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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VIROLOGY

Proficiency testing for SARS-CoV-2 in assuring the quality and overall performance in viral RNA detection in clinical and public health laboratories

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Summary

Diagnostic testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has undergone significant changes over the duration of the pandemic. In early 2020, SARS-CoV-2 specific nucleic acid testing (NAT) protocols were predominantly in-house assays developed based on protocols published in peer reviewed journals. As the pandemic has progressed, there has been an increase in the choice of testing platforms. A proficiency testing program for the detection of SARS-CoV-2 by NAT was provided to assist laboratories in assessing and improving test capabilities in the early stages of the pandemic. This was vital in quality assuring initial in-house assays, later commercially produced assays, and informing the public health response. The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) offered three rounds of proficiency testing for SARS-CoV-2 to Australian and New Zealand public and private laboratories in March, May, and November 2020. Each round included a panel of five specimens, consisting of positive (low, medium or high viral loads), inconclusive (technical specimen of selected SARS-CoV-2 specific genes) and negative specimens. Results were received for round 1 from 16, round 2 from 97 and round 3 from 101 participating laboratories. Improvement in the accuracy over time was shown, with the concordance of results in round 1 being 75.0%, in round 2 above 95.0% for all samples except one, and for round 3 above 95.0%. Overall, participants demonstrated high capabilities in detecting SARS-CoV-2, even in samples of low viral load, indicating excellent testing accuracy and therefore providing confidence in Australian and New Zealand public and private laboratories test results.

Key words: Quality assurance; SARS-CoV-2; viral detection; proficiency testing.

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INTRODUCTION

An outbreak of pneumonia of unknown cause detected in Wuhan, Hubei province, People's Republic of China was first reported to the World Health Organization (WHO) Country Office in China on 31 December 2019. Initially confined to Hubei and other parts of the People's Republic of China, the infection spread over January 2020 and involved all continents of the world (except Antarctica) by February 2020.

At the initial stage of the pandemic, a list of protocols for the detection of SARS-CoV-2 was shortlisted in the document available on the WHO website.[1] These were developed in various countries, including China, France, the USA, Japan, Germany, Hong Kong and Thailand. There was little information on the performance of these protocols; none of these had been validated through a WHO process nor endorsed by the WHO. As the pandemic has progressed, there has been a significant increase in the choice of commercially available kits or testing platforms used to test for the presence of SARS-CoV-2. This has highlighted the importance of an external proficiency testing program (PTP), as described here, to assess the accuracy and reliability of test results and to evaluate the performance of laboratories.

In response to the rapid global spread of the COVID-19 outbreak and the increased need for introduction of SARS-CoV-2 testing, the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) developed a PTP for the detection of SARS-CoV-2 by nucleic acid test. Three rounds of this PTP were offered on 4 March, 12 May, and 10 November 2020 to public health and private clinical laboratories across Australia and New Zealand. In this report, we capture a snapshot of the SARS-CoV-2 diagnostic outlook from the start of the pandemic and how it changed as the pandemic progressed in terms of the performance of the laboratories and the assays.

MATERIAL AND METHODS

Organisation and participating laboratories

Round 1 of the SARS-CoV-2 PTP was offered by the RCPAQAP in March 2020 to 12 Australian public health and private clinical laboratories, as well as four New Zealand laboratories, as approved by the Australian Government Department of Health. Participation in rounds 2 and 3 of the PTP was open to any Australian laboratory. A total of 85 and 94 Australian, as well as 12 and seven approved New Zealand laboratories, participated in rounds 2 and 3, respectively.

Survey specimens and survey instructions

Participating laboratories were supplied with a specimen panel consisting of five samples, including positive (low, medium or high viral loads), inconclusive

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(technical specimen of selected SARS-CoV-2 specific genes) and negative SARS-CoV-2 specimens as shown in Table 1. All samples were confirmed stable over the period of the PTP (a maximum of 14 days) and homogenous using an in-house process,[2] and an external laboratory validated the content of each sample before dispatch. Samples in the specimen panel for round 1, and sample S8 in round 2 were prepared by adding a total of 8 μL of 25X RNAsecure Reagent (Catalog number AM7005; Ambion, Life Technologies, USA), and the final volume was adjusted to 200 μL using RNase-free water. The mixtures were incubated at 60°C for 10 min to ensure complete inactivation of any contaminating RNases. These mixtures were then frozen and lyophilised, with the samples being dispatched in lyophilised form. Participants were instructed to reconstitute lyophilised samples in 500 μL (1.5 mL for sample S8) RNase-free water upon receipt. All other specimens included in panels prepared for rounds 2 and 3 were prepared by adding AssayAssureMultilock (Sierra-Molecular Corporation, USA) to the diluted SARS-CoV-2 or human coronavirus 229E (hCoV-229E, used in round 3) gamma-irradiated, inactivated culture supernatant. The final volume of these specimens was adjusted to 1.5 mL using 0.5% gelatin/phosphate buffered saline (PBSG). Participating laboratories in all three rounds of the PTP received the survey specimen panels within 24–48 hours, depending on their location. Participants were instructed to perform NAT on all specimens in the same manner as clinical specimens, using their established protocols, and report results within a 1-week (for round 1) and 2-week (for rounds 2 and 3) timeframe.

Reporting of PT results

All laboratories were required to return the electronic result sheet (round 1) or to report their results using an online data entry portal (rounds 2 and 3). Participants in all rounds of this PTP were requested to report cycle threshold (Ct) values and associated results (detected, not detected, equivocal or not tested) for SARS-CoV-2 and other unspecified gene targets included in their testing protocols. They were to provide details of their extraction method, the amplification and detection kit and the PCR system used and include any other comments or additional tests (if applicable). Further information related to the type of NAT assay used to perform the test (primers/probe designed in-house, primers/probe designed externally or commercial kit) was captured in round 1. As for rounds 2 and 3, additional information was collected, including extraction kit lot number, extraction and elution volumes, the amplification/detection kit lot number, master mix and sample volumes, whether a single round or nested PCR was performed, whether the inhibitor, negative and positive controls were used, the inhibitor control lot number and details of in-house assay primers/probe design.

Participants in round 1 were required to rule out or confirm the presence of SARS-CoV-2 and to report all available NAT assays (and not separate results for each assay) used in detecting SARS-CoV-2. This section was expanded in rounds 2 and 3, and participants were given the option to indicate the final interpretation whether a specimen was: (1) SARS-CoV-2 positive; (2) SARS-CoV-2 negative; (3) SARS-CoV-2 presumptive positive (as indicated by the testing platform, only some SARS-CoV-2 specific-gene was detected); (4) inconclusive (as indicated by the testing platform); (5) invalid (as indicated by the testing platform); or (6) not tested. Participants in rounds 2 and 3 could report results from more than one testing platform.

Assessment of PT results

Results returned in round 1 were not assessed individually at the close of round 1 due to the limitation of the participant reporting process using an electronic result sheet. However, these results were re-analysed and re-assessed manually for this study, using an in-house grading process. Assessment of the results recorded from the online data entry portal in rounds 2 and 3 was performed automatically by RCPAQAP's online data management system. The grading for the final interpretation of each sample is described here, in comparison to the final interpretation determined in-house: (1) concordant result: result consistent with the in-house result; (2) discordant result: result inconsistent with the in-house result. The definition of discordant results was different across each sample, depending on the type of specimen. The grading for each round is available in Table 2.

Participant report

After each round, participating laboratories were provided with a report. The report issued after round 1 of this PTP consisted of a summary of results submitted for each sample, a summary of the most commonly used extraction methods, amplification kits and PCR systems. Results reported for individual specimens were discussed, and participants were provided with details of the protocol used to prepare the survey specimen panel. Reports that were distributed to participants in rounds 2 and 3 were individual reports. They included a list of the method used by participants, and a performance assessment of individual laboratories, with an independent assessment of each sample in the survey specimen panel. Participant results were marked as concordant, discordant, or not assessed. The report contained a statement on the overall performance, a request to review results for samples with discordant results (if any), and a cumulative assessment for rounds 2 and 3.

Table 1 List of specimen panels for round 1, 2 and 3 of SARS-CoV-2 proficiency testing program

Round	RCPAQAP sample ID	Type of specimen	Copy number (copies per mL)	NAT SARS-CoV-2 specific genes	Final interpretation
1 (Mar 2020)	S1	Diluted SARS-CoV-2 (high viral load)	8.5×10^7	All positive	SARS-CoV-2 positive
	S2	Synthetic DNA fragment of SARS-CoV-2 N & E genes	4.5×10^7	N & E positive	SARS-CoV-2 presumptive positive
	S3	Negative sample (diluted MDCK cells)	NA	All negative	SARS-CoV-2 negative
	S4	Synthetic DNA fragment of SARS-CoV-2 N & E genes	4.3×10^2	N & E positive	SARS-CoV-2 presumptive positive
	S5	Synthetic DNA fragment of SARS-CoV-2 N & E genes	2.1×10^4	N & E positive	SARS-CoV-2 presumptive positive
2 (May 2020)	S6	Diluted SARS-CoV-2 (medium viral load)	5.0×10^5	All positive	SARS-CoV-2 positive
	S7	Diluted SARS-CoV-2 (high viral load)	3.5×10^7	All positive	SARS-CoV-2 positive
	S8	Synthetic DNA fragment of SARS-CoV-2 E gene	3.4×10^7	E positive	SARS-CoV-2 presumptive positive
	S9	Negative sample (diluted MDCK cells)	NA	All negative	SARS-CoV-2 negative
	S10	Diluted SARS-CoV-2 (low viral load)	9.4×10^3	All positive	SARS-CoV-2 positive
3 (Nov 2020)	S11	Negative sample (diluted MDCK cells)	NA	All negative	SARS-CoV-2 negative
	S12	Diluted hCoV-229E (medium viral load)	6.3×10^4	All negative	SARS-CoV-2 negative
	S13	Diluted hCoV-229E (high viral load)	3.4×10^6	All negative	SARS-CoV-2 negative
	S14	Diluted SARS-CoV-2 (low viral load)	5.4×10^3	All positive	SARS-CoV-2 positive
	S15	Diluted SARS-CoV-2 (medium viral load)	3.0×10^5	All positive	SARS-CoV-2 positive

E, envelope; N, nucleocapsid; NA, not applicable.

RESULTS

Demographics of participating laboratories

Participants in rounds 2 and 3 could submit results for more than one testing platform. The total result entries submitted were as follows; round 1 (n=16), round 2 (n=145) and round 3 (n=175). Australian participants made up a total of 12, 124 and 160 result entries, while the total of result entries from New Zealand participants were 4, 21 and 15.

Performance assessment

Participants' performance was assessed based on the results reported for each specimen, which was marked as either concordant, discordant or not assessed, as summarised in Table 3. The average total concordance for each round was considered independently, based on the results reported for all five specimens. The average total concordance in round 1 was low at 75.0%. However, the performance of participants improved in subsequent rounds, with total concordance above 95.0% on average for all samples in round 2, except S8, which represented a SARS-CoV-2 presumptive positive

Table 2 Grading of results for rounds 1, 2 and 3 of the SARS-CoV-2 proficiency testing program

Round	RCPAQAP Sample ID	Concordant	Discordant	Not assessed
1 (Mar 2020)	S1	SARS-CoV-2 positive	SARS-CoV-2 negative SARS-CoV-2 presumptive positive Inconclusive	Invalid Not tested
	S2	SARS-CoV-2 positive SARS-CoV-2 presumptive positive Inconclusive	N/A	SARS-CoV-2 negative (recorded either SARS-CoV-2 N or E genes detected) Invalid Not tested
	S3	SARS-CoV-2 negative	SARS-CoV-2 positive SARS-CoV-2 presumptive positive Inconclusive	Invalid Not tested
	S4	SARS-CoV-2 positive SARS-CoV-2 presumptive positive Inconclusive	SARS-CoV-2 negative (recorded either SARS-CoV-2 N or E genes not detected)	Invalid Not tested
	S5	SARS-CoV-2 positive SARS-CoV-2 presumptive positive Inconclusive	SARS-CoV-2 negative (recorded either SARS-CoV-2 N or E genes not detected)	Invalid Not tested
2 (May 2020)	S6	SARS-CoV-2 positive	SARS-CoV-2 negative SARS-CoV-2 presumptive positive Inconclusive	Invalid Not tested
	S7	SARS-CoV-2 positive	SARS-CoV-2 negative SARS-CoV-2 presumptive positive Inconclusive	Invalid Not tested
	S8	SARS-CoV-2 presumptive positive Inconclusive	SARS-CoV-2 positive	SARS-CoV-2 negative Invalid Not tested
	S9	SARS-CoV-2 negative	SARS-CoV-2 positive SARS-CoV-2 presumptive positive Inconclusive	Invalid Not tested
	S10	SARS-CoV-2 positive	SARS-CoV-2 negative SARS-CoV-2 presumptive positive Inconclusive	Invalid Not tested
3 (Nov 2020)	S11	SARS-CoV-2 negative	SARS-CoV-2 positive SARS-CoV-2 presumptive positive	Inconclusive (automated result generated by NAT assay)[a] Invalid Not tested
	S12	SARS-CoV-2 negative	SARS-CoV-2 positive SARS-CoV-2 presumptive positive	Inconclusive (automated result generated by NAT assay)[a] Invalid Not tested
	S13	SARS-CoV-2 negative	SARS-CoV-2 positive SARS-CoV-2 presumptive positive	Inconclusive (automated result generated by NAT assay)[a] Invalid Not tested
	S14	SARS-CoV-2 positive	SARS-CoV-2 negative SARS-CoV-2 presumptive positive Inconclusive	Invalid Not tested
	S15	SARS-CoV-2 positive	SARS-CoV-2 negative SARS-CoV-2 presumptive positive Inconclusive	Invalid Not tested

[a] Inconclusive and invalid results reported by participants were based on a nucleic acid test (NAT) assay that included an internal control, i.e., tested for the presence of RNaseP. The lack of RNaseP target resulted in an inconclusive or invalid result. These results were either manually interpreted by the participant or reported as unresolved by the system. Other participants using similar assays had disregarded the results for the RNaseP while acknowledging the potential of inadequate or lack of human cellular material in a proficiency testing material. Some participants who performed testing on the BD MAX platform commented that while the automated result generated by the system of the platform was invalid, their submitted results were manually interpreted and reported as SARS-CoV-2 negative as the system requires the presence of RNase-P in the survey specimen to automatically interpret the results.

(inconclusive) sample, part of the submitted results of which were not assessed. The average total concordance for all specimens included in round 3 was also above 95.0%.

Most participants in round 1 reported a concordant result for all samples, except samples S4 and S5 (Table 3). In round 2, sample S8 (SARS-CoV-2 presumptive positive sample) recorded low concordance. Only 58.6% of all results reported for S8 were concordant, 29.7% were not assessed, and the remaining 11.7% of results were discordant. In contrast, none of the samples in round 3 recorded low concordance (Table 3). The details of the concordance, discordance and not assessed results are available in Fig. 1. The participants' performance was analysed by specimen type, i.e., SARS-CoV-2 positive or negative samples (Fig. 2). While results for SARS-CoV-2 negative samples in round 1 (S3) and round 3 (S11) were 100% concordant, there were a few discordant results (n=6) (Fig. 1) for this sample in round 2 (S9). Similarly, there were a small number of discordant results in round 2 (S7) in comparison with round 1 (S1) for the SARS-CoV-2 positive sample (high viral load). Comparison of the SARS-CoV-2 positive sample (medium viral load) also revealed an increase in discordant results in round 3 (S15) compared to that of round 2 (S6). However, in round 3 we saw a slight improvement in the testing of the sample with low viral load, with more participants reporting concordant results in this sample (S14), in comparison with round 2 (S10).

Reporting of discordant results

False negative results with high discordance were reported in round 1 for samples S4 (62.5%) and S5 (31.3%) (Fig. 1). Both samples represented SARS-CoV-2 presumptive positive (inconclusive) samples with low and medium copies of SARS-CoV-2 N and E genes, respectively. Participants who recorded false negative results had tested these samples with NAT assays specific for either SARS-CoV-2 N or E genes and had incorrectly returned negative results.

All five samples in the round 2 specimen panel had recorded discordant results. False negative results were recorded for the SARS-CoV-2 positive sample (low viral load), S10 (3.5%), while false positive results were recorded for the SARS-CoV-2 presumptive positive (inconclusive) sample, S8 (11.7%). Amongst all 15 samples, S8 was the only sample with false positive results. Discordant results were also recorded in another two SARS-CoV-2 positive samples (medium and high viral loads, respectively), S6 and S7; these samples were incorrectly reported as SARS-CoV-2 presumptive positive (1.4%), while invalid results were also reported for S6 (0.7%). The negative sample, S9 in round 2, was also incorrectly reported as inconclusive (2.8%) and invalid (1.4%).

The lowest percentage of discordant results across all samples was recorded in the round 3 specimen panel. Only 2.9% of participants in this round incorrectly reported a SARS-CoV-2 presumptive positive result for the two SARS-CoV-2 positive samples S14 and S15.

Testing platforms

In round 1 of the SARS-CoV-2 PTP, the 16 participating laboratories used 26 assays for the detection of SARS-CoV-2, with the majority of participants (n=15) having implemented assays that were developed externally. A single testing platform was used by 62 and 59 laboratories that participated in rounds 2 and 3, respectively. Results from multiple platforms were reported from 32 participants in round 2 and 38 participants in round 3, including two (n=16 and n=17), three (n=14 and n=10), four (n=1 and n=4), five (n=1 and n=6) and six (n=1 in round 3 only). Results from assays that detected only a single SARS-CoV-2 target were reported by 12.5%, 4.8% and 16.6% of participants that submitted results for round 1, 2 and 3, respectively. Testing in round 2 was predominantly performed using commercial kits or platforms, with 77.9% of participants reporting the use of commercial kits or platforms. In-house assays and other unspecified assays were used by 17.2% and 4.8% of participants, respectively. The NAT assays most commonly used in round 3 were commercially available kits or platforms (92.6%), while only 7.4% of participants reported results performed using in-house developed assays. The data are summarised in Fig. 3.

The majority of participants in round 1 used a testing protocol published by Corman *et al.*[3] (n=13), followed by NAT assays developed in-house (n=6). The Roche LightMix Modular SARS kit (TIB Molbiol, Germany) was used by three participants and two participants used the AusDiagnostics Coronavirus Typing Panel. Other published external protocols that participants used included the study by Chu *et al.*,[4] (n=1)

Table 3 Total concordant, discordant and not assessed results in rounds 1, 2 and 3 of the SARS-CoV-2 proficiency testing program

Round	RCPAQAP Sample ID	Final interpretation	Total concordance *n* (%)	Total discordance *n* (%)	Total not assessed *n* (%)
1 (Mar 2020)	S1	SARS-CoV-2 positive	16 (100)	–	–
	S2	SARS-CoV-2 presumptive positive	13 (81.3)	–	3 (18.7)
	S3	SARS-CoV-2 negative	16 (100)	–	–
	S4	SARS-CoV-2 presumptive positive	5 (31.2)	10 (62.5)	1 (6.3)
	S5	SARS-CoV-2 presumptive positive	10 (62.5)	5 (31.2)	1 (6.3)
2 (May 2020)	S6	SARS-CoV-2 positive	140 (96.5)	3 (2.1)	2 (1.4)
	S7	SARS-CoV-2 positive	141 (97.2)	2 (1.4)	2 (1.4)
	S8	SARS-CoV-2 presumptive positive	85 (58.6)	17 (11.7)	42 (29.7)
	S9	SARS-CoV-2 negative	135 (93.1)	6 (4.1)	4 (2.8)
	S10	SARS-CoV-2 positive	136 (93.8)	5 (3.4)	4 (2.8)
3 (Nov 2020)	S11	SARS-CoV-2 negative	166 (94.9)	–	9 (5.1)
	S12	SARS-CoV-2 negative	167 (95.4)	–	8 (4.6)
	S13	SARS-CoV-2 negative	168 (96.0)	–	7 (4.0)
	S14	SARS-CoV-2 positive	167 (95.4)	5 (2.9)	3 (1.7)
	S15	SARS-CoV-2 positive	167 (95.4)	5 (2.9)	3 (1.7)

Round	RCPAQAP Sample ID	Final interpretation	SARS-CoV-2 positive *n* (%)	SARS-CoV-2 negative *n* (%)	SARS-CoV-2 presumptive positive *n* (%)	Inconclusive *n* (%)	Invalid *n* (%)	Not tested *n* (%)
1 (Mar 2020)	S1	SARS-CoV-2 positive	16 (100)	–	–	–	–	–
	S2	SARS-CoV-2 presumptive positive	9 (56.2)	2 (12.5)	–	4 (25.0)	–	1 (6.3)
	S3	SARS-CoV-2 negative	–	16 (100)	–	–	–	–
	S4	SARS-CoV-2 presumptive positive	3 (18.7)	10 (62.5)	–	2 (12.5)	–	1 (6.3)
	S5	SARS-CoV-2 presumptive positive	7 (43.8)	5 (31.2)	–	3 (18.7)	–	1 (6.3)
2 (May 2020)	S6	SARS-CoV-2 positive	140 (96.5)	-	2 (1.4)	–	1 (0.7)	2 (1.4)
	S7	SARS-CoV-2 positive	141 (97.2)	–	2 (1.4)	–	–	2 (1.4)
	S8	SARS-CoV-2 presumptive positive	17 (11.7)	37 (25.5)	77 (53.1)	8 (5.5)	4 (2.8)	2 (1.4)
	S9	SARS-CoV-2 negative	–	135 (93.0)	–	4 (2.8)	2 (1.4)	4 (2.8)
	S10	SARS-CoV-2 positive	133 (91.7)	5 (3.4)	3 (2.1)	–	–	4 (2.8)
3 (Nov 2020)	S11	SARS-CoV-2 negative	–	166 (94.8)	–	1 (0.6)	4 (2.3)	4 (2.3)
	S12	SARS-CoV-2 negative	–	167 (95.4)	–	1 (0.6)	3 (1.7)	4 (2.3)
	S13	SARS-CoV-2 negative	–	168 (96.0)	–	1 (0.6)	3 (1.7)	3 (1.7)
	S14	SARS-CoV-2 positive	167 (95.4)	–	5 (2.9)	–	–	3 (1.7)
	S15	SARS-CoV-2 positive	167 (95.4)	–	5 (2.9)	–	–	3 (1.7)

Fig. 1 Performance of participants in rounds 1, 2 and 3 of the SARS-CoV-2 proficiency testing program. Green, concordant result; yellow, not assessed; orange, discordant result.

and United States Centers for Disease Control and Prevention[5] (*n*=1) (Supplementary Table 1, Appendix A).

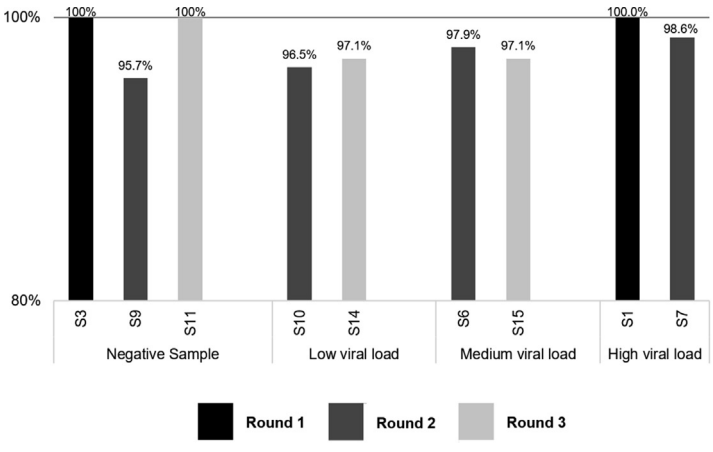
In round 2, the top five most frequently used assay were the Cepheid GeneXpert Xpert Xpress SARS-CoV-2 (*n*=47), followed by in-house NAT assays (*n*=25), Seegene Allplex 2019-nCoV (*n*=14), AusDiagnostics SARS-CoV-2, Influenza and RSV (8-Well) (*n*=13) and Roche Diagnostics Cobas SARS-CoV-2 (*n*=13). In round 3, the top five most frequently used assays were the Cepheid GeneXpert Xpert Xpress SARS-CoV-2 (*n*=68), followed by Seegene Allplex 2019-nCoV (*n*=14), in-house NAT assays (*n*=13), Roche Diagnostics Cobas SARS-CoV-2 (*n*=11) and AusDiagnostics SARS-CoV-2, Influenza and RSV (8-Well) (*n*=8).

Performance of commercial kit/platform

Overall performance (concordant, discordant and not assessed results) of rounds 2 and 3 participants using the top five commercial kit/platforms is summarised in Table 4. This assessment was not performed for round 1, due to the low number of participants using commercial kits. The majority of the listed top five commercial kits/platforms used in round 2 recorded high concordance (above 90%) across the entire specimen panel. The Seegene Allplex 2019-nCoV kit recorded only 69.2% of concordance, followed by Roche Diagnostics Cobas SARS-CoV-2 (81.8%) for sample S8 (SARS-CoV-2 presumptive positive or inconclusive sample). All the top five commercial kits/platforms used to detect SARS-CoV-2 in round 3 recorded 100% concordance across the entire specimen panel, except Cepheid GeneXpert Xpert Xpress SARS-CoV-2, which scored a 94% concordance for both SARS-CoV-2 positive samples, S14 and S15.

DISCUSSION

Participation in the PTP is fundamental for laboratory accreditation and critical where new tests are rapidly introduced, particularly using in-house assays. The performance of laboratories in PTP has become a crucial and objective indicator of the testing quality in the clinical setting.[6–8] At the beginning of the SARS-CoV-2 pandemic, little was known about the efficiency,



Type of specimen	RCPAQAP Sample ID	Copy number (copies per ml)
SARS-CoV-2 Negative Sample	S3	NA
	S9	NA
	S11	NA
SARS-CoV-2 Positive Sample (Low Viral Load)	S10	9.4×10^3
	S14	5.4×10^3
SARS-CoV-2 Positive Sample (Medium Viral Load)	S6	5.0×10^5
	S15	3.0×10^5
SARS-CoV-2 Positive Sample (High Viral Load)	S1	8.5×10^7
	S7	3.5×10^7

Fig. 2 Comparison of concordant results for SARS-CoV-2 negative and positive (low, medium and high viral loads) samples in rounds 1, 2 and 3 of the SARS-CoV-2 proficiency testing program.

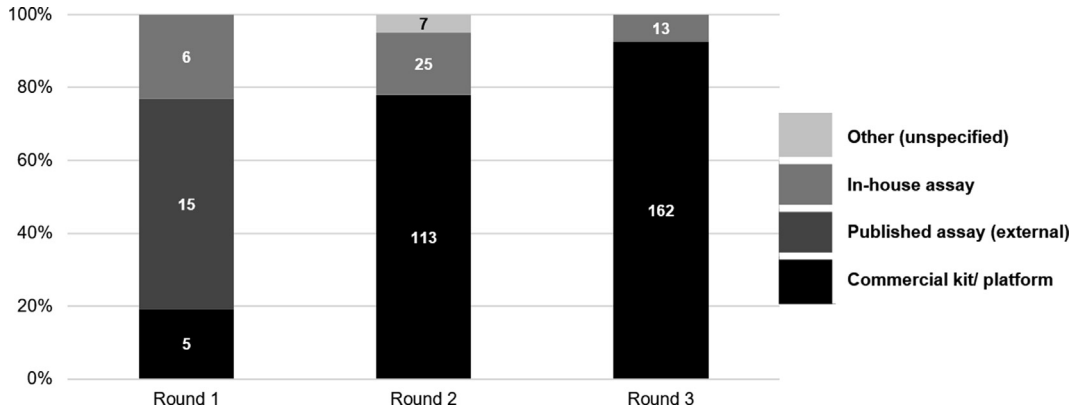


Fig. 3 Summary of reported nucleic acid test assays used in testing the specimen panels for rounds 1, 2 and 3 of the SARS-CoV-2 proficiency testing program.

accuracy and sensitivity of in-house developed NAT assays or published protocols in a variety of settings.[9] This required the rapid development of a SARS-CoV-2 PTP to assess these assays and the performance of individual laboratories.

The PTP described in this study was the first PTP (March 2020) for the detection of SARS-CoV-2 using NAT offered worldwide. Similar PTPs, such as those reported by Edson *et al.*,[10] Matheeussen *et al.*,[11] and Sung *et al.*,[12] followed. The results submitted by participants of the RCPAQAP SARS-CoV-2 PTP demonstrated the capability of laboratories across Australia and New Zealand in the detection of SARS-CoV-2. A variety of testing protocols were used. The round 1 of the SARS-CoV-2 PTP was offered in early March 2020, 9 weeks after the first cases of COVID-19 emerged in the People's Republic of China and 5 weeks after the first Australian case of the disease. The number of Australian and New Zealand laboratories participating in round 1 was low, as very few laboratories had commenced SARS-CoV-2 specific diagnostic workflows in such a short timeframe. By the time the second and third rounds of the PTP were offered in May 2020 and November 2020, the total number of participants had increased significantly. The majority of participants were from New South Wales (NSW) public and private laboratories.

Overall, the performance of the participating laboratories improved over time, confirming the potential benefits of ongoing participation in external quality assurance programs. We found that the correct detection and identification of SARS-CoV-2 presumptive positive (inconclusive) samples in rounds 1 and 2 were particularly challenging to participating laboratories. These samples were designed to represent inconclusive patient samples that may be presented to the laboratory as positive cases with low viral load in a clinical setting. Testing performed on these samples may return positive results for one but not the other SARS-CoV-2 targets. The results submitted for these inconclusive samples highlighted the potential weaknesses in some SARS-CoV-2 molecular testing protocols. Comparable findings were also observed in other similar external quality assurance (EQA) schemes, such as those reported previously.[11,12] Reporting such results had been inconsistent across all participating laboratories in this PTP. The initial PTP results highlighted the lack of a testing algorithm and reporting workflow in some laboratories. In particular, the importance of interpreting results based on a second NAT as a confirmatory assay to rule out or confirm the presence of SARS-CoV-2 was undermined. Laboratories with discordant results for inconclusive samples should aim to increase the quality of results

Table 4 Overall performance of rounds 2 and 3 participants using top five commercial kits or platforms in SARS-CoV-2 proficiency testing program

Round	Assay	Concordant (%)					Discordant (%)				
		S6	S7	S8	S9	S10	S6	S7	S8	S9	S10
2 (May 2020)	Cepheid GeneXpert Xpert Xpress SARS-CoV-2	97.8	100	91.5	97.8	100	2.2	–	8.5	2.2	–
	Seegene Allplex 2019-nCoV	100	100	69.2	92.9	100	–	–	30.8	7.1	–
	AusDiagnostics SARS-CoV-2, Influenza and RSV (8-Well)	100	100	100	100	100	–	–	–	–	–
	Roche Diagnostics Cobas SARS-CoV-2	100	100	81.8	100	100			18.2	–	–
	Hologic Panther Fusion SARS-CoV-2 assay	100	100	NA	100	100	–	–	NA	–	–
3 (Nov 2020)		S11	S12	S13	S14	S15	S11	S12	S13	S14	S15
	Cepheid GeneXpert Xpert Xpress SARS-CoV-2	100	100	100	94.0	94.0	–	–	–	6.0	6.0
	Seegene Allplex 2019-nCoV	100	100	100	100	100	–	–	–	–	–
	Roche Diagnostics Cobas SARS-CoV-2	100	100	100	100	100	–	–	–	–	–
	AusDiagnostics SARS-CoV-2, Influenza and RSV (8-Well)	100	100	100	100	100	–	–	–	–	–
	Seegene Allplex SARS-CoV-2 assay	100	100	100	100	100					

NA, results not assessed; S6, SARS-CoV-2 positive; S7, SARS-CoV-2 positive; S8, SARS-CoV-2 presumptive positive (inconclusive); S9, SARS-CoV-2 negative; S10, SARS-CoV-2 positive; S11, SARS-CoV-2 negative; S12, SARS-CoV-2 negative; S13, SARS-CoV-2 negative; S14, SARS-CoV-2 positive; S15, SARS-CoV-2 positive.

by modifying their testing algorithm to prevent interpreting results only based on a single assay. Similarly, if a laboratory interprets the results based on a single platform, this platform should have a NAT assay that detects at least two SARS-CoV-2 targets.

While the participating laboratories were not required to disclose information on whether specimen pooling was performed on specimens in all rounds, laboratories were required to test the specimens in the same manner as routine patient samples. Therefore, any false negative results for the SARS-CoV-2 positive samples included in this PTP, particularly those with lower viral loads, reflected the need to review the laboratory testing process. For instance, the correct approach in sample pooling strategies to minimise false negativity of the assay, for example based on the predictive algorithm recommended by Mulu *et al.*[13]

The commercially available testing platforms had varying sensitivity and accuracy in detecting the presence of SARS-CoV-2 in the specimen panel, which improved over time in some platforms. Throughout the three rounds of the SARS-CoV-2 PTP, participating laboratories generally increased the number of testing platforms. The majority of participants performed testing on more than one platform by round 3.

Upon analysing the results submitted by participants, we identified the following limitations of our study. First, a low concentration of MDCK cells in the negative specimen and its associated disadvantage was evident in round 2. A total of six out of 145 result entries for the negative specimen in round 2 were inconclusive or invalid, based on the testing system, which reported it as unresolved. These assays incorporated testing for the presence of internal control (e.g., RNaseP), returning an invalid (inconclusive) result if the internal control was not detected, usually either from sample inhibition or the absence of the internal control gene target. Although adding MDCK cells to the negative specimen should have prevented a negative result for internal control, the concentration of the cells may be too low and was missed by the testing system. Given the number of inconclusive or invalid results reported for the negative specimen, the concentration of MDCK cells in the negative specimen should be increased in future PTP. This will allow for the accurate assessment of the performance of the laboratory in reporting the results for a negative specimen. Second, the survey specimens included in this PTP were not tested to determine whether they are suitable for platforms used to detect SARS-CoV-2 in a saliva specimen. The cross reaction of the added sample stabiliser, AssayAssure Multilock, with the transport media or other reagent in processing the saliva specimen has yet to be studied. In future, extensive studies can be performed to determine the type of sample that will be suitable for saliva testing.

CONCLUSION

Our study highlighted the importance of participation in PTP, especially those for newly emerging organisms that are capable of causing a major outbreak, such as the PTP for the detection of SARS-CoV-2 described here. Ongoing participation of clinical and public health laboratories in a PTP will improve the quality of testing for SARS-CoV-2.

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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.2022.01.006.

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