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The Royal Melbourne Hospital

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Updated Report

Post-market validation of serological assays for COVID-19

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Executive Summary

Here, we present results of our post-market validation of a further three serological assays for the detection of SARS-CoV-2 antibodies and comparison with findings for assays already reported. 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 overall sensitivities of these three point-of-care tests (PoCT) tested are below that reported by the manufacturer in the instructions for use (IFU). However, both the Wondfo SARS-CoV-2 Antibody Test and Hightop SARS-CoV-2 IgM/IgG Antibody Rapid Test assays achieve the stated performance characteristics if only serum samples collected greater than 14 days following the onset of COVID-19 related symptoms are included in the analysis. In this situation, the sensitivity for the Hangzhou IgG/IgM Rapid Test remained below that reported in the IFU, specificity was comparable to the IFU. None of the respective IFU qualify the reported sensitivity on the basis of when samples are collected.

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

One of the fundamental pillars in the prevention and control of COVID-19 is timely, scalable and accurate diagnostic testing. Diagnostic testing plays a critical role in defining the epidemiology of the disease, informing case and contact management, and ultimately in reducing viral transmission. Initial laboratory responses included early characterisation and release of the viral whole genome sequence by Chinese investigators in early January 2020,

which enabled rapid development of real-time RT-PCR workflows for detection of SARS-COV-2 (1). To date, diagnostic testing for SARS-CoV-2 has relied on real-time RT-PCR testing, with the conventional testing paradigm of sample collection, nucleic acid extraction and RT-PCR (2). However, over the past few months, there have been rapid development of serological assays for COVID-19 (3-6). The most publicised serological tests for COVID-19 have been lateral flow immunoassays, also known as serological PoCT which have been manufactured at scale in many countries, particularly China. The urgent need for diagnostic testing has meant that many testing kits have not gone through the usual stringent regulatory pathways due to COVID-19 emergency exemptions 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. Here, we present findings from a post-market validation study of three further serological PoCT (all listed on the ARTG), to supplement the initial report (28th April 2020) of two PoCT and one enzyme immunoassay (EIA).

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. To assess the kinetics of the antibody response, serum was obtained from patients at numerous time points post-symptom onset.

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 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	137/91
2	Other non-COVID-19 infections	Specificity analysis	36/36
3	Pre-pandemic controls	Specificity analysis	56/56

2.2 Test descriptions

2.2.1 Point of care lateral flow serological assays

Five lateral flow serological assays in total have been assessed, two were described in detail in report date 28th April, three are additionally described here. Common features are that:

i. they are single used 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 2, 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
OnSite COVID-19 IgG/IgM Rapid Test*	IgG 96.86% (95% CI: 93.66-98.47) IgM 78.03% (95% CI: 72.14-82.96%) IgM or IgG positive: 96.86% (95%CI: 93.66-98.47%)	IgG 100% (95% CI: 98.84-100%) IgM 99.39% (95% CI97.8-99.83%) IgM or IgG positive: 99.39% (95% CI: 97.8-99.83%)
VivaDiag COVID-19 IgM/IgG Rapid Test*	4-10 days of infection: IgM 81.25%; IgG 37.5%; IgM or IgG 81.25% 11-24 days of infection: IgM 97.1%; IgG 95.7%; IgM or IgG 97.1%	IgM 100% IgG 100% IgM or IgG: 100%
Hangzhou IgG/IgM Rapid Test	IgM 85.0% (95%CI: 62.1-96.8%) IgG 100% (95% CI: 86.0-100%	IgM 96.0% (95% CI: 86.3-99.5%) IgG 98.0% (95%CI: 89.4-99.9%)
Wondfo SARS-CoV-2 Antibody Test	86.43% (95% CI: 82.51-89.58%)	99.57% (95% CI: 97.63-99.92%)
Hightop SARS-CoV-2 IgM/IgG Antibody Rapid Test	IgM or IgG positive: 94.15%	IgM or IgG positive: 93.91%
EUROIMMUN Anti-SARS-CoV-2 ELISA (IgA)* Anti-SARS-CoV-2 ELISA (IgG)*	≤10 days from symptom onset: IgA 44.8%; IgG 22.4% >10-20 days from symptom onset: IgA 100.0%; IgG 87.5% ≥ 21 days from symptom onset: IgA 100.0%; IgG 100%	IgA 90.5% IgG 99.3%

* Reported in detail in Final report dated 28th April 2020

Of note, the Hangzhou IgG/IgM Rapid Test was received in two alternative packaging styles from the Therapeutic Goods Australia. The first was badged as the 'AllTest', the second had no markings on the outside of the packet, each was a different lot number. Both devices appeared identical and appeared to be a single use lateral flow test, similar to those previously evaluated. A small pictorial card was included as instructions for use for the 'AllTest' packaging, with no formal IFU included. A copy of the IFU was downloaded from the

manufacturer's website. The Wondfo SARS-CoV-2 Antibody Test does not differentiate between antibody class, with only a single test line indicative of a positive test (IgM/IgG).

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 (2).

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, or as per the small pictorial card in the instance of the Hangzhou assays. Testing was undertaken in duplicate for the Wondfo SARS-CoV-2 Antibody Test and the Hightop SARS-CoV-2 IgM/IgG Antibody Rapid Test, with a third test undertaken for discordant results. One sample was excluded from testing in the Hightop SARS-CoV-2 IgM/IgG Antibody Rapid Test assay as results were discordant and insufficient test kits remained to test in triplicate, and one included negative cohort sample (testing negative) was tested once only in the Wondfo SARS-CoV-2 Antibody Test due to insufficient sample for replicate testing. The majority result (i.e. 2/3) was taken as the final result, any faint line present at test termination was considered a positive result. Due to the significant difference in packaging, with no accompanying IFU, for the Hangzhou Alltest IgG/IgM Rapid Test and the Hangzhou plain

packaging, these results are presented separately. There were insufficient test kits to repeat each packaging style in duplicate.

All testing was undertaken in a blinded manner with results collated by an independent investigator at the conclusion. Clinical and epidemiological details were retrieved from the medical record.

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).
- Positive predictive value specifically for this validation cohort (i.e. not taking into account the population prevalence) was calculated as the number of true positive results (according to RT-PCR) which tested positive in the test assay, as a proportion the total number of samples that tested positive in the assay.
- Positive predictive value specifically for this validation cohort (i.e. not taking into account the population prevalence) was calculated as the number of true negative results (negative cohort samples) which tested negative in the test assay, as a proportion of the total number of samples that tested negative in the assay.

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

In total, 229 samples from 183 patients were included in this analysis (Table 1), with 137 samples from 91 patients included in the sensitivity analysis and 92 samples from 92 patients in the specificity analysis. Sensitivity findings are reported in Tables 3 to 6; sensitivity increased with increasing time post-symptom onset for all assays assessed.

Table 3: Comparison of the Hangzhou Alltest IgG/IgM Rapid Test with RT-PCR for 91 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]
0-3	23	1 (4.3) [1.1, 22.0]	0 (0) [0, 14.8]	1 (4.3) [1.1, 22.0]
4-8	28	5 (17.9) [6.1, 36.9]	9 (32.1) [15.9, 52.4]	9 (32.1) [15.9, 52.4]
9-14	21	5 (23.8) [8.2, 47.2]	14 (66.7) [43.0, 84.5]	14 (66.7) [43.0, 84.5]
15-20	8	4 (50.0) [15.7, 84.3]	8 (100) [63.1, 100]	8 (100) [63.1, 100]
21-30	27	2 (7.4) [0.9, 24.3]	25 (92.6) [75.7, 99.1]	25 (92.6) [75.7, 99.1]
>30	30	1 (3.3) [0.1, 17.2]	26 (86.7) [69.3, 96.2]	26 (86.7) [69.3, 96.2]
Total	**137**	**18 (13.1) [8.0, 20.0]**	**82 (59.9) [51.1, 68.1]**	**83 (60.6) [51.9, 69.8]**

CI = Confidence interval (Clopper-Pearson)

Table 4: Comparison of the Hangzhou unlabelled packaging with RT-PCR for 91 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]
0-3	23	0 (0.0) [0.0, 14.8]	2 (8.7) [1.1, 28.0]	2 (8.7) [1.1, 28.0]
4-8	28	7 (25.0) [10.7, 44.9]	10 (35.7) [18.6, 55.9]	11 (39.3) [21.5, 59.4]
9-14	21	5 (23.8) [8.2, 47.2]	15 (71.4) [47.8, 88.7]	15 (71.4) [47.8, 88.7]
15-20	8	4 (50.0) [15.7, 84.3]	6 (75.0) [34.9, 96.8]	6 (75.0) [34.9, 96.8]
21-30	27	4 (14.8) [4.2, 33.7]	25 (92.6) [75.7, 99.1]	25 (92.6) [75.7, 99.1]
>30	30	1 (3.3) [0.1, 17.2]	25 (83.3) [65.3, 94.4]	25 (83.3) [65.3, 94.4]
Total	**137**	**21 (15.3) [9.8, 22.5]**	**83 (60.6) [51.9, 68.8]**	**84 (61.3) [52.6, 69.5]**

CI = Confidence interval (Clopper-Pearson)

Table 5: Comparison of the Wondfo SARS-CoV-2 Antibody Test with RT-PCR for 91 patients with confirmed COVID-19 infection, stratified by days post-symptom onset.

Days post-symptom onset	Samples (n)	Positive Test Result (%) [95% CI]
0-3	23	3 (13.0) [2.8, 38.6]
4-8	28	14 (50.0) [30.7, 69.4]
9-14	21	16 (76.2) [52.8, 91.8]
15-20	8	8 (100) [63.1, 100]
21-30	27	26 (96.3) [81.0, 99.9]
>30	30	27 (90.0) [73.5, 97.9]
Total	**137**	**94 (68.6) [60.1, 76.3]**

CI = Confidence interval (Clopper-Pearson)

Table 6: Comparison of the Hightop SARS-COV-2 IgM/IgG Antibody Rapid Test with RT-PCR for 91 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]
0-3	23	0 (0.0) [0.0, 14.8]	0 (0.0) [0.0, 14.8]	0 (0.0) [0.0, 14.8]
4-8	28	5 (17.9) [6.1, 36.9]	7 (25.0) [10.7, 44.9]	8 (28.6) [13.2, 48.7]
9-14	21	10 (47.6) [25.7, 70.2]	13 (61.9) [38.4, 81.9]	15 (71.4) [47.8, 88.7]
15-20	8	6 (75.0) [34.9, 96.8]	7 (87.5) [47.4, 99.7]	7 (87.5) [47.4, 99.7]
21-30	26	15 (57.7) [36.9, 76.7]	25 (96.2) [80.4, 99.9]	25 (96.2) [80.4, 99.9]
>30	30	17 (56.7) [37.4, 74.5]	28 (93.3) [77.9, 99.1]	28 (93.3) [77.9, 99.1]
Total	**136**	**53 (39.0) [30.7, 47.7]**	**80 (58.8) [50.1, 67.2]**	**83 (61.0) [52.3, 69.3]**

CI = Confidence interval (Clopper-Pearson)

When only samples collected more than 14 days following symptom onset were considered, the sensitivity of the Hangzhou AllTest IgG/IgM Rapid Test was 90.8% (95% CI: 81.0-96.5%), the Hangzhou unlabelled test was 86.2% (95% CI 75.3-93.5%), the Wondfo SARS-CoV-2 Antibody Test was 93.8% (95% CI: 85.0-98.3%) and the Hightop SARS-CoV-2 IgM/IgG Antibody Rapid Test was 93.8% (95% CI: 84.8-98.3%) (Table 8).

Sample cohorts 2 and 3 (Table 1) were used to assess specificity. The specificity of the respective assays was as follows: Hangzhou AllTest IgG/IgM Rapid Test 96.7 % (95% CI: 90.8-99.3%); Hangzhou unlabelled test 94.6% (95% CI: 87.8-98.2%); Wondfo SARS-CoV-2 Antibody Test 97.8% (95% CI: 92.4-99.7%); Hightop SARS-CoV-2 IgM/IgG Antibody Rapid Test 100% (96.1-100%) (Tables 7, 8).

Summary tables of overall performances characteristics (Table 7), and performance characteristics for samples collect more than 14 days post symptoms onset (Table 8) are presented, allowing comparisons with assays previously described (OnSite IgG/IgM Rapid

Test, VivaDiag COVID-19 IgM/IgG Rapid Test and the EUROIMMUN EIA SARS-CoV-2 IgA and IgG (S protein target)). Comparison of the IgG component of each assay tested is also included (Table 9); Wondfo SARS-CoV-2 Antibody Test is included for completeness but note this is an IgM/IgG combined assay.

Concordance between different lot numbers was as follows (Appendix 2):

- Hangzhou Alltest IgG/IgM Rapid Test compared to the Hangzhou unlabelled test kit: IgM 92.6% (95% CI: 88.4-95.6%); IgG 91.7% (95% CI: 87.4-94.9%)
- Wondfo SARS-CoV-2 Antibody Test: 91.7% (95% CI: 87.3-94.9%)
- Hightop SARS-CoV-2 IgM/IgG Antibody Rapid Test: IgM 93.4 % (95% CI: 89.4-96.3%); IgG 96.1% (95% CI: 92.6-98.2%)

Table 7: Comparative performance of serological assays with RT-PCR for 229 samples from 183 patients, regardless of days post-symptom onset.

Performance Characteristic / Test Assay	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	Positive Predictive Value (%) [95% CI]	Negative Predictive Value (%) [95% CI]	Total (samples/ patients)
OnSite IgM	49.6 [41.0, 58.3]	96.7 [90.8, 99.3]	95.8 [88.1, 99.1]	56.3 [48.2, 64.2]	229/183
OnSite IgG	46.7 [38.2, 55.4]	98.9 [94.1, 99.97]	98.5 [91.7, 99.96]	55.5 [47.5, 63.2]	229/183
Onsite IgM or IgG	**56.9 [48.2, 65.4]**	**95.6 [89.2, 98.8]**	**95.1 [88.0, 98.7]**	**59.9 [51.5, 67.9]**	229/183
VivaDiag IgM	51.8 [43.1, 60.4]	97.8 [92.4, 99.7]	97.3 [90.5, 99.7]	57.6 [49.5, 65.6]	229/183
VivaDiag IgG	51.8 [43.1, 60.4]	98.9 [94.1, 99.97]	98.6 [92.5, 99.96]	58.0 [49.8, 65.8]	229/183
VivaDiag IgM or IgG	**51.8 [43.1, 60.4]**	**97.8 [92.4, 99.7]**	**97.3 [90.5, 99.7]**	**59.1 [49.6, 68.2]**	229/183
ELISA IgA	65.7 [57.1, 73.6]	73.9 [63.7, 82.5]	78.3 [69.6, 85.4]	58.7 [49.2, 67.9]	229/183
ELISA IgG	56.2 [47.5, 64.7]	97.8 [92.4, 99.7]	97.5 [91.2, 99.7]	60.0 [51.7, 67.9]	229/183

ELISA IgA or IgG	**67.9 [59.4, 75.6]**	**72.8 [62.6, 81.6]**	**78.8 [70.3, 85.8]**	**60.4 [50.6, 69.5]**	229/183
Hangzhou AllTest IgM	13.1 [8.0, 20.0]	96.7 [90.8, 99.3]	85.7 [63.7, 97.0]	42.8 [36.0, 49.8]	229/183
Hangzhou AllTest IgG	59.9 [51.1, 68.1]	100 [96.1, 100]	100 [95.6, 100]	62.6 [54.2, 70.4]	229/183
Hangzhou AllTest IgM or IgG	**60.6 [51.9, 68.8]**	**96.7 [90.8, 99.3]**	**96.5 [90.1, 99.3]**	**62.2 [53.8, 70.2]**	229/183
Hangzhou Unlabelled IgM	15.3 [9.8, 22.5]	96.7 [90.8, 99.3]	87.5 [67.3, 97.3]	43.4 [36.5, 50.5]	229/183
Hangzhou Unlabelled IgG	60.6 [51.9, 68.8]	97.8 [2.4, 99.7]	97.6 [91.8, 99.7]	62.5 [54.0, 70.4]	229/183
Hangzhou Unlabelled IgM or IgG	**61.3 [52.6, 69.5]**	**94.6 [87.8, 98.2]**	**94.4 [87.4, 98.2]**	**62.1 [53.6, 70.2]**	229/183
Wondfo SARS-CoV-2 Antibody Test	**68.6 [60.1, 76.3]**	**97.8 [92.4, 97.9]**	**97.9 [92.7, 99.8]**	**67.7 [59.0, 75.5]**	229/183
Hightop IgM	39.0 [30.7, 47.7]	100 [96.1, 100]	100 [93.3, 100]	52.6 [44.9, 60.2]	228/182
Hightop IgG	58.8 [50.7, 67.2]	100 [96.1, 100]	100 [95.6, 100]	62.2 [53.8, 70.0]	228/182
Hightop IgM or IgG	**61.0 [52.3, 69.3]**	**100 [96.1, 100]**	**100 (95.7, 100]**	**63.4 [55.1, 71.3]**	228/182

Table 8: Comparative performance of serological assays with RT-PCR for 157 samples from 155 patients, collected >14 days post symptom onset.

Performance Characteristic / **Test Assay**	**Sensitivity (%) [95% CI]**	**Specificity (%) [95% CI]**	**Positive Predictive Value (%) [95% CI]**	**Negative Predictive Value (%) [95% CI]**	**Total (samples/ patients)**
OnSite IgM	69.2 [56.6, 80.1]	96.7 [90.8, 99.3]	93.8 [82.8, 98.7]	81.7 [73.1, 88.4]	157/155
OnSite IgG	80.0 [68.2, 88.9]	98.9 [94.1, 99.97]	98.1 [89.9, 99.95]	87.5 [79.6, 93.2]	157/155
Onsite IgM or IgG	**84.6 [73.5, 92.4]**	**95.6 [89.2, 98.8]**	**93.2 [83.5, 98.1]**	**89.8 [82.0, 95.0]**	157/155

VivaDiag IgM	78.5 [66.5, 87.7]	97.8 [92.4, 99.7]	96.2 [87.0, 99.5]	86.5 [78.5, 92.4]	157/155
VivaDiag IgG	78.5 [66.5, 87.7]	98.9 [94.1, 99.97]	98.1 [89.9, 99.95]	86.7 [78.6, 92.5]	157/155
VivaDiag IgM or IgG	**78.5 [66.5, 87.7]**	**97.8 [92.4, 99.7]**	**96.2 [87.0, 99.5]**	**86.5 [78.5, 92.4]**	157/155
ELISA IgA	89.2 [79.1, 95.6]	73.9 [63.7, 82.5]	70.7 [59.7, 80.3]	90.7 [81.7, 96.2]	157/155
ELISA IgG	92.3 [83.0, 97.5]	97.8 [92.4, 99.7]	96.8 [88.8, 99.6]	94.7 [88.1, 98.3]	157/155
ELISA IgA or IgG	**93.8 [85.0, 98.3]**	**72.8 [62.6, 81.6]**	**70.9 [60.1, 80.2]**	**94.4 [86.2, 98.4]**	157/155
Hangzhou AllTest IgM	10.8 [4.4, 20.9]	96.7 [90.8, 99.3]	70 [34.8, 93.3]	60.5 [52.5, 68.5]	157/155
Hangzhou AllTest IgG	90.8 [81.0, 96.5]	100 [96.1, 100]	100 [93.9, 100]	93.9 [87.2, 97.7]	157/155
Hangzhou AllTest IgM or IgG	**90.8 [81.0, 96.5]**	**96.7 [90.8, 99.3]**	**95.2 [86.5, 99.0]**	**93.7 [86.8, 97.7]**	157/155
Hangzhou Unlabelled IgM	13.8 [6.5, 24.7]	96.7 [90.8, 99.3]	75.0 [42.8, 94.5]	61.4 [52.9, 69.3]	157/155
Hangzhou Unlabelled IgG	86.2 [75.3, 93.5]	97.8 [92.4, 99.7]	96.6 [88.1, 99.6]	90.9 [83.4, 95.8]	157/155
Hangzhou Unlabelled IgM or IgG	**86.2 [75.3, 93.5]**	**94.6 [87.8, 98.2]**	**91.8 [81.9,97.3]**	**90.6 [83.0, 95.6]**	157/155
Wondfo SARS-CoV-2 Antibody Test	**93.8 [85.0, 98.3]**	**97.8 [92.4, 99.7]**	**96.8 [89.0, 99.6]**	**95.7 [89.5, 98.7]**	157/155
Hightop IgM	59.4 [46.4, 71.5]	100 [96.1, 100]	100 [90.8, 100]	77.3 [68.7, 84.5]	156/154
Hightop IgG	93.8 [85.0, 98.3]	100 [96.1, 100]	100 [94.1, 100]	95.8 [89.7, 98.9]	156/154
Hightop IgM or IgG	**93.8 [84.8, 98.3]**	**100 [96.1, 100]**	**100 [94.1, 100]**	**95.8 [89.7, 98.9]**	156/154

Table 9: Comparative performance of IgG testing for 91 RT-PCR positive patients with confirmed COVID-19 infection, stratified by days post-symptom onset.

Days post-symptom onset	Total (samples)	Onsite IgG (%) [95% CI]	VivaDiag IgG (%) [95% CI]	EUROIMMUN EIA IgG (%) [95% CI]	Hangzhou AllTest IgG (%) [95% CI]	Hangzhou Unlabelled IgG (%) [95% CI]	Wondfo Test Result* (%) [95% CI]	Hightop IgG (%) [95% CI]
0-3	23	0 (0.0) [0.0, 14.8]	0 (0.0) [0.0, 14.8]	0 (0.0) [0.0, 14.8]	0 (0) [0, 14.8]	2 (8.7) [1.1, 28.0]	3 (13.0) [2.8, 38.6]	0 (0.0) [0.0, 14.8]
4-8	28	6 (21.4) [8.3, 41.0]	8 (28.6) [13.2, 48.7]	7 (25.0) [10.7, 44.9]	9 (32.1) [15.9, 52.4]	10 (35.7) [18.6, 55.9]	14 (50.0) [30.7, 69.4]	7 (25.0) [10.7, 44.9]
9-14	21	6 (28.6) [11.3, 52.2]	12 (57.1) [34.0, 78.2]	10 (47.6) [25.7, 70.2]	14 (66.7) [43, 84.5]	15 (8.7) [1.1, 28.0]	16 (76.2) [52.8, 91.8]	13 (61.9) [38.4, 81.9]
15-20	8	6 (75.0) [34.9, 96.8]	6 (75.0) [34.9.0, 96.8]	7 (87.5) [47.4, 99.7]	8 (100) [63.1, 100]	6 (75.0) [34.9, 96.8]	8 (100) [63.1, 100]	7 (87.5) [47.4, 99.7]
21-30	27	23 (85.2) [66.3, 95.8]	21 (77.8) [57.7, 91.4]	27 (100) [87.2, 100]	25 (92.6) [75.7, 99.1]	25 (92.6) [75.7, 99.1]	26 (96.3) [81.0, 99.9]	25 (96.2) [80.4, 99.9][#]
>30	30	23 (76.7) [76.7, 57.7]	24 (80.0) [61.4, 92.3]	26 (86.7) [69.3, 96.2]	26 (86.7) [69.3, 96.2]	25 (83.3) [65.3, 94.4]	27 (90.0) [73.5, 97.9]	28 (93.3) [77.9, 99.1]
Total	**137**	**64 (46.7) [38.2, 55.4]**	**71 (51.8) [43.1, 60.4]**	**77 (56.2) [47.5, 64.7]**	**82 (59.9) [51.1, 68.1]**	**83 (60.6) [51.9, 68.8]**	**94 (68.6) [60.1, 76.3]**	**80 (58.8) [50.1, 67.2]**

CI = Confidence interval (Clopper-Pearson), * = Combined IgM/IgG, [#] = only 26 samples included for this test in this category

3.2 Comparison of Specimen Type for PoCT

A subset of 20 serum and plasma samples, collected simultaneously from participants, were tested in the Hangzhou IgG/IgM Rapid Test assays, Wondfo SARS-CoV-2 Antibody Test and the Hightop SARS-CoV-2 IgM/IgG Antibody Rapid Test. Concordance between serum and plasma samples ranged from 80 - 100% (95% CI: 56.3-100%), (Table 10).

Table 10: 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]
Hangzhou AllTest IgM	5 (25.0) [8.7, 49.1]	3 (15.0) [3.2, 37.9]	80% [56.3, 94.3]
Hangzhou AllTest IgG	15 (75.0) [50.9, 91.3]	15 (75.0) [50.9, 91.3]	100% [83.2, 100]
Hangzhou Unlabelled IgM	7 (35.0) [15.4, 59.2]	5 (25.0) [8.7, 49.1]	80% [56.3, 94.3]
Hangzhou Unlabelled IgG	14 (70.0) [45.7, 88.1]	15 (75.0) [50.9, 91.3]	95% [75.1, 99.9]
Wondfo SARS-CoV-2 Antibody Test	18 (90.0) [68.3, 98.8]	19 (95.0) [75.1, 99.9]	95% [75.1, 99.9]
Hightop IgM	12 (60.0) [36.1, 80.9]	10 (50.0) [27.2, 72.8]	80% [56.3, 94.3]
Hightop IgG	14 (70.0) [45.7, 88.1]	15 (75.0) [50.9, 91.3]	95% [75.1, 99.9]

4. Discussion

Here, we present results of our post-market validation of the Hangzhou IgG/IgM Rapid Test assays, the Wondfo SARS-CoV-2 Antibody Test and the Hightop SARS-CoV-2 IgM/IgG Antibody Rapid Test. Our findings suggest that the performance characteristics of the Wondfo SARS-CoV-2 Antibody Test and the Hightop SARS-CoV-2 IgM/IgG Antibody Rapid Test are only in keeping with those reported in the IFU if samples collected 14 days or earlier following

symptom onset are excluded from the analysis. However even in this situation, the sensitivity for the Hangzhou IgG/IgM Rapid Test assays fell short of that reported, although specificity did approach that of the IFU. Direct comparison with the manufacturers IFU is limited as information regarding the patient / sample cohort used for validation is not provided in the IFUs. Poor sensitivity was found for all assays for samples collected early following symptom onset, again confirming the limited role for PoCT in acute infection.

One of the strengths of this study is the testing of a consistent serum panel across a number of different assays, allowing standardisation and comparison of findings. The large collection of convalescent samples from different time points post infection, for patients who have recovered from COVID-19, highlights the strengths and limitations of these assays. Work is ongoing to determine the performance characteristics of additional assays supplied by the Therapeutic Goods of Australia.

In summary, our data describes the performance characteristics of three further PoCT devices. Overall, our findings remain in keeping with the position statements by the Public Health Laboratory Network (PHLN) and the Royal College of Pathologists Australasia (RCPA) that note that serological assays have limited, if any, role in the diagnosis of acute COVID-19 infection. Our findings strongly suggest that PoCT devices should not be used in the diagnosis of acute COVID-19, and have limited, if any, role in clinical management of individual patients. The role of PoCT in population-level serosurveys remains to be seen in the context of other emerging serological tests for SARS-CoV-2. The curated panel of samples assembled for this study is being expanded and provides a valuable repository for rapid validation of new serological assays as they become available on the Australian market.

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Appendix 1: Summary of test results by cohort tested for Hangzhou AllTest, Hangzhou unlabelled packaging,Wondfo SARS-CoV-2 Antibody Test and the Hightop SARS-COV-2 IgM/IgG Antibody Rapid Test

Overall results for the Hangzhou AllTest IgG/IgM Rapid Test versus RT-PCR for 183 patients.

Cohort	Hangzhou AllTest IgM		Hangzhou AllTest IgG		Hangzhou AllTest IgM or IgG		Total (samples)
	Positive	Negative	Positive	Negative	Positive	Negative	
RT-PCR Positive	18	119	82	55	83	54	**137**
Controls	3	89	0	92	3	89	**92**
Total	**21**	**208**	**82**	**147**	**86**	**143**	**229**

Overall results for the Hangzhou unlabelled packaging Rapid Test versus RT-PCR for 183 patients.

Cohort	Hangzhou unlabelled IgM		Hangzhou unlabelled IgG		Hangzhou unlabelled IgM or IgG		Total (samples)
	Positive	Negative	Positive	Negative	Positive	Negative	
RT-PCR Positive	21	116	83	54	84	53	**137**
Controls	3	89	2	90	5	87	**92**
Total	**24**	**205**	**85**	**144**	**89**	**140**	**229**

Overall results for the Wondfo SARS-CoV-2 Antibody Test versus RT-PCR for 183 patients.

Cohort	Wondfo Test Result		Total (samples)
	Positive	Negative	
RT-PCR Positive	94	43	**137**
Controls	2	90	**92**
Total	**96**	**133**	**229**

Overall results for the Hightop SARS-CoV-2 IgM/IgG Antibody Rapid Test versus RT-PCR for 182 patients.

Cohort	Hightop unlabelled IgM		Hightop unlabelled IgG		Hightop unlabelled IgM or IgG		Total (samples)
	Positive	Negative	Positive	Negative	Positive	Negative	
RT-PCR Positive	53	83	80	56	83	53	**136**
Controls	0	92	0	92	0	92	**92**
Total	**53**	**175**	**80**	**148**	**83**	**145**	**228**

Appendix 2. Summary of discordant results for the Hanzhou AllTest IgG/IgM Rapid Test and Hangzhou unlabelled test; the Wondfo SARS-CoV-2 Antibody Test and the Hightop SARS-CoV-2 IgM/IgG

Test Assay	IgM		IgM Concordant (%) [95% CI]	IgG ^		IgG Concordant (%) [95% CI]	Total (samples)
	Positive	Negative		Positive	Negative		
Hanzhou AllTest	21	208	212 (92.6) [88.4, 95.6]	82	147	210 (91.7) [87.4, 94.9]	229
Hangzhou unlabelled	24	205		85	144		
Wondfo Lot 1	n/a	n/a	n/a	100	128	209 (91.7) [87.3, 94.9]	228 #
Wondfo Lot 2	n/a	n/a		95	133		
Hightop Lot 1	59	169	213 (93.4) [89.4, 96.3]	81	147	219 (96.1) [92.6, 98.2]	228 %
Hightop Lot 2	57	171		82	146		

n/a = not applicable; ^ IgM/IgG combined test line for Wondfo; # 1 negative cohort sample insufficient for testing in duplicate; % 1 discordant sample excluded from dataset as insufficient kits to test in triplicate

Appendix 3. Manufacturer's instructions for use for serological assays included in this evaluation (attached)