhelming laboratory capacity. To protect testing of high-risk populations, the aims of testing with respect to the phase of the pandemic must be considered.

Key words: Testing; COVID-19; SARS-CoV-2.

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INTRODUCTION

As the COVID-19 pandemic has progressed and diagnostic needs have evolved, a range of emerging testing technologies for detection of SARS-CoV-2 have become available. These include rapid diagnostic and point-of-care (POC) tests and those facilitating high-throughput laboratory-based testing. The quality of the evaluations for emerging tests are variable and often insufficient, especially for performance related to the intended use.[1–3] The majority of available information regarding emerging tests and testing strategies is derived from high-prevalence scenarios and the relevance to the Australian context is unclear. We summarise the currently available information for emerging testing strategies and technologies to elucidate their potential use in the Australian context.

IDENTIFYING TESTING APPROACHES USING MODELLING

The characteristics of emerging assays relative to standard laboratory-based reverse transcription polymerase chain reaction (RT-PCR) vary considerably with respect to performance, turnaround time (TAT) and throughput (Table 1). Mathematical modelling studies examining the relative contributions of various testing strategies show that approaches with high test numbers and short TAT have the greatest impact.[4–6]

Modelling has shown that minimising testing delay had the largest impact on reducing onward transmissions and optimising testing coverage further enhanced contact tracing effectiveness.[4] Keeping the time between symptom onset and testing and isolation of an index case at 2 days or less is imperative for success in reducing the reproductive number, noting that rapid testing of symptomatic people is at least as important as the efficiency of contact tracing.[4] Weekly PCR screening of healthcare workers (HCWs) and other high-risk groups, irrespective of symptoms was estimated to reduce their contribution to SARS-CoV-2 transmission (R) by 23%.[5]

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Table 1 Comparison of characteristics of emerging assays relative to standard laboratory-based RT-PCR

Assay type	Available literature[a]	TAT	Sensitivity	Specificity	Ease of use at POC	Scalability	Cost	Supply chain
Standard laboratory-based RT-PCR	+++/++++	Hours	++++	++++	n/a	+++	+++	++/+++
1. Innovations of molecular assays								
Rapid or near POC RT-PCR	++++	Under 1 hour	++++	++++	++	+	++++	+/++
POC NAAT	+/++	Minutes	+++/++++	++++	++++	+	++++	+/++
Extraction-free LAMP	+/++	Minutes–hours	++/+++	+++/++++	+	++	+	+++
Saliva RT-PCR	+/++	Hours	++/+++	++++	n/a	+++	+++	+++
Pooling	++	Hours	+++	++++	n/a	++++	+	++
Extraction-free RT-PCR	+	Hours	+++	++++	n/a	+++	++	++
RT-PCR with WGS	–	Hours–days	–	–	–	–/++++	++++	–
2. Non-molecular assays								
POC Antigen	+/+++	Minutes	++/+++	+++	+++/++++	++	+	–/++
3. Emerging technology								
CRISPR	+	Hours	+++	+++	–	–	+	–
Microfluidics	–	Hours	–/+	–	–	–	–	–
Virolens	–	Seconds	–	–	–	–	–	–
4. Antibody assays								
POC Antibody	++	Minutes	+/++	++	++++	++	+++	++

–, unknown/insufficient data; +, minimal; ++, moderate; +++, high; ++++, very high; n/a not applicable; CRISPR, clustered regularly interspaced short palindromic repeats; LAMP, loop-mediated isothermal amplification; NAAT, nucleic acid amplification techniques; POC, point-of-care; RT-PCR, reverse-transcription polymerase chain reaction; TAT, turnaround time; WGS, whole genome sequencing.
[a] Peer-reviewed literature or independent evaluation of clinical performance.

This reduction is in addition to the reductions achieved when people self-isolate following symptoms, assuming test results are available at 24 hours. A 15% reduction in test sensitivity reduces the effectiveness of weekly screening from 23% to 19%. If 80% of cases and contacts are identified and there is immediate testing following symptom onset and quarantine of contacts occurs within 24 hours, R can be reduced by 26%. This result is on top of reductions achieved by self-isolating following symptoms. For 50% coverage and a 48-hour delay in quarantining, the reduction in transmission is just 8%.

One model found that effective screening depends largely on testing frequency and the speed of reporting and is only marginally improved by high test sensitivity.[6] A notable assumption in this model is that during the exponential growth phase of the virus, the time between 10^3 and 10^5 copies/mL is short, allowing a limited window in which only the more sensitive test could diagnose individuals. A limitation of this study is it did not consider test specificity: specificity considerations must be taken into account given that, for example, if a test is 80% sensitive and 98% specific, at 1% prevalence the positive predictive value would only be 28.8%.[7]

It has been suggested that people would possibly use negative low-sensitivity test results (conceivably falsely negative) to justify abandoning more proven interventions, such as wearing a mask and social distancing. It is probable that people who have high levels of SARS-CoV-2 in their respiratory secretions are more likely to be infectious than those with low levels, but whether lower-sensitivity tests can reliably detect persons who are likely to be infectious remains to be proven. It is clear that samples with lower PCR cycle threshold (Ct) values are more likely to contain SARS-CoV-2 that can be detected in viral culture, but there is no clear separation among samples that are culture positive or not when using the Ct value. Furthermore, there remain no robust clinical data linking viral quantity to transmissibility.[7]

Fundamentally, optimisation of SARS-CoV-2 testing is paramount to controlling the pandemic. Here we summarise the current literature for emerging testing technologies and approaches and discuss their potential in the Australian context. We have included peer-reviewed literature, independent evaluations and selected widely-cited pre-prints.

INNOVATIONS IN MOLECULAR ASSAYS

Commercial point-of-care (POC) or near-POC molecular assays

Commercial POC rapid nucleic acid amplification techniques (NAATs) fall into two categories: those with instrumentation widely available in Australia and those where instrumentation is less available (Table 2). Of the former group, the Xpert Xpress SARS-CoV-2 assay has shown reliable performance but has had severe limitations on test cartridge supply. Therefore, there is a need to investigate the performance of alternative assays for which there are less available data.

A March 2021 Cochrane review[8] found that sensitivities of rapid molecular assays varied according to test brand. Most of the data relate to the ID NOW COVID-19 (Abbott) and Xpert Xpress (Cepheid) assays. Using data from evaluations following the instructions for use (IFU), the average sensitivity of ID NOW was 73.0% [95% confidence interval (CI) 66.8–78.4%] and average specificity 99.7% (95% CI 98.7–99.9%). For Xpert Xpress, the average sensitivity was 100% (95% CI 88.1–100%) and average specificity 97.2% (95% CI 89.4–99.3%). A Foundation for Innovative New Diagnostics (FINDdx) independent evaluation determined the Xpert assay had 100% sensitivity and 99% specificity when compared to the Cobas assay (Roche) which is widely used in Australia.[9]

The variable performance in sensitivity of the ID NOW SARS-CoV-2 assay may be attributed to the type of swab used, the time of testing following onset of symptoms and the reference assay used in the different studies.[10–14] Several of the early evaluations of ID NOW used viral transport media/universal transport media rather than dry swabs, so did not follow the manufacturer's IFU, thus requiring further evaluation.

Table 2 Rapid or near POC nucleic acid amplification tests (as of 12 April 2021)

Assay	FDA Reference panel LOD (NDU/mL)	Approximate turnaround time
TGA listed		
Cepheid Xpert Xpress	5400	30 min
Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV	n/a	
BioFire FilmArray Respiratory Panel 2.1plus SARS-CoV-2 assay	6000	45 min[111]
Abbott ID NOW COVID-19	300,000	15–20 min
Roche Cobas SARS-CoV-2 and Influenza A/B Liat	5400	20 min
Veri-Q PCR 316 COVID-19	n/a	35 min (n=1), 1 h (n=16)
Sansure 2019-nCoV Nucleic Acid Diagnostic Kit	Did not provide shipping information	30 min
USTAR EasyNat Diagnostic Kit	n/a	79 min
Not TGA listed (FDA approved/CE marked)		
Credo SARS-CoV-2 VitaPCR	n/a	20 min
Aries SARS-CoV-2 Assay	180,000	
Accula SARS-CoV-2	Under interactive review	30 min
Talis One COVID-19 Assay	n/a	30 min
Visby Medical Instrument-free PCR	54,000	30 min
MobileDetect-Bio BCC19	Data not returned	Up to 96 tests per machine in 30 min
LumiraDx RNAstar	5400	12 min following extraction
Atila BioSystems iAMP	180,000	~1 hour from dry swab to final result
Cue COVID-19 Test	60,000	20 min
T2 Biosystems T2SARS-CoV-2 Panel	18,000	Results in <2 h; throughput up to 60 samples/day
Quidel Solana SARS-CoV-2 assay	n/a	
Pro-AmpRT SARS-CoV-2 test	Did not provide shipping information	
Color Genomics SARS-CoV-2 RT-LAMP Diagnostic Assay	18,000	
Seasun AQ-TOP COVID-19 Rapid Detection Kit	6000	~1 h ('fast extraction' of ~5 min plus 30 min for detection)
Lucira	n/a	
COVIDNudge	Sensitivity 94% (95% CI 86–98) Specificity 100% (99–100)[112]	90 min
SAMBA II	n/a	<90 min
OptiGene	n/a	<20 min
Novodiag	n/a	1 h 20 min
POCKIT Central	n/a	85 min

n/a, not applicable; POC, point-of-care; RT-PCR, reverse-transcription polymerase chain reaction.

To allow comparison of analytical performance of different molecular assays, the US Food and Drug Administration (FDA) developed a SARS-CoV-2 Reference Panel.[15] Results of various concentrations of inactivated SARS-CoV-2 as blinded samples for a number of different POC molecular assays are shown in Supplementary Table 1 (Appendix A). Other TGA listed POC or near-POC assays with significant performance data available in peer-reviewed literature and showing similar performance to Xpert assay include the Liat (Roche) and the FilmArray (BioFire).[16,17]

Loop-mediated isothermal amplification (LAMP)

LAMP was developed as a rapid and cheap method to amplify DNA/RNA target at a single reaction temperature, bypassing the need for sophisticated thermal cycling equipment. Several studies have evaluated various novel LAMP methods for SARS-CoV-2 using extraction-free methods, with results available within minutes.[18–26] Extraction of RNA improves sensitivity approaching that of laboratory-based RT-PCR but increases testing complexity, TAT and the need for extraction reagents, which at times during the pandemic have been in short supply. This rapid assay can potentially be used at POC with medium throughput capability.[22] Currently, there are limited studies on LAMP at POC; therefore, its feasibility, particularly with and without an extraction step, requires further study.

Saliva testing

Collecting saliva specimens for SARS-CoV-2 testing has several advantages over nasopharyngeal swab (NPS): less patient discomfort, is amenable to self-collection and poses less risk to HCWs.[27] There are 17 US FDA Emergency Use Authorisation assays for SARS-CoV-2 RNA detection in saliva: four are authorised for use in asymptomatic populations. The limit of detection (LOD) for evaluated assays ranges from 600 NDU/mL to 180,000 NDU/mL in FDA Reference Panel data,[15] but limited clinical performance evaluations are available. There are currently three TGA approved COVID-19 tests (NeumoDx, PerkinElmer and Seegene) for use with saliva, meaning that most Australian laboratories are required to perform their own validation of assays for saliva testing.

Six published meta-analyses using various RT-PCR assays have generally found saliva testing to be less sensitive than testing of nasopharyngeal swabs.[27–32] Studies using saliva testing for SARS-CoV-2 detection have employed various collection methods, processing methods, assays and patient populations and therefore have yielded variable results.[33–39]

Two studies testing saliva samples used the Xpert assay.[40,41] In one, there was good correlation with validated assays in a mix of emergency department (ED), inpatients and outpatients with positive agreement (PPA) of 98.9% (87/88 specimens) and negative agreement (NPA) of 100%.[40] The other found good correlation with paired NPS in ED

and suspected COVID-19 patients, with a PPA of 96% (47/49 patients) and NPA of 99%.[41]

Given the advantages of saliva testing, and as several reasonably sized studies show moderately high sensitivity, further studies are warranted to evaluate the optimum specimen collection and processing methods for saliva testing. In the interim, the current Australian Testing Framework (updated 25 June 2021) states that Australian laboratories are using saliva samples for RT-PCR to facilitate expanded surveillance, but that saliva sample testing is not intended to replace well validated, gold-standard swab-based RT-PCR for diagnosis of disease in symptomatic people.[42]

Currently there are very few clinical validation studies on the use of saliva, as sample material for rapid antigen tests and data on the sensitivity of the tests are lacking.[43] Limited available information suggests poor sensitivity compared to nasal swabs.[44]

Sample pooling

Testing pooled specimens rather than individual testing saves on resources and time.[45,46] The European Centre for Disease Prevention and Control (ECDC) guidelines suggest large-scale population-wide testing by means of pool-based strategies.[45,47] The disease prevalence in a population affects the efficiency of pooled testing strategies, with lower prevalence potentially enabling a larger pool size. The US Centers for Disease Control and Prevention (CDC) found that NAAT for SARS-CoV-2 reliably returned a positive result when one positive sample was mixed with four negatives and could reduce the number of tests needed by >50% in scenarios such as COVID-19 prevalence of <5%.[48] As pooled specimens are diluted when the pool is larger, there is a higher likelihood of generating false-negative results so monitoring prevalence is also important to limit this.[49]

Extraction-free RT-PCR

The extraction step of RT-PCR improves sensitivity by purifying DNA/RNA. The advantages of extraction-free PCR are that it is rapid, cost-effective and not reliant on extraction reagents that have been in short supply. The main disadvantage of extraction-free RT-PCR is reduction in sensitivity, which ranges between 81.3 and 94.6%.[50–52]

RT-PCR with whole-genome sequencing (WGS)

WGS of SARS-CoV-2 can improve the resolution of outbreak clusters. It can better define possible transmission networks and therefore inform public health responses. Combining diagnostic testing with WGS has the potential to improve TAT for the genomics results since high throughput detection of SARS-CoV-2 can be combined with WGS of positive samples such as with COVIDSeq assay (Supplementary Table 2, Appendix A).[53–56]

ANTIGEN TESTS

There is a paucity of peer-reviewed literature evaluating the clinical performance of the various antigen assays, particularly in low-prevalence settings (Table 3). A 2020 Cochrane review found the sensitivity of included antigen tests (Beijing Savant, Coris Bioconcept, Liming Bio, RapiGEN, Shenzhen Bioeasy and one in-house assay) varied considerably across studies. Based on five studies (n=943), the average sensitivity was 56.2% (95% CI 29.5–79.8%) and average specificity was 99.5% (95% CI 98.1–99.9%).[8] Data for individual antigen tests were limited, with no more than two studies for any test. This review identified that early evaluations of POC tests are largely based on remnant laboratory samples. It is uncertain whether tests will perform similarly in clinical practice, and with regards to symptom type, duration of symptoms, or in asymptomatic people.[8]

A March 2021 update to the Cochrane review found that estimates of antigen sensitivity varied considerably between studies and between brands. There were differences between symptomatic (72.0%, 95% CI 63.7–79.0%) and asymptomatic participants (58.1%, 95% CI 40.2–74.1%). Average sensitivity was higher in the first week after symptom onset (78.3%, 95% CI 71.1–84.1%) than in the second week of symptoms (51.0%, 95% CI 40.8–61.0%). Using data from manufacturer's instructions for use (IFU) compliant evaluations in symptomatic participants, summary sensitivities ranged from 34.1% (95% CI 29.7–38.8%; Coris Bioconcept) to 88.1% (95% CI 84.2–91.1%; SD Biosensor STANDARD Q). Average specificities were high in symptomatic and asymptomatic participants, and for most brands (overall summary specificity 99.6%, 95% CI 99.0–99.8%).[8]

Of the 13 TGA approved antigen tests, the Panbio COVID-19 Ag rapid test (Abbott) currently has the most peer-reviewed literature, including for children.[57–68] Two early studies of the Panbio COVID-19 Ag rapid test found sensitivity between 73–81% and 100% specificity.[61,62] In addition, FINDdx independent evaluations found Panbio sensitivity for NPS in symptomatic persons to be 85.5% (95% CI 78.2–90.6%) with clinical specificity of 100% (95%CI 99.1–100%).[9] An Australian multi-site assessment of the Panbio antigen test in a low-prevalence setting found a specificity of 99.96% in 2413 individuals (95% CI 99.73–100%).[69]

Of the other TGA approved antigen tests, the SD Biosensor Standard Q (supplied in Australia by Roche Diagnostics) also has a significant amount of peer-reviewed literature.[66,68,70–76] FINDdx evaluation found sensitivities for NPS in symptomatic persons between 88.7–89% with specificity 97.6–99.7%.[9]

Continuously emerging results from FINDdx independent evaluations are likely to substantially inform performance in real-life scenarios (Table 4).[9] For example, recent FINDdx results comparing performance of nasal swabs (NS) to NPS found for the the PanBio antigen test a sensitivity of 86.4% (95% CI 73.3–93.9%) for NS and 90.9% (95% CI 78.8–96.4%) for NPS in a small study with 44 positive cases; for SD Biosensor a sensitivity of 80.5% (95% CI 66.0–89.8%) for NS and 73.2% (95%CI 58.1–84.3%) for NPS in 41 positive patients; and for NowCheck a sensitivity of 89.9% (95%CI 81.3–94.8%) for both NS and NPS in 79 positive patients.

OTHER NOVEL EMERGING TECHNOLOGIES

CRISPR

Two assays for detection of SARS-CoV-2 RNA combining isothermal amplification and CRISPR technology have been approved by the US FDA: SHERLOCK and DETECTR (Mammoth Biosciences). The SHERLOCK assay is portable, and testing can be performed on a heat block or water bath. CRISPR assay reagents can cost <$1 per test.[77]

Detection can be performed by lateral flow assay (portable, read by eye) or fluorescence. The reaction TAT for the SHERLOCK assay is 1 hour, whereas it is 45 minutes for the DETECTR assay. According to the IFU, the DETECTR assay LOD is 12,000 copies/mL UTM. The SHERLOCK assay was included in the recent FDA Reference Panel testing and LOD was found to be 6000 NDU/mL, which was more sensitive than the CDC assay (Supplementary Table 1, Appendix A).

Overall, available information suggests CRISPR may be less sensitive when compared to most commercial RT-PCR. However, studies have shown specificity between 71.4% and 98.5% for CRISPR,[78,79] so further evaluations are required since a recent review has concluded that although CRISPR technology is still in its infancy it is potentially revolutionary as tests are rapid and portable.[80]

Microfluidics

Currently there is one FDA approved assay for SARS-CoV-2 detection in saliva using microfluidics: Advanta Dx (Fluidigm Corporation). Saliva is a validated sample type

Table 3 Peer-reviewed literature for TGA listed SARS-CoV-2 antigen tests (as of 12 April 2021)

Assay	TAT, minutes	Specimen type	Sensitivity/PPA (*n*=positive samples/95% CI)	Specificity/NPA (*n*=negative samples/95% CI)	References
Assure Tech Antigen Rapid Test Device	15	NPS/OPS	–	–	
BD Veritor System for rapid detection	15	NS	76.3% (*n*=38) 66.4% (*n*=116)	98.8% (*n*=1268)	113 114
BIOHIT Antigen Rapid Test Kit	15		–	–	
BTNX Antigen Rapid Test Cassette	15	NPS/OPS	–	–	
Carestart Antigen/Atomo Antigen Test	10	NS/NPS	Adults 84.8% (71.1–93.7) Children 85.7% (42.1–99.6)	Adults 97.2% (92.0–99.4) Children 89.5% (66.9–98.7)	115 (preprint)
GenBody Ag	15	NPS	–	–	
InnoScreen Antigen Rapid Test Device	15	NS/NPS/OPS/NA	–	–	
NowCheck Antigen Test	15–30	NS/NPS	89.2% (81.7–93.9), *n*=102 55.6% (21.2–86.3), *n*=9 89.9% (81.3–94.8), *n*=79	97.3% (94.8–98.6) 100% (99.7–100), *n*=1317 98.6% (94.9–99.6), *n*=139	9 116 9
Onsite Ag Point of care test	15	NS/NPS	–	–	
Abbott Panbio Ag Rapid Test Device	15–20	NPS	85.5% (78.2–90.6), *n*=124 86.8% (79–92), *n*=106 72.6% (64.5–79.9%) 81.0% (69.0–89.8%) 73.3% (62.2–83.8) Asymptomatic contacts 54.5 % (25–84) 75.5% (69.5–81.5) Asymptomatic patients 45.4% (*n*=22) Asymptomatic contacts 48.1% (37.4–58.9) 79.6% (67.0–88.8%) 90.5% (87.5–93.6), (*n*=325) Children 77.7% (*n*=18) Children 45.4% 71.4% (63.1%, 78.7%) 85.5% (78.0–91.2), (*n*=124) NPS in saline 83% (*n*=158)	100% (99.1–100), (*n*=411) 99.9% (99.4–100), (*n*=1002) 100% (99.7–100%) 100% (99.7–100%) 94.9% (91.2–98.6) 100% (99.3–100) 100% (98.7–100%) 98.8% (98–99.7) Children 100% (*n*=422) Children 99.8% 99.8% (99.4%, 99.9%) 100.0% (99.1–100) 100% (*n*=40)	9 9 61 62 62 57 57 59 60 58 117 66 32 65 118
		NS	86.4% (73.3, 93.9), (*n*=44) 78.9% (*n*=26)	99.2% 99.96% (99.73–100%)	9 69
SD Biosensor Standard Q Ag Test/ Roche Rapid Antigen Test	15–30	NPS	88.7% (*n*=106) 76.6% (*n*=47) 89% (*n*=191) 70.7% (*n*=75) 72.5% (*n*=149) 79.5% (64.5–89.2), (*n*=39) 98.33% (91.06–99.96%), (*n*=60) 70.6% (*n*=109) 71.4% (NPS & OPS) 70.0% (60–79) 89.0% (83.7–93.1), (*n*=191) NPS in saline 81% (*n*=158)	97.6% 99.3% 99.7% 96% (*n*=75) 99.4% 99.6% (97.8–100) 98.73% (97.06–99.59%), (*n*=394) 100% 92% (87–96) 99.7% (98.4–100) 100% (*n*=40)	9 9 9 119 72 71 73 67 74 75 65 118
		NS	74.4% (58.9–85.4), (*n*=29)	99.2% (97.1–99.8)	71
Quidel Sofia Antigen FIA	15	NS	77.0% (*n*=61) 80.0% (*n*=40)	99.6% (*n*=285) 98.9% (*n*=187)	120 121
		NPS	NPS in saline 80% (*n*=158)	100% (*n*=40)	118
VivaDiag Ag Rapid Test	15	NS/OPS	–	–	

NA, nasal aspirate; NPA, negative agreement; NPS, nasopharyngeal swab; NS, nasal swab; OPS, oropharyngeal swab; PPA, positive agreement; TAT, turnaround time; 95% CI, 95% confidence interval.
Nil peer-reviewed literature in PubMed or independent evaluation in FINDdx or FDA reference panel.

Table 4 Foundation for Innovative New Diagnostics (FIND) independent evaluations for TGA listed antigen assays (as of 14 April 2021)

Bionote NowCheck COVID-19 Ag Test		
Brazil, community testing clinic, adults in community meeting national suspect definition Reference method: oropharyngeal swab, lab-developed assay based on the US CDC protocol Antigen test: nasopharyngeal swab		
Symptoms present [%Yes, (n/N)]		100% (382/382)
Days from symptom onset [median (Q1–Q3); N]		4 (3–6), 390
Clinical sensitivity (95% CI), N		89.2% (81.7, 93.9), 102
Sensitivity days ≤7 (95% CI), N		92.2% (84.8, 96.2), 90
Sensitivity Ct ≤33 (95% CI), N		90.8% (82.9, 95.3), 87
Sensitivity Ct ≤25 (95% CI), N		94.3% (84.6, 98.1), 53
Clinical specificity (95% CI), N		97.6% (95.1, 98.8), 288
SD Biosensor STANDARD Q COVID-19 Ag Test		
Country	Germany	Brazil
Reference method	Various: Cobas, Abbott, etc	CDC
Antigen test/PCR	NPS or combined NPS/OPS	NPS
Symptoms present	84.7% (1039/1227)	98.7% (392/397)
Days from symptom onset	3 (2–4); 1002	5 (4–6); 397
Clinical sensitivity (95% CI), N	76.6% (62.8, 86.4), 47	88.7% (81.3, 93.4), 106
Sensitivity days ≤7 (95% CI), N	80.0% (64.1, 90.1), 35	90.7% (83.3, 95.0), 97
Sensitivity Ct ≤33 (95% CI), N	87.8% (74.5, 94.7), 41	91.9% (84.9, 95.9), 99
Sensitivity Ct ≤ 25 (95% CI), N	100% (84.5, 100), 21	95.9% (86.3, 98.9), 49
Clinical specificity (95% CI), N	99.3% (98.6, 99.6), 1212	97.6% (95.2, 98.8), 294
Ease of use 86 out of 100		
Abbott Panbio COVID-19 Ag		
Country	Switzerland	Germany
Reference method	Roche Cobas	various
Antigen test/PCR	NPS	NPS
Symptoms present	99.8% (534/535)	64.5% (709/1100)
Clinical sensitivity (95% CI), N	85.5% (78.2, 90.6), 124	(79, 92), 106
Sensitivity days ≤7 (95% CI), N	85.6% (77.9, 90.9), 111	90.8% (82.2, 95.5), 76
Clinical specificity (95% CI), N	100% (99.1,100), 411	99.9% (99.4, 100), 1002
Ease of use 86 out of 100		

CDC, Centers for Disease Control; NPS, nasopharyngeal swab; OPS, oropharyngeal swab.

and the test duration is 2 hours 32 minutes. No published evaluations are currently available. The LOD was 54,000 NAAT detectable units (NDU)/mL in the FDA Comparative Data (Supplementary Table 1, Appendix A).

Virolens

The Virolens system uses a self-administered mouth swab, which is placed inside a cartridge and inserted into a portable tabletop device. Inside the system is a holographic microscope designed to look at nano-scale structures and the light diffracted off the surface of each cell in the sample. This data is run through a computer trained by artificial intelligence to identify the unique pattern of the virus from other cells. The Virolens system is self-contained and can give a result in 20 seconds. Following the first round of field testing carried out in partnership with London Heathrow Airport, Virolens is about to embark on clinical trials (including Heathrow Airport and Leidos in the USA).[81] The UK Medicines and Healthcare products Regulatory Agency have approved the device and submission has been made to the TGA, however no peer-reviewed performance data are publicly available. Ultra-rapid tests with novel mechanisms of action such as this test may have a unique role in the pandemic, subject to performance evaluations.

Breathalysers

Several countries are at various stages of large clinical evaluations of instruments for breath detection of SARS-CoV-2 infection: examples include GeNose, BreathPass, SpiroNose, Brethonix.[82–90] Detection technologies employed include gas chromatography, different forms of mass spectrometry, and nanosensors to either detect volatile organic compounds or directly detect the virus.[91] There is a paucity of peer-reviewed literature evaluating large-scale clinical performance of the various assays. In May 2021 Singapore's Health Sciences Authority (HSA) granted provisional authorisation for two COVID-19 breathalysers. One of these, the TracieX Breathalyser is reported to have a sensitivity of 95% and specificity of 97.8% and the HSA will continue to closely monitor the clinical performance of these tests as they are deployed locally.[92] However, another Dutch assay was found to have lower specificity of 82% when tested on asymptomatic persons and not long after it was launched its use was halted by Amsterdam's Public Health Service after a concern about sensitivity.[93,94] Assays with good performance would have the potential to rapidly screen large populations as these assays screen individuals within minutes, but assays with high specificity would be more useful in low-prevalence settings such as Australia.

Trained sniffer dogs

A March 2021 World Health Organization (WHO) blueprint has reviewed the global use of sniffer dogs for SARS-CoV-2 detection and found variable sensitivity between 65.4–100% and specificity between 85.2–98%. The blueprint describes that one dog is able to screen 250–300 persons a day. Challenges include the need for standardisation and validation of approaches and difficulties in training dogs in low-prevalence settings.[95]

Environmental testing

Researchers in the Netherlands, France and the USA have demonstrated a correlation between wastewater SARS-CoV-2 concentrations and clinical cases.[96] Some of the results suggested that the RNA concentrations could provide advanced notice of infections (4–7 days) before confirmed cases[96] and retrospective testing has shown that SARS-CoV-2 is present several months before large outbreaks.[97] There is potential to use environmental surveillance for early warning of outbreaks in countries that have already contained transmission but this may be challenged by detection of viral RNA shedding from past infection. In the first confirmed detection of SARS-CoV-2 in untreated wastewater in the Australian community, two positive detections of SARS-CoV-2 RNA were found within a 6-day period from the same wastewater treatment plant.[98] Estimated RNA copy numbers in the wastewater were then used to estimate the number of infected individuals in the catchment via Monte Carlo simulation. As the proportion of infected patients shedding viral RNA in stool is subject to substantial variation (27% in a Chinese cohort, 88% in a German cohort) and given the uncertainty and variation in other input parameters, the model estimated a median range of 171–1090 infected people in the catchment.[98] In view of these limitations, the authors concluded that one of the biggest challenges is to establish predictions from the sewage RNA concentrations to the actual case numbers in the community.

There is a paucity of clinical studies of air sampling for SARS-CoV-2 detection: for example, one recent preprint comparing a commercial air sampler (not TGA listed) to other environmental testing in 32 hospital rooms of COVID-19 positive patients found that among positive rooms, 32% had only active air samples that returned positive results, while ~27% and ~9% had only one or more surface swabs or passive settling plates that returned a positive result respectively; 32% of rooms had more than one sample type that returned a positive result.[99] Therefore, the utility of air sampling in various prevalence contexts relevant to Australia requires further research.[99]

POC ANTIBODY TESTS

The Peter Doherty Institute for Infection and Immunity has so far evaluated 23 different serology-based point of care tests with results on the TGA website. This validation suggests that manufacturers have claimed a better sensitivity compared to that observed in the Doherty Institute studies (when compared to a molecular-based method). The Doherty Institute studies did demonstrate that the sensitivity of most tests improved with increasing duration (i.e., longer time) between sample collection and symptom onset, up to approximately 20–30 days post-symptom onset.[100] The Australian Testing Framework indicates that POC serology tests are not recommended as first line tests for the diagnosis of acute infection and that the role of these tests is uncertain in the context of Australia's broadly low prevalence setting. These recommendations may change with changing prevalence, vaccination rates and border opening.

CONSIDERATIONS FOR 'OUT OF LAB' OR POC TESTING

POC testing may be located at the bedside or may be 'near' POC and performed in a mobile laboratory (e.g., laboratory in a van) or in a 'pop-up' laboratory that can be in a marquee or a local room. For mobile laboratories, the effects of transportation and weather extremes on equipment performance need to be considered. Biosafety requirements will depend on the assay used: biosafety cabinets may be required (in addition to personal protective equipment) particularly if virus in samples is not inactivated or if testing is performed in 'open' instrumentation or may generate aerosols.

Some assay manufacturer's instructions require a short time (e.g., 15 minutes) between sample collection and processing and this will influence the location of POC testing. Some assays are easier to use than others and the number of sample manipulation steps required will also impact testing location, the required training and qualifications of staff.

In-field recording and reporting of results may require dedicated equipment for sample accessioning and result management with capacity to integrate remote devices with laboratory information systems that can facilitate reporting of results to patients, requesting practitioners and public health units.

OVERSEAS EXPERIENCE AND TESTING STRATEGIES

Information detailing population-wide testing strategies for SARS-COV-2 is largely missing from the literature. Overall, a variety of strategies are being considered overseas and the relevance of these to the Australian context is unclear.

Strategies used in high prevalence settings include a Liverpool testing pilot of over 3000 people using the Innova lateral flow antigen test which missed 60% of infected asymptomatic people, including 33% of those with high viral loads. Of note, Public Health England's evaluation of the Innova test showed that its sensitivity was 79.2% when used by trained laboratory scientists, 73% when used by trained healthcare staff, but only 57.5% when used by track and trace centre staff employed by the pharmacy chain Boots.[101,102] Mass antigen testing (of the entire adult population) in Slovakia has generated controversy and a recent modelling study has concluded that while it was impossible to disentangle the precise contribution of infection control measures and mass testing, mass testing is likely to have had a substantial effect in curbing the pandemic.[103,104] A hospital preprocedural surveillance PCR testing study in Seattle found low rates of positivity despite a community-wide outbreak.[105]

The European Centre for Disease Prevention and Control (ECDC) suggests that rapid antigen tests can contribute to overall COVID-19 testing capacity, especially in situations in which RT-PCR testing capacity is limited and the ECDC agrees with the minimum performance requirements set by the WHO at $\geq$80% sensitivity and $\geq$97% specificity.[47,106]

Strategies used in low-prevalence countries include rapid antigen test pre-event testing, recently introduced in Singapore for large gatherings such as tradeshows, live performances, spectator sports and weddings.[107] The effect of this testing strategy will be of interest to other low-prevalence countries such as Australia.[108]

The WHO implementation guide for SARS-CoV-2 rapid antigen tests suggests that appropriate scenarios for their use include outbreak or high-risk scenarios, particularly during widespread community transmission, but only limited use in low-prevalence populations or in asymptomatic individuals.[109]

Australia's testing strategy is guided by the Communicable Diseases Network Australia (CDNA)/Public Health Laboratory Network (PHLN) Testing Framework. The role of each test is dependent on the 'Epidemiological Zone' or phase of the pandemic which takes into account local prevalence and public health objectives of testing in that context.[42] The role of diagnostic tests for symptomatic people (POC molecular, antigen and antibody tests) and screening tests for asymptomatic people (such as breathalysers, wastewater/sewerage surveillance or air sampling) will change depending on the presence and degree of local community transmission as this will affect the predictive value of these tests. For example, The Australian Testing Framework indicates that for Epidemiological Zone 1 (no community transmission) POC RT-PCR tests may be useful in rural and remote communities when rapid TAT is required or that for Epidemiological Zone 3 (community transmission placing burden on response capacity) rapid antigen tests may prove useful as a screening test for individuals in high risk settings where the pre-test probability is high. The Framework recommends that positive antigen tests would require RT-PCR for confirmation. PHLN and CDNA also recommend reflex RT-PCR of suspected COVID-19 cases that return a negative rapid antigen test result. Although for some tests this may require the collection of two swabs, there are emerging data evaluating the performance of using the universal transport media from standard-of-care swabs in the antigen test buffer instead of collecting a second swab.[110]

As the pandemic evolves, with increasing vaccination rates and plans to open Australian borders, the roles of each test will evolve in the context of emerging performance data of the test in each prevalence context.

THE FUTURE OF DIAGNOSTIC TEST DEVELOPMENT

In Australia, the classification of assays as TGA listed or not (Table 2; Supplementary Table 3, Appendix A) will determine their availability for use in Australia. Assays that are not TGA listed but are available overseas, or that have received funding for development, are required to go through an approval process to ensure that minimum quality and performance requirements are met. Various similar processes are in place overseas (e.g., FDA approval for US or CE-marking for Europe). Due to differences in submissions from manufacturers and approval requirements this leads to varying availability of tests between countries.

For example, the US National Institutes of Health (NIH) awarded $248.7 million in contracts to seven diagnostic companies under its first round of the Rapid Acceleration of COVID-19 Diagnostics Initiative.[90] More than 650 applications were submitted and hundreds of experts from government, academia, and industry helped evaluate applications. NIH selected approximately 100 of the best concepts to enter a 1-week evaluation process. Thirty-one of these moved to a 4–6-week period of initial validation. The seven tests to receive this funding were the first to be chosen for scale-up and delivery to the marketplace (Supplementary Table 3, Appendix A). It is hoped that such initiatives may facilitate rapid development and approval of novel diagnostics to enable timely response during emergency scenarios such as the current COVID-19 pandemic.

CONCLUSIONS

New technologies for detection of SARS-CoV-2 are emerging at an unprecedented pace but reliable information on individual assay performance, availability and supply chains is highly variable. Large-scale evaluations of the performance of available assays in the context of intended use are required to inform optimal deployment for control of the pandemic in Australia.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2021.08.001.

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