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Clinical evaluation of AusDiagnostics SARS-CoV-2 multiplex tandem PCR assay

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ABSTRACT

Background: In the context of the pandemic, the rapid emergency use authorisation of diagnostic assays for SARS-CoV-2 has meant there are few peer-reviewed published studies of clinical performance of commercial assays.

Aims: To evaluate the clinical performance of AusDiagnostics respiratory multiplex tandem PCR assay including SARS-CoV-2.

Methods: We reviewed the results following implementation of AusDiagnostics respiratory multiplex tandem PCR assay including SARS-CoV-2, and compared with an in-house RT-PCR assay at our State Reference Laboratory.

Results: Initial validation using AusDiagnostics coronavirus multiplex tandem PCR assay including SARS-CoV-2 demonstrated good concordance with the State Reference Laboratory. After implementing the AusDiagnostics respiratory multiplex tandem PCR assay including SARS-CoV-2, we tested 7839 samples. 127 samples in which SARS-CoV-2 was detected using the AusDiagnostics assay were referred for testing at the State Reference Laboratory, with concordant results in 118/127 (92.9%) of samples. After resolution of discrepancies, 125/127 (98.4%) of AusDiagnostics results were determined to be true positive results. Out of 7839 samples tested for SARS-CoV-2 during this period, only 2 tests (0.02%) were indeterminate results.

Conclusion: The AusDiagnostics respiratory MT-PCR assay is a reliable assay for detection of SARS-CoV-2.

1. Introduction

In December 2019, a novel strain of coronavirus, severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2), was recognized in Hubei Province, China [1–3]. This rapidly spread throughout other countries and was subsequently declared a pandemic by the World Health Organisation (WHO) in March 2020 [4].

The symptoms expressed by COVID-19 patients are non-specific and cannot be used alone for an accurate diagnosis. These symptoms include fever, cough, fatigue, sputum production and shortness of breath, all of which can be associated with many other respiratory infections [5]. Accurate and rapid diagnostics is crucial for clinical management and to allow for epidemiological tracking and rapid implementation of control measures that limit the spread of SARS-CoV-2 through case identification, isolation and contact tracing.

Nucleic acid testing (NAT) using real time polymerase chain reaction (RT-PCR) is the method of choice for detection of SARS-CoV-2 [6]. Specific RT-PCR primer sets to detect SARS-CoV-2 are available [6–8]. In order to rapidly respond to the pandemic needs, regulatory bodies approved Emergency Use Authorization of commercial nucleic acid amplification diagnostic assays [9,10]. However, few large evaluations of clinical performance of these commercial assays have been published [11–13].

Respiratory multiplex PCR testing is an established method for diagnosis of respiratory viral infections. Our laboratory was using the AusDiagnostics Respiratory multiplex PCR, which has comparable

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performance to other commercial and in-house RT-PCR assays for detection of non-SARS-CoV-2 pathogens [14–16].

Rapid commencement of testing for SARS-CoV-2 was required in response to the COVID-19 pandemic. The first reported case of COVID-19 in Australia presented to our health service on 24 January 2020 and SARS-CoV-2 was detected by RT-PCR at the Victorian Infectious Diseases Reference Laboratory [18]. Integration of SARS-CoV-2 testing as part of routine respiratory multiplex PCR testing was our preferred strategy for SARS-CoV-2 diagnosis. AusDiagnostics developed a molecular diagnostic test to detect SARS-CoV-2 and the assay received European CE marking and Australian Therapeutic Goods Administration registration in March 2020 [9,17].

This study evaluated the AusDiagnostics Respiratory multiplex tandem PCR [14,15] including SARS-CoV-2 and compared results to an in-house real-time TaqMan PCR at our State Reference Laboratory [18].

2. Materials and methods

2.1. Setting

Our laboratory services Monash Health, a large metropolitan health care network in Melbourne, Australia with 2150 inpatient beds, three emergency departments and four community respiratory assessment clinics that opened in response to the COVID-19 pandemic.

2.2. Specimen collection

Combined oropharyngeal and nasopharyngeal swabs were collected according to national guidelines [19] using FLOQSwabs® and transported in UTM medium (Copan, Brescia, Italy). Sputum and endotracheal aspirates were also accepted for testing for suspected SARS-CoV-2. Samples were stored at 4 °C before processing.

2.3. Molecular testing

AusDiagnostics (Mascot, Australia) tests utilise a multiplex-tandem polymerase chain reaction (MT-PCR). The assays consist of two amplification steps where the primary amplification involves “target enrichment” using target-specific outer primer sets with a small number of PCR cycles, followed by secondary amplification where inner primers amplify a target region within the product from the primary amplification. Extraction occurred on AusDiagnostics MT-Prep extraction system as per manufacturer’s instructions.

Initial validation (16–19 March 2020) used the AusDiagnostics 8-well coronavirus assay (catalogue number 20081) including SARS-CoV-2 open reading frames ORF1 gene & ORF8 gene targets. The Victorian Infectious Diseases Reference Laboratory provided a proficiency-testing panel that represented a titration series of gamma-irradiated virus isolate diluted 1/1,000–1/256,000.

Subsequent testing (20 March to 17 April 2020) utilised the AusDiagnostics Respiratory Pathogens 12-well assay (catalogue number 80,618) with targets for SARS-CoV-2 (ORF1 gene), influenza A, influenza B, respiratory syncytial virus, human parainfluenza 1–4, rhinovirus/enterovirus, adenovirus, human metapneumovirus and *Bordetella pertussis*. The AusDiagnostics platform uses SYBR Green detection and reports a semi-quantitative result using 1+, 2+ detection up to the maximum of 5+ rather than a cycle threshold (Ct) value. Molecular target concentrations, expressed as arbitrary units, are calculated relative to the internal control SPIKE, which amplifies a known amount of target molecules.

The AusDiagnostics assay was compared to SARS-CoV-2 in-house real-time TaqMan PCR performed at the Victorian Infectious Diseases Reference Laboratory [18]. RNA extraction was performed at the reference laboratory using the QIAamp 96 Virus QIAcube HT Kit (Qiagen, Hilden, Germany) and the genetic target of was the RNA-dependent RNA polymerase (RdRP) gene. Further testing of discordant samples was undertaken using assays targeting the envelope (E), nucleocapsid (N) and spike (S) genes.

Results discordant between the two assays were further assessed by analysing results from other specimens from the same patient. Amplicons generated from discordant samples were referred to AusDiagnostics for pyrosequencing. All samples from patients that had SARS-CoV-2 detected by more than one assay from any sample or by pyrosequencing were determined to be true positive results. A sample from a patient that was positive by only one RT-PCR assay and had no specific product detected by pyrosequencing was determined to be an indeterminate result if no other samples from that patient were determined to be a true positive result.

2.4. Data collection

A review of microbiological and medical records of patients tested for SARS-CoV-2 between March 16, 2020 and April 17, 2020 was performed. Patients were tested for suspected SARS-CoV-2 if they met criteria set out by national guidelines. This included clinical criteria (fever ≥38 °C) or history of fever (e.g. night sweats, chills) OR acute respiratory infection (e.g. cough, shortness of breath, sore throat) AND epidemiological criteria including close contact with a known case, interstate or overseas travel in the previous 14 days or being a health care worker with direct patient contact [20].

All patients positive for SARS-CoV-2 were notified to the Victorian Department of Health and information collected included age, sex, presentation, place of sample collection and epidemiological risk factors including overseas travel within the previous 14 days, contact with a known COVID-19 case and if they were a healthcare workers. For patients who presented to hospital, notes were reviewed for epidemiological risk factors, requirement for admission, intensive care admission, mechanical ventilation and mortality.

2.5. Ethics approval

Low risk Quality Assurance approval as defined by the National Statement on Ethical Conduct in Human Research was obtained from Monash Health Human Research Ethics Committee (reference number RES-20-0000260Q-63945.)

3. Results

In our initial validation of the AusDiagnostics MT-PCR including SARS-CoV-2, we did not detect SARS-CoV-2 in 59 of 60 (98.3%) samples were negative for SARS-CoV-2 at the reference laboratory. The one discordant sample was from a patient with characteristic clinical and radiological features of COVID-19 who had returned from travel in Iran. SARS-CoV-2 was detected at the reference laboratory from a repeat specimen collected the following day. (See Table 1, Patient A.)

The AusDiagnostics assay including the SARS-CoV-2 target achieved 100% concordance of results using the proficiency-testing panel with blinded samples.

Following the initial validation, the AusDiagnostics Respiratory Pathogens multiplex assay including SARS-CoV-2 was implemented and 7839 samples were tested during the one-month study period.

The most common respiratory pathogen identified was rhinovirus/enterovirus with 1049 positive samples (13.4%), followed by parainfluenza 1 (1.6%), parainfluenza 4 (1.2%), adenovirus (1.2%) and RSV (0.8%). 24 samples were positive for influenza A (0.3%) and 3 for influenza B (0.04%). Parainfluenza 2 and 3, human metapneumovirus and *B. pertussis* were detected in ≤0.5% of samples.

SARS-CoV-2 was detected in 127 samples (1.6%) from 116 patients. Median age was 44 years (IQR 27–58 years), 56% were female. Most (88%) presented to the community respiratory assessment clinics, with 12% from emergency department presentations. 54% of patients had a history of overseas travel in the previous 14 days, 57% had contact with

Table 1
Summary of discordant SARS-CoV-2 results.

	Date	AusDx result	Repeat AusDx result	Reference laboratory result	Pyrosequencing result	Risk factors	Resolution
A[a]	*6 March*	*DET*	–	*NDET (RdRP)*	–	*Travel history*	*True positive*
	7 March	–	–	*DET (RdRP and E)*			
1	16 March	DET	DET 1+	NDET (RdRP and E)	*SARS-CoV-2*	Travel history Known contact	True positive
2	25 March	DET 1+	NDET	NDET 4 genes[b]	Insufficient amplicon for sequencing	Travel history Known contact	Indeterminate
3	30 March	DET 2+	DET 2+	NDET 4 genes	*SARS-CoV-2*	Travel history	True positive
	4 April	DET 2+	DET 2+	DET			
4	31 March	DET 1+	NDET	NDET 4 genes	*SARS-CoV-2*	Healthcare worker	True positive
	13 April	NDET	–	–			
5	2 April	DET 1+	NDET	NDET 4 genes	*SARS-CoV-2*	Healthcare worker	True positive
	6 April	NDET	–	–			
	10 April	–	–	NDET (RdRP)			
	12 April	NDET	–	–			
6	6 April	DET 1+	NDET	NDET 4 genes	*SARS-CoV-2*	Healthcare worker	True positive
	7 April	NDET		–			
	9 April	NDET	–	–			
	13 April	NDET	–	–			
7	12 April	DET 1+	NDET	NDET 4 genes	Insufficient amplicon for sequencing	ICU patient nil risk factors	Indeterminate
	13 April	NDET	–	–			
	15 April	NDET	–	–			
8	13 April	DET 1+	–	NDET 4 genes	–	Healthcare worker	True positive
	19 April	DET	–	*DET*			
9	13 April	DET 1+	–	NDET 4 genes	–	Healthcare worker	True positive
	19 April	DET	–	*DET*			

Combination of oropharyngeal and nasopharyngeal swabs.
DET – detected, NDET – not detected.
[a] Patient A – was tested as part of test validation prior to commencement of the study period.
[b] 4 genes = RdRP, E, N and S genes.

a known positive case and 14% were healthcare workers. Less than 15% of cases required admission, however 40% of admitted patients required intensive care admission with half of those requiring mechanical ventilation. Three patients died during the admission, two of whom were > 75 years of age and were not admitted to the intensive care unit.

SARS-CoV-2 was detected at the reference laboratory in 118/127 (92.9%) specimens in which SARS-CoV-2 was detected using the AusDiagnostics assay.

Nine tests (7.1%) had discordant results with all being a positive SARS-CoV-2 result on the AusDiagnostics assay and a negative result on the reference laboratory assay. Table 1 summarises the nine discordant results during the study period. Two of these tests were repeat swabs for health care workers undergoing clearance swabs to return to work (patients 8 & 9). They both subsequently had a further positive test on repeat swabs at the reference laboratory and therefore were classified as true positive results. Pyrosequencing demonstrated that five out of the nine discordant results were positive for SARS-CoV-2 RNA (patients 1, 3, 4, 5 and 6.) Therefore, seven of nine (77.8%) discordant results were classified as true positives.

Two patients were classified as having indeterminate results as there was insufficient amplicon for pyrosequencing and no further positive samples. Thus, only two of 7839 (0.02%) samples tested for SARS-CoV-2 were indeterminate results.

4. Discussion

Laboratories have been required to implement new testing platforms rapidly to respond to the SARS-CoV-2 public health crisis. This study demonstrates that the AusDiagnostics MT-PCR assay is a reliable assay for detection of SARS-CoV-2 with a high level of correlation with the reference laboratory in-house RT-PCR. The AusDiagnostics multiplex assay performed well when rapidly implemented in a busy hospital laboratory, also allowing the benefit of detection of other respiratory pathogens. This is the largest study of a non-manufacturer sponsored review of a commercial assay for SARS-CoV-2 and supports its use in this pandemic [13,21–23].

Several assays targeting different regions of the SARS-CoV-2 genome have been proposed but there is still limited data as to the performance of the various assays. This study provides evidence for the use of the AusDiagnostics MT-PCR for the detection of SARS-CoV-2 using the ORF1 gene. There was excellent concordance with a real-time TaqMan PCR performed at the reference laboratory.

Several of the discordant results in our study did demonstrate detection of SARS-CoV-2 on the AusDiagnostics assay prior to subsequent detection on the reference laboratory assay indicating similar performance of the two assays around the limit of detection. Performance of the AusDiagnostics assay on the proficiency panel serial dilutions indicated high analytic sensitivity. Furthermore, any person with ongoing symptoms during this study period who fulfilled testing criteria was encouraged to have a repeat test as per national guidelines [19] in order to minimise the risk of false negative results and no further positive patients were identified.

After resolution of discrepancies by pyrosequencing or incorporation of other results from the patients, only two of 7839 (0.02%) samples tested for SARS-CoV-2 were indeterminate results, as there was insufficient amplicon for pyrosequencing and no further positive samples. There are multiple possible reasons for discordant results. This includes RNA degradation (for example, during transport or storage between the two assays being performed), differences in genetic targets, primer sequences, reagents, or amplification conditions. The two assays target different SARS-CoV-2-specific genes, which could lead to false negative results if a mutation prevents primer binding. Natural variability at the limit of detection of PCR assays may also be explained by sampling differences. Several of the samples in our study were from patients having clearance swabs collected at least a week after symptom onset, thus the viral load would be expected to be at or near the limit of detection for many of these samples.

As with all nucleic acid amplification assays there is the possibility of contamination and this may explain the two samples with

indeterminate results since these were both detected at low level and not confirmed on repeat testing. In particular one patient (patient 7) had no clear epidemiological risk factors for SARS-CoV-2 and the virus was not detected in two other specimens from this patient, however there were no other positive patient samples on the initial runs that included these two patient's indeterminate results.

The large number of clinical specimens tested (7839) with only two potential false positive samples indicates high specificity of the AusDiagnostics assay.

5. Conclusion

The AusDiagnostics SARS-CoV-2 PCR assay is a reliable assay for detection of SARS-CoV-2. It allows for the integration of a multiplex respiratory PCR assay in the SARS-CoV-2 response, aiding with the detection of other respiratory pathogens as well as SARS CoV-2.

CRediT authorship contribution statement

Lucy O Attwood: Investigation, Writing - original draft, Visualization. **Michelle J Francis:** Conceptualization, Methodology, Validation, Investigation. **John Hamblin:** Resources, Writing - review & editing. **Tony M Korman:** Writing - review & editing, Supervision. **Julian Druce:** Writing - review & editing. **Maryza Graham:** Conceptualization, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2020.104448.

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