Doherty Institute

THE UNIVERSITY OF MELBOURNE

The Royal Melbourne Hospital

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

Updated Report

Post-market validation of a further five serological assays for COVID-19

–

24th September 2020

Report prepared for:

Office of Health Protection, Commonwealth Government of Australia

The Therapeutics Goods Administration (TGA) of Australia

Report prepared by:

Dr Katherine Bond

Ms Suellen Nicholson

Ms Tuyet Hoang

Dr Mike Catton

Professor Benjamin Howden

Professor Deborah Williamson

Contents

Executive Summary

Here, we present results of our post-market validation of a further five serological assays for the detection of SARS-CoV-2 antibodies. Testing was undertaken on a cohort of stored serum prior to the COVID-19 outbreak in Australia, and on samples of serum collected from patients with SARS-CoV-2 infection confirmed by molecular testing.

Our findings suggest that the overall sensitivities vary markedly depending on the point-of-care tests (PoCT) assessed, and most often fall below that reported by the manufacturer in the instructions for use (IFU). Specificity findings are more often consistent with those reported by the manufacturer. Careful test selection and consideration of clinical utility remain critical in the appropriate utilisation of these assays.

Overall, our findings continue to support a recent position statements by the Public Health Laboratory Network (PHLN) and the Royal College of Pathologists Australasia (RCPA) that serological assays have limited, if any, role in the diagnosis of acute COVID-19 infection. The role of PoCT in population-level serosurveys remains to be seen in the context of other emerging serological tests for SARS-CoV-2.

1. Introduction

This work continues the post-market validation work previously reported on 28th April, 2nd June and 3rd August 2020. Following the Initial laboratory responses and release of the viral whole genome sequence by Chinese investigators in early January 2020, there was initially a rapid development of serological assays for COVID-19.[1–3] The most publicised serological tests for COVID-19 have been lateral flow immunoassays, also known as serological point of care tests (PoCT). The urgent need for diagnostic testing has meant that many test kits have had an expedited assessment from the Australian Therapeutic Goods Administration (TGA). As such, robust post-market

validation of COVID-19 diagnostic kits that are listed on the Australian Register of Therapeutic Goods (ARTG) is essential. This emergency exemption ended on July 31st 2020.

Here, we present findings from a post-market validation study of five further serological PoCT (all listed on the ARTG), to supplement the reports dated 28th April 2020, 2nd June 2020 and 10th August 2020. This brings the total number of assays evaluated to thirteen PoCT and one ELISA.

2. Methods

2.1 Establishment of patient cohorts and serum samples

In order to test sensitivity and specificity of the included lateral flow assays, a testing panel was developed consisting of the following three patient cohorts:

Sensitivity analysis

1. Serum from patients with SARS-CoV-2 detected by RT-PCR from upper and / or lower respiratory tract specimens.

Specificity analysis

2. Serum from patients with infections with the potential for cross-reactivity in serological assays, namely (i) patients with respiratory viral infections, including seasonal coronavirus infections and (ii) patients with other acute infections (e.g. dengue; CMV; EBV).
3. Serum from a representative sample of the Victorian population collected in 2018 and 2019 ('pre-pandemic controls').

All serum samples were obtained from a tertiary hospital (Royal Melbourne Hospital, RMH) or the state reference laboratory for virology (Victorian Infectious Diseases

Reference Laboratory, VIDRL). Serum samples were aliquoted into 100uL aliquots for processing and storage at time of entry into the study.

Table 1: Number and type of samples included in ongoing post-market validation of serological PoCT assays.

Cohort	Characteristics	Purpose of samples	Total (samples / patients)
1	SARS-CoV-2 RT PCR-positive patients	Sensitivity analysis	50/49
2	Other non-COVID-19 infections	Specificity analysis	30/30
3	Pre-pandemic controls	Specificity analysis	70/70

2.2 Test descriptions

2.2.1 Point of care lateral flow serological assays

Thirteen lateral flow serological assays in total have been assessed, two were described in detail in report date 28th April, three were described in an updated report on the 2nd June, three were described in report dated 10th August, and five are additionally described here. Common features are that:

i. they are single use immunochromatographic lateral flow tests, for the detection of IgM and/or IgG in serum, plasma or whole blood
ii. the specific SARS-CoV-2 recombinant antigen(s) incorporated into the assay are not described in the IFU
iii. IFUs indicate that test results should not be used as the sole basis for clinical management decisions, requiring interpretation alongside clinical features and other diagnostic (molecular) assays

Immunochromatographic assays involve detection of anti-SARS-CoV-2 IgM or IgG antibodies through binding to immobilised recombinant antigen attached to colloidal gold, followed by detection of the conjugates by an anti-human IgM or IgG antibody.

A control line is also incorporated, which measures adequacy of fluid flow along the test strip. Reported manufacturer reported characteristics are summarised in Table 3 and include details for assays described in previous reports. In general, with respect to the generation of reported performance characteristics limited information was supplied regarding:

i. where validation samples were sourced from
ii. whether plasma, serum, whole blood or a combination of these were used for validation
iii. what proportion of patients included were confirmed by a result from RT-PCR
iv. what the time frame was for collection of samples post the onset of clinical symptoms.

Table 2: Reported performance characteristics of included serological assays according to manufacturer's instructions for use

Assay	Sensitivity	Specificity
PCL COVID19 IgG/IgM Rapid Gold	Not listed[%]	Not listed[%]
Cellex qSARS-CoV-2 IgG/IgM Cassette Rapid Test kit	IgM = 82.0% [74.3, 88.3] IgG = 60.2% [51.1, 68.7] IgM or IgG = 93.8% [88.1, 97.3] (n = 128)	IgM = 98.4% [96.0, 99.6] IgG = 97.6% [94.9, 99.1] IgM or IgG = 95.6% [92.3, 97.8] (n = 250)
COVID-19 IgG IgM Rapid Test Cassette (Zhejiang Orient Gene)	IgM = 87.9% [79.8, 93.6] (n = 113) IgG* = 97.2% [85.5, 99.9] (n = 36)	IgM = 100% [76.8, 100] IgG = 100% [76.8, 100] (n = 14)
LYHER Novel Coronavirus (2019-nCoV) IgM/IgG Antibody Combo Test kit	IgM or IgG = 96.98% [94.51, 98.54] (n = 331)	IgM or IgG = 99.29% [97.94, 99.85] (n = 422)
Diagnostic Kit for IgM/IgG Antibody to Coronavirus (SARS-CoV-2) (Zhuhai Livzon Diagnostics)	IgM = 79.0% [7.39, 83.3] IgG = 84.3% [79.6, 88.0] IgM or IgG = 90.6% [86.6, 93.4] (n = 644)[#]	IgM = 99.7% [98.4, 100] IgG = 99.4% [90.4. 94.5] IgM or IgG = 99.2% [97.6, 99.7] (n = 644)[#]

[%] 3 positive samples tested in dilution series, no figures given for specificity analysis; * Only samples in 'convalescent period' included (36 of 113 patients); [#] Total number for both sensitivity and specificity analysis

2.2.2 RT-PCR

Patients with confirmed COVID-19 infection had SARS-CoV-2 detected using the Coronavirus Typing assay (AusDiagnostics, Mascot, NSW). This is a two-step, hemi-nested multiplex tandem PCR, with seven coronavirus RNA targets plus a proprietary artificial sequence as an internal control. In addition, all positive samples had SARS-CoV-2 detected at VIDRL where testing was first conducted using an in-house assay for the SARS-CoV-2 RdRp gene. If positive, subsequent testing for the SARS-CoV-2 E gene was performed, using previously published primers.[4]

2.2.3 MICRONEUTRALISATION ASSAY

The microneutralisation assay is an in-house assay performed in the Subbarao laboratory, based in the Doherty Institute, University of Melbourne. SARS-CoV-2 virus, initially isolated from a clinical specimen from a patient in Melbourne, Australia,[5] is propagated in Vero cells, before being incubated with dilutions of test sera. This solution is subsequently inoculated onto a monolayer of Vero cells. Cell cultures are reviewed at five days, with cytopathic effect scored and compared between test and control wells. The ability of test sera to inhibit viral invasion and replication is reported as a titre, calculated by the Reed and Muench method, with titres above 40 considered positive. The assay has been validated against an initial panel of serum from SARS-CoV-2 PCR confirmed patients and a representative serum cohort from 2016 with the assay cut-off of 40 determined by a receiver operating curve (ROC) analysis.

2.3 Testing protocol

Testing of the lateral flow assays was performed in the Clinical Trials Research Laboratory in the Department of Pathology RMH, by three laboratory research technicians, all of whom had undergone previous training in the use of lateral flow assays. Testing was performed exactly as per the IFU using the previously described serum panel (Table 1) for the PCL COVID19 IgG/IgM Rapid Gold (PCL Inc, sponsored by Haemokinesis Pty Ltd, Lot number COV03-200318); the Cellex qSARS-CoV-2 IgG/IgM Rapid Test kit (Cellex Inc distributed by Medicision, Lot numbers 20200409W & 20200418); the COVID-19 IgG/IgM Rapid Test Cassette (Zhejiang Orient Gene Biotech Co Ltd distributed by Expia lot numbers 2004231 & S2004232); the LYHER Novel Coronavirus (2019-nCoV) IgM/IgG Antibody Combo Test Kits (Hanzhou Laihe Biotech Co Ltd distributed by Complementary Medicines Group Pty Ltd, lot numbers 2004031 & 2005037) and the Diagnostic Kit for IgM/IgG Antibody to Coronavirus

(SARS-CoV-2) (Zhuhai Livzon Diagnostics Inc distributed by Marcel Equity Pty Limited/Avania PL, Lot numbers CK2004350410 & CK2004390410).

Subsequent lot numbers of kits for which two different lot numbers had already been assessed in one of the test serum panels, were tested for lot to lot variation in a dilution series of 3 high positive samples (with microneutralisation titres 1016, 905 and 1280; positive titre is any titre above 40). Doubling dilutions were undertaken from neat samples to 1/256 dilution, for a total of 10 test sera per sample (neat, ½, ¼, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256 and normal saline control). The last dilution at which the test kit detected antibody is reported here. Kits assessed in this dilution panel included the SARS-CoV-2 Antibody Test (Wondfo, distributed by Advangen International Pty Ltd lot numbers W19500309 & W195004131 and distributed by Allsafe Medical Pty Ltd W19500490); and the COVID-19 IgG/IgM Rapid Test Cassette (Zhejiang Orient Gene Biotech Co Ltd distributed by Onsite Diagnostics Pty Ltd lot numbers S2004020, S2004021; and distributed by Amandla China Pty Ltd Lot number S2004025).

For all testing, lateral flow test strips were read in duplicate, a third read was undertaken if the first two were discordant, with the third read taken as the final result. A sample with discordant results for different lot numbers was tested a third time, with the third test result taken as the final result. All testing was undertaken in a blinded manner with results collated by an independent investigator at the conclusion.

2.4 Statistical analysis

Statistical analysis was carried out using GraphPad Prism (version 8.4.2). Binomial 95% confidence intervals (CI) were calculated for all proportions.

- Sensitivity of the serological assays was calculated as the number of positive results for each component of the test, divided by the number of samples from patients with confirmed COVID-19 as determined by RT-PCR.

- Specificity was calculated as the number of negative results for each component of the test, divided by the number of samples from patients without confirmed COVID-19 as determined by RT-PCR and clinical end point (Cohort 2 and 3).

2.5 Ethics

Ethical approval for this project was obtained from the RMH Human Research Ethics Committee (RMH HREC QA2020052). This ethics approval allows for prospective serum collection following discharge from hospital, thus enabling longitudinal assessment of the performance of serological assays. Patients recruited into this project also provided specimens to assess the performance of plasma samples.

3. Results

3.1 Comparison of serological PoCT with RT-PCR

Serum samples tested in this analysis included 50 samples for the sensitivity analysis, and 100 samples for the specificity analysis (Table 1). Sensitivity findings according to time of collection relative to sample onset are reported in Tables 3 to 7; lot to lot variation is reported in Tables 8 and 9. Summary performance characteristics for each lot number tested, with respect to overall sensitivity, sensitivity for samples collected more than 14 days from symptom onset and specificity, can be found in Table 10.

Table 3: Comparison of the PCL COVID19 IgG/IgM Rapid Gold with RT-PCR for 49 patients with confirmed COVID-19 infection, stratified by days post-symptom onset.

Days post-symptom onset	Samples (n)	IgM detected (%) [95% CI]	IgG detected (%) [95% CI]	IgM or IgG (%) [95% CI]
4-8	6	1 (16.7) [0.4, 64.1]	1 (16.7) [0.4, 64.1]	1 (16.7) [0.4, 64.1]
9-14	6	2 (33.3) [4.3, 77.7]	3 (50.0) [11.8, 88.2]	4 (66.7) [22.3, 95.7]
15-20	6	3 (50.0) [11.8, 88.2]	5 (83.3) [35.9, 99.6]	5 (83.3) [35.9, 99.6]
21-30	16	3 (18.8) [4.1, 45.7]	7 (43.8) [19.8, 70.1]	7 (43.8) [19.8, 70.1]
>30	16	3 (18.8) [4.1, 45.7]	7 (43.8) [19.8, 70.1]	7 (43.8) [19.8, 70.1]
Total	**50**	**12 (24.0) [13.1, 38.2]**	**23 (46.0) [31.8, 60.7]**	**24 (48.0) [33.7, 62.6]**

CI = Confidence interval (Clopper-Pearson)

Table 4: Comparison of the Cellex qSARS-CoV-2 IgG/IgM Cassette Rapid Test Kit with RT-PCR for 49 patients with confirmed COVID-19 infection, stratified by days post-symptom onset.

Days post-symptom onset	Samples (n)	IgM detected (%) [95% CI]	IgG detected (%) [95% CI]	IgM or IgG (%) [95% CI]
4-8	6	0 (0.0) [0.0, 45.9]	2 (33.3) [4.3, 77.7]	2 (33.3) [4.3, 77.7]
9-14	6	1 (16.7) [0.4, 64.1]	3 (50.0) [11.8, 88.2]	3 (50.0) [11.8, 88.2]
15-20	6	2 (33.3) [4.3, 77.7]	5 (83.3) [35.9, 99.6]	5 (83.3) [35.9, 99.6]
21-30	16	1 (16.7) [0.4, 64.1]	15 (93.8) [69.8, 99.8]	15 (93.8) [69.8, 99.8]
>30	16	2 (12.5) [1.55, 38.4]	13 (81.3) [54.4, 96.0]	13 (81.3) [54.4, 96.0]
Total	**50**	**6 (14.0) [5.8, 26.7]**	**38 (76.0) [61.8, 86.9]**	**38 (76.0) [61.8, 86.9]**

CI = Confidence interval (Clopper-Pearson)

Table 5: Comparison of the COVID-19 IgG/IgM Rapid Test Cassette (Zhejiang Orient Gene) with RT-PCR for 49 patients with confirmed COVID-19 infection, stratified by days post-symptom onset.

Days post-symptom onset	Samples (n)	IgM detected (%) [95% CI]	IgG detected (%) [95% CI]	IgM or IgG (%) [95% CI]
4-8	6	3 (50.0) [11.8, 88.2]	3 (50.0) [11.8, 88.2]	3 (50.0) [11.8, 88.2]
9-14	6	4 (66.7) [22.3, 95.7]	5 (83.3) [35.9, 99.6]	5 (83.3) [35.9, 99.6]
15-20	6	5 (83.3) [35.9, 99.6]	6 (100) [54.1, 100]	6 (100) [54.1, 100]
21-30	16	9 (56.3) [29.9, 80.3]	16 (100) [79.4, 100]	16 (100) [79.4, 100]
>30	16	9 (56.3) [29.9, 80.3]	15 (93.8) [69.8, 99.8]	15 (93.8) [69.8, 99.8]
Total	**50**	**30 (60.0) [45.2, 73.6]**	**45 (90.0) [78.2, 96.7]**	**45 (90.0) [78.2, 96.7]**

CI = Confidence interval (Clopper-Pearson)

Table 6: Comparison of the LYHER Novel Coronavirus (2019-nCoV) IgM/IgG Antibody Combo Test Kit with RT-PCR for 49 patients with confirmed COVID-19 infection, stratified by days post-symptom onset.

Days post-symptom onset	Samples (n)	IgM detected (%) [95% CI]	IgG detected (%) [95% CI]	IgM or IgG (%) [95% CI]
4-8	6	3 (50.0) [11.8, 88.2]	1 (16.7) [0.4, 64.1]	3 (50.0) [11.8, 88.2]
9-14	6	4 (66.7) [22.3, 95.7]	1 (16.7) [0.4, 64.1]	4 (66.7) [22.3, 95.7]
15-20	6	5 (83.3) [35.9, 99.6]	4 (66.7) [22.3, 95.7]	5 (83.3) [35.9, 99.6]
21-30	16	13 (81.3) [54.4, 96.0]	14 (87.5) [61.7, 98.5]	14 (87.5) [61.7, 98.5]
>30	16	12 (75.0) [47.6, 92.7]	13 (81.3) [54.4, 96.0]	14 (87.5) [61.7, 98.5]
Total	**50**	**37 (74.0) [59.7, 85.4]**	**33 (66.0) [51.2, 78.8]**	**40 (80.0) [66.3, 90.0]**

CI = Confidence interval (Clopper-Pearson)

Table 7: Comparison of the Diagnostic Kit for IgM/IgG Antibody to Coronavirus (SARS-CoV-2) (Zhuhai Livzon Diagnostics) with RT-PCR for 49 patients with confirmed COVID-19 infection, stratified by days post-symptom onset.

Days post-symptom onset	Samples (n)	IgM detected (%) [95% CI]	IgG detected (%) [95% CI]	IgM or IgG (%) [95% CI]
4-8	6	1 (16.7) [0.4, 64.1]	2 (33.3) [4.3, 77.7]	2 (50.0) [11.8, 88.2]
9-14	6	5 (83.3) [35.9, 99.6]	3 50.0) [11.8, 88.2]	5 (83.3) [35.9, 99.6]
15-20	6	5 (83.3) [35.9, 99.6]	5 (83.3) [35.9, 99.6]	5 (83.3) [35.9, 99.6]
21-30	16	6 (37.5) [15.2, 64.6]	7 (43.8) [19.8, 70.1]	8 (50.0) [24.7, 75.4]
>30	16	8 (50.0) [24.7, 75.4]	8 (50.0) [24.7, 75.4]	9 (56.3) [29.9, 80.3]
Total	**50**	**25 (50.0) [35.5, 64.5]**	**25 (50.0) [35.5, 64.5]**	**29 (58.0) [43.2, 71.8]**

CI = Confidence interval (Clopper-Pearson)

Table 8: RT-PCR positive serum dilution series for lot to lot comparison of the COVID-19 IgG/IgM Rapid Test Cassette (Zhejiang Orient Gene Biotech Co Ltd), highest dilution recording a positive test result

Assay, Sponsor and lot number	Test Sample 1 [MN titre 1016 at 1:1]		Test Sample 2 [MN titre 905 at 1:1]		Test Sample 3 [MN titre 1280 at 1:1]	
	IgM	**IgG**	**IgM**	**IgG**	**IgM**	**IgG**
Zhejiang Orient Gene, Onsite Diagnostics, S2004020	1:256	1:256	1:32	1:32	1:64	1:64
Zhejiang Orient Gene, Onsite Diagnostics, S2004021	1:128	1:128	1:64	1:64	1:64	1:64
Zhejiang Orient Gene, Amandla China Pty Ltd, S2004025	1:256	1:128	1:32	1:32	1:64	1:64

MN = Microneutralisation

Table 9: RT-PCR positive serum dilution series for lot to lot comparison of the SARS-CoV-2 Antibody Test (Wondfo), highest dilution recording a positive test result

Assay, Sponsor and lot number	Test Sample 1 [MN titre 1016 at 1:1]	Test Sample 2 [MN titre 905 at 1:1]	Test Sample 3 [MN titre 1280 at 1:1]
	Test Result	**Test Result**	**Test Result**
Wondfo, Advangen International Pty Ltd, W19500309	1:128	1:32	1:16
Wondfo, Allsafe Medical Pty Ltd, W195004131	1:128	1:16	1:32
Wondfo, Allsafe Medical Pty Ltd, W19500490	1:128	1:16	1:32

MN = Microneutralisation

When only samples collected more than 14 days following symptom onset were considered, the sensitivity of the PCL COVID19 IgG/IgM Rapid Gold was 50% (95% CI: 33.4-66.6%), the Cellex qSARS-CoV-2 IgG/IgM Rapid Test Kit was 86.8% (95% CI:71.9-95.6), the COVID-19 IgG/IgM Rapid Test Cassette (Zhejiang Gene Orient) was 97.4% (95%CI: 86.2-99.9%), the LYHER Novel Coronavirus (2019-n) IgM/IgG Antibody Combo Test Kit was 86.8% (95% CI: 71.9-95.6%) and the Diagnostic Kit for IgM/IgG Antibody to Coronavirus (SARS-CoV-2) (Zhuhai Livzon) was 57.9% (95% CI:40.8-73.7%) (Table 10).

There was no significant difference in lot to lot sensitivity for the COVID-19 IgG/IgM Rapid Test Cassette (Zhejiang Gene Orient) or the SARS-CoV-2 Antibody Test (Wondfo) tested in the dilution series (Tables 8 and 9).

The specificity of the respective assays was as follows: PCL COVID19 IgG/IgM Rapid Gold was 100% (95% CI: 96.4-100%), the Cellex qSARS-CoV-2 IgG/IgM Rapid Test

Kit was 97.0% (95% CI:91.5-99.4%), the COVID-19 IgG/IgM Rapid Test Cassette (Zhejiang Gene Orient) was 99.0% (95%CI: 94.6- >99.9%), the LYHER Novel Coronavirus (2019-n) IgM/IgG Antibody Combo Test Kit was 97.0% (95% CI:91.5-99.4%) and the Diagnostic Kit for IgM/IgG Antibody to Coronavirus (SARS-CoV-2) (Zhuhai Livzon) was 99% (95% CI:94.6- >99.9%).

Table 10: Comparative performance of serological assays with RT-PCR, for 150 samples from 149 patients

Performance Characteristic / Test Assay	Sensitivity, all samples (%) [95% CI]	Sensitivity, >14 days# (%) [95% CI]	Specificity (%) [95% CI]
PCL IgM	24.0 [13.1, 38.2]	23.7 [11.4, 40.2]	100.0 [96.4, 100]
PCL IgG	46.0 [31.8, 60.7]	50.0 [33.4, 66.6]	100.0 [96.4, 100]
PCL IgM or IgG	**48.0 [33.7, 62.6]**	**50.0 [33.4, 66.6]**	**100.0 [96.4, 100]**
Cellex IgM	12.0 [4.5, 24.3]	13.2 [4.4, 28.1]	98.0 [93.0, 99.8]
Cellex IgG	76.0 [61.8, 86.9]	86.8 [71.9, 95.6]	99.0 [94.6, >99.9]
Cellex IgM or IgG	**76.0 [61.8, 86.9]**	**86.8 [71.9, 95.6]**	**97.0 [91.5, 99.4]**
Zhejiang IgM	60.0 [45.2, 73.6]	60.5 [43.4, 76.0]	100.0 [96.4, 100]
Zhejiang IgG	90.0 [78.2, 96.7]	97.4 [86.2, 99.9]	99.0 [94.6, >99.9]
Zhejiang IgM or IgG	**90.0 [78.2, 96.7]**	**97.4 [86.2, 99.9]**	**99.0 [94.6, >99.9]**
Lyher IgM	74.0 [59.7, 85.4]	78.9 [62.7, 90.5]	98.0 [93.0, 99.8]
Lyher IgG	66.0 [51.2, 78.8]	81.6 [65.7, 92.3]	99.0 [94.6, >99.9]
Lyher IgM or IgG	**80.0 [66.3, 90.0]**	**86.8 [71.9, 95.6]**	**97.0 [91.5, 99.4]**
Zhuhai IgM	56.0 [41.3, 70.0]	50.0 [33.4, 66.6]	99.0 [94.6, >99.9]
Zhuhai IgG	50.0 [35.5, 64.5]	52.6 [35.8, 69.0]	100 [96.4, 100]
Zhuhai IgM or IgG	**58.0 [43.2, 71.8]**	**57.9 [40.8, 73.7]**	**99.0 [94.6, >99.9]**

Samples collected more than 14 days from symptom onset

3.2 Comparison of Specimen Type for PoCT

A subset of 20 serum and plasma samples, collected simultaneously from participants, were tested in each assay. Concordance between serum and plasma samples ranged from 85 - 100% (95% CI: 62.1-100%), (Table 15)

Table 11: Comparison of positive results for 20 patients with RT-PCR confirmed COVID-19 infection for serum and plasma sample types

Sample Type / Test Assay	Positive Serum Samples (%) [95% CI]	Positive Plasma Samples (%) [95% CI]	Concordance (%) [95% CI]
PCL IgM	5 (25%) [8.7, 49.1]	6 (30%) [11.9, 54.3]	85.0% [62.1, 96.8]
PCL IgG	8 (40%) [19.1, 64.0]	11 (55%) [31.5, 76.9]	85.0% [62.1, 96.8]
Cellex IgM[a]	2 (10%) [1.2, 31.7]	2 (10%) [1.2, 31.7]	100% [83.2, 100]
Cellex IgG[a]	14 (70%) [45.7, 88.1]	15 (75%) [50.9, 91.3]	95.0% [75.1, 99.9]
Zhejiang IgM[b]	13 (65%) [40.8, 84.6]	12 (60%) [36.1, 80.9]	95.0% [75.1, 99.9]
Zhejiang IgG[b]	18 (90%) [68.3, 98.8]	18 (90%) [68.3, 98.8]	100% [83.2, 100]
Lyher IgM[c]	14 (70%) [45.7, 88.1]	11 (55%) [31.5, 76.9]	95.0% [75.1, 99.9]
Lyher IgG[c]	13 (65%) [40.8, 84.6]	10 (50%) [27.2, 72.8]	85.0% [62.1, 96.8]
Zhuhai IgM[d]	10 (50%) [27.2, 72.8]	10 (50%) [27.2, 72.8]	90.0% [68.3, 98.8]
Zhuhai IgG[d]	9 (45%) [23.1, 68.5]	9 (45%) [23.1, 68.5]	100% [83.2, 100]

[a] Assessed for lot 20200409; [b] Assessed for lot 2004231; [c] Assessed for lot 2004031; [d] Assessed for lot CK2004350410

4. Discussion

Here, we present results of our post-market validation of the PCL COVID19 IgG/IgM Rapid, the Cellex qSARS-CoV-2 IgG/IgM Rapid Test Kit, the COVID-19 IgG/IgM Rapid Test Cassette (Zhejiang Gene Orient), the LYHER Novel Coronavirus (2019-n) IgM/IgG Antibody Combo Test Kit, and the Diagnostic Kit for IgM/IgG Antibody to Coronavirus (SARS-CoV-2) (Zhuhai Livzon). Not all tests met their stated performance

characteristics with respect to sensitivity, but all were within the stated IFU range for specificity.

The sensitivity for both components of the assay, IgM and IgG, was found to be low for the PCL COVID19 IgG/IgM Rapid and the Diagnostic Kit for IgM/IgG Antibody to Coronavirus (SARS-CoV-2) (Zhuhai Livzon), even for specimens collected more than 14 days following symptom onset. Performance characteristics were not stated in the manufacturer's IFU for the PCL COVID19 IgG/IgM Rapid, and for the Diagnostic Kit for IgM/IgG Antibody to Coronavirus (SARS-CoV-2) (Zhuhai Livzon) the sensitivity fell significantly below that reported by the manufacturer.

Sensitivity for IgM component was significantly lower than that reported by the manufacturer for both the Cellex qSARS-CoV-2 IgG/IgM Rapid Test Kit and the COVID-19 IgG/IgM Rapid Test Cassette (Zhejiang Gene Orient). IgG components for both assays were either in agreement with, or within the confidence intervals reported by the manufacturer, with the COVID-19 IgG/IgM Rapid Test Cassette (Zhejiang Gene Orient) reporting the higher sensitivity, 97.4% (95%CI: 86.2-99.9%) versus 86.8% (95% CI: 71.9-95.6%), when analysing convalescent samples.

Sensitivity results for the IgM or IgG component alone were not available from the manufacturer for the LYHER Novel Coronavirus (2019-n) IgM/IgG Antibody Combo Test Kit. Combined sensitivity results for either IgM or IgG fell just short, but with overlapping confidence intervals, of the manufacturers reported values for convalescent samples, at 86.8% (95%CI: 71.9-95.6%) compared to 96.98% (95%CI: 94.51-98.54%).

In summary, our data describe the performance characteristics of five further PoCT devices. Specificity findings are more often consistent with those reported by the manufacturer in the accompanying IFU, compared to sensitivity findings which are quite variable depending on the assay. Careful test selection and consideration of clinical utility remain critical in the appropriate utilisation of these assays.

5. Acknowledgements

We thank staff of the Pathology department at RMH, the Serology department of the Victorian Infectious Diseases Reference Laboratory (VIDRL) and the Hospital in the Home medical and nursing staff at RMH. We also thank patients and their families who have contributed to this study.

6. References

1. Nisreen M.A. Okba *et al.* Severe Acute Respiratory Syndrome Coronavirus 2−Specific Antibody Responses in Coronavirus Disease 2019 Patients. *Emerging Infectious Disease journal* **26**, (2020).
2. Petherick, A. Developing antibody tests for SARS-CoV-2. *The Lancet* **395**, 1101–1102 (2020).
3. Wu, F. *et al.* Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. *Pre-Print MedRxiv* 20 doi:10.1101/2020/03/30.20047365.
4. Corman, V. M. *et al.* Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance* **25**, (2020).
5. Caly, L. *et al.* Isolation and rapid sharing of the 2019 novel coronavirus (SARS - CoV-2) from the first patient diagnosed with COVID -19 in Australia. *Medical Journal of Australia* **212**, 459–462 (2020).