Doherty Institute

THE UNIVERSITY OF MELBOURNE

The Royal Melbourne Hospital

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

Final Report

Post-market validation of three serological assays for COVID-19

–

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Executive Summary

Here, we present results of our post-market validation of 3 serological assays for the detection of SARS-CoV-2 antibodies. Two lateral flow assays were included, the Onsite IgM/IgG Rapid Test and the VivaDiag IgM/IgG Rapid Test, as well as one laboratory based enzyme immunoassay, the EUROIMMUN EIA. 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 performance characteristics of both PoCT are below that reported by the manufacturer in the IFU. Although the sensitivities of the two PoCT devices improved with increasing duration between sample collection and symptom onset, they did not match the manufacturer's stated performance criteria at any time point. Our observed sensitivities and specificities of the EUROIMMUN EIA assay are broadly in keeping with the manufacturer's updated performance criteria (updated on 22nd April to reflect the inclusion of additional samples), but further highlight the poor sensitivity of serology in acute infection.

Overall, our findings are in keeping with recent 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

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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 two 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 point of care tests (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 two serological PoCT (both listed on the ARTG) and one enzyme immunoassay (EIA), not yet listed on the ARTG but commercially available to Australian laboratories.

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 the 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	137/90
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 OnSite COVID-19 IgG/IgM Rapid Test

The OnSite IgG/IgM Rapid Test is a single use lateral flow test, which involves 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. According to the accompanying manufacturer's instructions for use (IFU), the test is invalid if this line does not display a colour change. The specific SARS-CoV-2 recombinant antigen(s) incorporated into the assay are not described in the IFU. The manufacturer recommends taking either a positive IgM or a positive IgG as indication of COVID-19 infection, advising that the test result should be interpreted in conjunction with clinical findings and alternative test methods should be considered to confirm results. The reported performance characteristics are provided in the IFU (Appendix 2), but it is not mentioned in the IFU: (i) where validation samples are sourced from; (ii) whether plasma, serum, whole blood or a combination of these were used for validation (iii) when in the course of COVID-19 infection each sample was taken, or (iv) which RT-PCR assay was used as a gold-standard, and (v) where RT-PCR testing was performed.

2.2.2 VivaDiag COVID-19 IgM/IgG Rapid Test

The VivaDiag COVID-19 IgM/IgG Rapid Test is a single use lateral flow test, of the same overall design as the OnSite IgG/IgM Rapid Test. The specific SARS-CoV-2 recombinant antigen(s) incorporated into the assay are not described in the IFU. The manufacturer recommends taking either a positive IgM or a positive IgG as indication of COVID-19 infection, advising that the test result should be interpreted in conjunction with clinical findings and cannot be alone used for the diagnosis of COVID-19. The related performance characteristics provide in the IFU are described relative to time elapsed since infection (4-10

days and 11-24 days, Appendix 2), but it is not mentioned in the IFU: (i) where validation samples are sourced from; (ii) whether plasma, serum, whole blood or a combination of these were used for validation; (iii) which RT-PCR assay was used as a gold-standard, and (iv) where RT-PCR testing was performed.

2.2.3 EUROIMMUN EIA

The EUROIMMUN EIA is an enzyme immunoassay (EIA) that involves semi-quantitative detection of anti-SARS-CoV-2 IgA or IgG antibodies in serum, through binding to a recombinant structural antigen (S1 domain of the Spike protein) fixed to reagent wells. If test sera contain anti-SARS-CoV-2 antibodies, a second incubation step using enzyme-labelled anti-IgA or anti-IgG will catalyse a colour reaction, detected by an optical density reader. Results are reported relative to the control sample with negative, borderline or positive findings. The manufacturer reports sensitivity for IgG of 22.4% at 10 days or less following symptom onset; 87.5% between 10 and 20 days post symptom onset and 100% sensitivity after 20 days, for a cohort of 71 samples from 64 European patients confirmed by SARS-CoV-2 RT-PCR molecular testing (Appendix 2). IgA sensitivity is quoted at 44.8%, 100% and 100% for the same time intervals, respectively. IgA was less specific than IgG (90.5% versus 99.3%, respectively).

2.2.4 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 (7), 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.

2.2.3 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, including use of the plastic droppers if provided in the kits. Testing of each sample was performed in triplicate for the Onsite IgM/IgG Rapid Test, unless precluded by low volume (2/188 samples; 1.1%, neither discordant). A subset of 35 RT-PCR positive samples were also tested in triplicate using OnSite IgM/IgG Rapid Test tests from a different lot number to assess lot variation. Discordant results were scored as per the majority finding (i.e. 2/3) and any faint line present at test termination was considered a positive result.

Testing of the VivaDiag was conducted as above, except that tests were undertaken in duplicate (due to limited kit provision), with a third test undertaken for discordant results.

Testing of the EUROIMMUN EIA was performed in the Serology Laboratory, VIDRL, by an experienced senior scientist with extensive experience in performing EIA testing. Testing was performed exactly as per the IFU, with the use of automated plate washers and an optical density reader. A single test was undertaken per isolate due to limitations on kit availability.

Microneutralisation assays were undertaken in the Subbarao Laboratory, as described above.

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 (true positives).
- 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) (true negatives).
- Positive predictive value was calculated as the number of true positive results as a proportion of all samples that tested positive in the serological test assay.

- Negative predictive value was calculated as the number of true negative results as a proportion of the number of samples that tested negative in the serological test 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 (i) plasma and (ii) whole blood from finger prick samples.

3. Results

3.1 Comparison of serological PoCT with RT-PCR

In total, 229 samples from 182 patients were included in this analysis (Table 1), with 137 samples from 90 patients in the sensitivity analysis and 92 samples from 92 patients in the specificity analysis. The overall sensitivity for the Onsite IgM/IgG Rapid Test for detection of IgM or IgG was 56.9% (95%CI 48,6 – 64.9) and for the VivaDiag IgM/IgG Rapid Test was 51.8% (95%CI 43.1 – 60.4). Sensitivity increased with increasing time post-symptom onset (Tables 2 and 3).

Table 2: Comparison of the Onsite IgM/IgG Rapid Test with RT-PCR for 90 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	22	0 (0.0) [0.0, 15.4]	0 (0.0) [0.0, 15.4]	0 (0.0) [0.0, 15.4]
4-8	28	10 (35.7) [18.6, 55.9]	6 (21.4) [8.3, 41.0]	10 (35.7) [18.6, 55.9]
9-14	21	12 (57.1) [34.0, 78.2]	6 (28.6) [11.3, 52.2]	12 (57.1) 34.0, 78.2]
15-20	9	7 (77.8) [40.0, 97.2]	6 (66.7) [29.9, 92.5]	7 (77.8) [40.0, 97.2]
21-30	27	20 (74.1) [53.7, 88.9]	23 (85.2) [66.3, 95.8]	24 (88.9) [70.8, 97.7]
>30	30	19 (63.3) [43.9, 80.1]	23 (76.7) [76.7, 57.7]	25 (83.3) [65.3, 94.4]
Total	**137**	**68 (49.6) [40.6, 58.0]**	**64 (46.7) [38.2, 55.4]**	**78 (56.9) [48.6, 64.9]**

CI = Confidence interval (Clopper-Pearson)

Table 3: Comparison of the VivaDiag IgM/IgG Rapid Test with RT-PCR for 90 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	22	0 (0.0) [0.0 15.4]	0 (0.0) [0.0 15.4]	0 (0.0) [0.0 15.4]
4-8	28	8 (28.6) [13.2, 48.7]	8 (28.6) [13.2, 48.7]	8 (28.6) [13.2, 48.7]
9-14	21	11 (52.4) [29.8, 74.3]	11 (52.4) [29.8, 74.3]	11 (52.4) [29.8, 74.3]
15-20	9	7 (77.8) [40.0, 97.2]	7 (77.8) [40.0, 97.2]	7 (77.8) [40.0, 97.2]
21-30	27	21 (77.8) [57.7, 91.4]	21 (77.8) [57.7, 91.4]	21 (77.8) [57.7, 91.4]
>30	30	24 (80.0) [61.4, 92.3]	24 (80.0) [61.4, 92.3]	24 (80.0) [61.4, 92.3]
Total	**137**	**71 (51.8) [43.1, 60.4]**	**71 (51.8) [43.1, 60.4]**	**71 (51.8) [43.1, 60.4]**

CI = Confidence interval

Of note, apart from one instance in the control cohort, the VivaDiag IgM and IgG returned exactly the same result for each component of the test (i.e. IgM and IgG were always concordant and never appeared separately).

Sample cohorts 2 and 3 (Table 1) were used to assess specificity. The specificity of the Onsite IgM/IgG Rapid Test was 95.6% [95% CI 89.2-98.8%] and the VivaDiag IgM/IgG Rapid Test was 97.8 [95% CI 92.4-99.7%] (Table 4).

When only samples collected after day 20 were considered, the sensitivity of the Onsite IgM/IgG Rapid Test was 84.8% (95% CI 73.9-92.5%), and the VivaDiag IgM/IgG Rapid Test was 78.8% (95% CI 67.0-87.9%) (Table 5).

3.2 Comparison of EIA, PoCT and RT-PCT

In total, 208 serum samples from 167 patients were assessed using the EUROIMMUN EIA. The overall sensitivity was 68.1% (95% CI 58.8-76.5) and specificity was 72.8% (95% CI 58.8-76.5) (Table 6). There was a significant increase in the sample / calibration ratio over time ($P < 0.001$) (Figure 1).

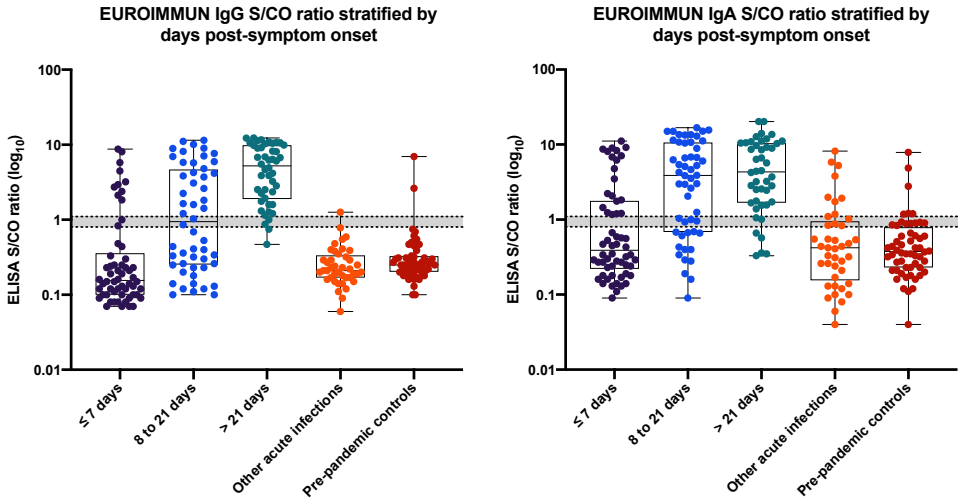

Figure 1. Comparison of sample / calibration ratio in the EUROIMMUN assay stratified by days post-symptom onset.

Table 4: Comparative performance of serological assays with RT-PCR, regardless of days post-symptom onset.

Performance Characteristic / Test Assay	Sensitivity (P/TP, %) [95% CI]	Specificity (N/TN, %) [95% CI]	Positive Predictive Value (TP/P, %) [95% CI]	Negative Predictive Value (TN/N, %) [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]	229/182
OnSite IgG	46.7 [38.2, 55.4]	98.9 [94.1, 99.97]	98.9 [91.7, 99.96]	55.5 [47.5, 63.2]	229/182
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]**	229/182
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]	229/182
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]	229/182
VivaDiag IgM or IgG	**51.8 [43.1, 60.4]**	**97.8 [92.4, 99.7]**	**97.3 [90.5, 99.7]**	**57.6 [49.5, 65.6]**	229/182
EIA IgA	65.5 [56.1, 74.1]	72.8 [62.6, 81.6]	75.2 [65.7, 83.3]	62.6 [52.7, 71.8]	208/167
EIA IgG	57.8 [48.2, 66.9]	97.8 [92.4, 99.7]	97.1 [89.9, 99.7]	64.7 [56.2, 72.7]	208/167
EIA IgA or IgG	**68.1 [58.8, 76.5]**	**72.8 [62.6, 81.6]**	**76.0 [66.6, 83.8]**	**64.4 [54.4, 73.6]**	**208/167**

P = Positive result in Onsite IgM/IgG Rapid test; TP = True positive (positive by RT-PCR);
N = Negative result in Onsite IgM/IgG Rapid Test; TN = True negative (not positive by RT-PCR)

Table 5: Comparative performance of serological assays with RT-PCR for samples collected >20 days post symptom onset.

Performance Characteristic / Test Assay	Sensitivity (P/TP) [95% CI]	Specificity (N/TN) [95% CI]	Positive Predictive Value (TP/P) [95% CI]	Negative Predictive Value (TN/N) [95% CI]	Total (samples/ patients)
OnSite IgM	69.7 [57.2, 80.4]	96.7 [90.8, 99.3]	93.9 [83.1, 98.7]	81.7 [73.1, 88.4]	158/132
OnSite IgG	78.8 [67.0, 87.9]	98.9 [94.1, 99.97]	98.1 [89.9, 99.95]	86.7 [78.6, 92.5]	158/132
Onsite IgM or IgG	**84.8 [73.9, 92.5]**	**95.6 [89.2, 98.8]**	**93.3 [83.8, 98.2]**	**89.8 [82.0, 95.0]**	**158/132**
VivaDiag IgM	78.8 [67.0, 87.9]	97.8 [92.4, 99.7]	96.3 [87.3, 99.6]	86.5 [78.5, 92.4]	158/132
VivaDiag IgG	78.8 [67.0, 87.9]	98.9 [94.1, 99.97]	98.1 89.9, 99.95]	86.7 [78.6, 92.5]	158/132
VivaDiag IgM or IgG	**78.8 [67.0, 87.9]**	**97.8 [92.4, 99.7]**	**96.3 [87.3, 99.6]**	**86.5 [78.5, 92.4]**	**158/132**
EIA IgA	90.9 [80.0, 97.0]	72.8 [62.6, 81.6]	75.8 [63.6, 85.5]	93.1 [84.5, 97.7]	147/125
EIA IgG	92.7 [82.4, 98.0]	97.8 [92.4, 99.7]	96.2 [87.0, 99.4]	95.7 [89.5, 98.8]	147/125
EIA IgA or IgG	**96.4 [87.5, 99.6]**	**72.8 [62.6, 81.6]**	**67.9 [56.4, 78.1]**	**97.1 [89.9, 99.8]**	**147/125**

P = Positive result in Onsite IgM/IgG Rapid test; TP = True positive (positive by RT-PCR);
N = Negative result in Onsite IgM/IgG Rapid Test; TN = True negative (not positive by RT-PCR)

Table 6: Comparison of the EUROIMMUN EIA with RT-PCR for 75 patients with confirmed COVID-19 infection, stratified by days post-symptom onset

Days post-symptom onset	Samples (n)	IgA detected (%) [95% CI]	IgG detected (%) [95% CI]	IgA or IgG (%) [95% CI]
0-3	15	2 (13.3) [1.7, 40.5]	0 (0.0) [0.0, 15.44]	2 (13.3) [1.7, 40.5]
4-8	25	11 (44.0) [24.4, 65.1]	6 (24.0) [9.4, 45.1]	11 (44.0) [24.4, 65.1]
9-14	21	13 (61.9) [38.4, 81.9]	10 (47.6) [25.7, 70.2]	13 (61.9) [38.4, 81.9]
15-20	9	9 (100) [66.4, 100]	7 (77.8) [40.0, 97.2]	9 (100) [66.4, 100]
21-30	23	21 (91.3) [72.0, 98.9]	23 (100) [85.2, 100]	23 (100) [85.2, 100]
>30	23	20 (87.0) [66.4, 97.2]	21 (91.3) [72.0, 98.9]	21 (91.3) [91.3, 98.9]
Total	**116**	**76 (65.5) [56.1, 74.1]**	**64 (57.1) [45.7, 64.4]**	**79 (68.1) [58.8. 76.5]**

CI = Confidence interval, * = 8 pending; # = 1 pending; ^ = 3 pending; % = 7 pending

When stratified by days post-symptom onset, the sensitivity of the EIA IgG was consistently higher than the two PoCT devices (Table 7); only IgG was compared as it was common to all three assays.

Table 7: Comparative performance of IgG testing for RT-PCR positive patients with confirmed COVID-19 infection, stratified by days post-symptom onset.

Days post-symptom onset	Onsite IgG (%) [95% CI] (n = 137)	VivaDiag IgG (%) [95% CI] (n = 137)	EUROIMMUN EIA IgG (%) [95% CI] (n = 116)
0-3	0 (0.0) [0.0, 15.4]	0 (0.0) [0.0 15.4]	0 (0.0) [0.0, 15.44]
4-8	6 (21.4) [8.3, 41.0]	8 (28.6) [13.2, 48.7]	6 (24.0) [9.4, 45.1]
9-14	6 (28.6) [11.3, 52.2]	11 (52.4) [29.8, 74.3]	10 (47.6) [25.7, 70.2]
15-20	6 (66.7) [29.9, 92.5]	7 (77.8) [40.0, 97.2]	7 (77.8) [40.0, 97.2]
21-30	23 (85.2) [66.3, 95.8]	21 (77.8) [57.7, 91.4]	23 (100) [85.2, 100]
>30	23 (76.7) [76.7, 57.7]	24 (80.0) [61.4, 92.3]	21 (91.3) [72.0, 98.9]
Total	**64 (46.7) [38.2, 55.4]**	**71 (51.8) [43.1, 60.4]**	**64 (57.1) [45.7, 64.4]**

CI = Confidence interva

One serum sample from a PCR confirmed SARS-CoV-1 infection and 2 serum samples from PCR confirmed MERS-CoV were tested in the OnSite IgM/IgG Rapid Test and the EUROIMMUN EIA. The SARS-CoV-1 serum was positive in both assays and both MERS-CoV samples were negative. There was insufficient sample to test in the VivaDiag IgM/IgG Rapid Test.

3.3. Sensitivity comparison with microneutralisation assay

In total, 65 serum samples from RT-PCR positive patients were tested in parallel on the EUROIMMUN EIA, Onsite IgM/IgG Rapid Test and microneutralisation assay, with a subset of 49 tested in the VivaDiag IgM/IgG Rapid Test. Using a positive microneutralisation result (i.e. titres over 40) as the reference standard instead of PCR, sensitivity for each of the serological assays was:

- Onsite IgM/IgG Rapid Test IgM or IgG: **88.6% [95%CI 75.4-96.2]**
- VivaDiag IgM/IgG Rapid Test IgM or IgG: **86.8 [95%CI 72.2-94.7]**
- EUROIMMUN EIA IgA or IgG: **97.7% [95%CI 88.0-99.9].**

3.4 Comparison of Specimen Type for PoCT

A subset of participants (67 patients for the OnSite IgM/IgG Rapid Test and 22 participants for the VivaDiag IgM/IgG Rapid Test) had simultaneous whole blood (fingerprick) testing, and venous blood drawn for serum and plasma testing. Concordance for sample type was 81.8% - 88.1%, (Tables 8 & 9).

Table 8: Overall results for the Onsite IgM/IgG Rapid Test versus RT-PCR, according to sample type

Test Assay	Onsite IgM/IgG Rapid Test IgM				Onsite IgM/IgG Rapid Test IgG			
Sample Type	Serum (%)	Plasma (%)	Whole Blood (%)	Concordance [95%CI]	Serum (%)	Plasma (%)	Whole Blood (%)	Concordance [95%CI]
Positive Tests	49 (73.1)	51 (76.1)	51 (76.1)	82.1% [70.8, 90.4]	50 (74.6)	52 (77.6)	43 (64.2)	88.1% [77.8, 94.7]

Table 9: Overall results for the VivaDiag IgM/IgG Rapid Test versus RT-PCR, according to sample type

Test Assay	VivaDiag IgM/IgG Rapid Test IgM				VivaDiag IgM/IgG Rapid Test IgG			
Sample Type	Serum (%)	Plasma (%)	Whole Blood (%)	Concordance [95%CI]	Serum (%)	Plasma (%)	Whole Blood (%)	Concordance [95%CI]
Positive Tests	13 (59.1)	14 (63.6)	12 (54.4)	86.4% [65.1, 97.1]	13 (59.1)	14 (63.6)	12 (54.5)	81.8% [59.7, 94.8]

4. Discussion

Here, we present results of our post-market validation of the Onsite IgM/IgG Rapid Test, VivaDiag IgM/IgG Rapid Test and the EUROIMMUN EIA. Our findings suggest that the performance characteristics of both PoCT are below that reported by the manufacturer in the IFU. Although the sensitivities of the two PoCT devices improved with increasing duration between sample collection and symptom onset, they did not match the manufacturer's stated performance criteria. However, direct comparison with the

manufacturers IFU is limited as information regarding the patient / sample cohort used for validation is not provided in the IFUs. Our observed sensitivities and specificities of the EUROIMMUN EIA assay are broadly in keeping with the manufacturer's updated performance criteria (updated on 22nd April to reflect the inclusion of additional samples), but further highlight the poor sensitivity in acute infection. Again, direct comparison of results is limited by differences in sample cohorts.

One of the strengths of this study is the collection of convalescent samples from patients who have recovered from COVID-19. By establishing a community collection platform, we tested over 50 patients who were more than 21 days post-symptom onset, representing one of the largest reported convalescent patient cohorts to date. Our sensitivity and specificity analysis of the two provided PoCT devices are in keeping with a recent evaluation of nine PoCT devices in the United Kingdom, which reported a range of PoCT sensitivities from 55% (95% CI 36-72%) to 70% (95% CI 51-84%) and specificity from 95% (95% CI 86-99%) to 100% (95% CI 94-100%) (8). Of note, we observed that the sensitivity of each assay in this study was different when comparing against microneutralisation as a reference standard, rather than RT-PCR. This highlights the need for standardised protocols, including reference standards, across laboratories when conducting evaluations of emerging serological assays.

In summary, our data describe the performance characteristics of two PoCT devices and a commercially available EIA assay. Overall, our findings are in keeping with recent 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.

5. Acknowledgements

We thank staff of the Pathology department at RMH, 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.

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Appendix 1: Summary of test results by cohort tested for Onsite IgM/IgG Rapid Test, VivaDiag IgM/IgG Rapid Test and EUROIMMUN EIA

Overall results for the Onsite IgM/IgG Rapid Test versus RT-PCR for 182 patients.

Cohort	Onsite IgM		Onsite IgG		Onsite IgM or IgG		Total (samples)
	Positive	Negative	Positive	Negative	Positive	Negative	
RT-PCR Positive	68	69	64	73	78	59	**137**
Controls	3	89	1	91	4	88	**92**
Total	**71**	**158**	**65**	**164**	**82**	**147**	**229**

Overall results for the VivaDiag IgM/IgG Rapid Test versus RT-PCR for 182 patients.

Cohort	VivaDiag IgM		VivaDiag IgG		VivaDiag IgM or IgG		Total (samples)
	Positive	Negative	Positive	Negative	Positive	Negative	
RT-PCR Positive	71	66	71	66	71	66	**137**
Controls	2	90	1	91	2	90	**92**
Total	**73**	**156**	**72**	**157**	**73**	**156**	**229**

Overall results for the EUROIMMUN EIA versus RT-PCR for 125 patients.

Cohort	EUROIMMUN EIA IgM		EUROIMMUN EIA IgG		EUROIMMUN EIA IgM or IgG		Total (samples)
	Positive	Negative	Positive	Negative	Positive	Negative	
RT-PCR Positive	76	40	67	49	79	37	**116**
Controls	25	67	2	90	25	67	**92**
Total	**101**	**107**	**69**	**139**	**104**	**143**	**208**

Appendix 2. Manufacturer's instructions for use for serological assays included in this evaluation (attached)

OnSite™ COVID-19 IgG/IgM Rapid Test

REF R0180C CE

Instructions for Use

INTENDED USE

The *OnSite* COVID-19 IgG/IgM Rapid Test is a lateral flow immunoassay for the detection of anti-SARS-CoV-2 IgG and IgM antibodies in human serum, plasma or whole blood. It is intended to be used by healthcare professionals as an aid in the diagnosis of infection with SARS-CoV-2 coronavirus.

Any interpretation or use of this preliminary test result must also rely on other clinical findings as well as on the professional judgment of healthcare providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

SUMMARY AND EXPLANATION OF THE TEST

SARS-CoV-2 belongs to the broad family of coronaviruses which are capable of causing illnesses ranging from the common cold to more severe diseases[1]. SARS-CoV-2 infections cause COVID-19 disease. The infected patients have a wide range of clinical symptoms, from little to no symptoms, to fever, tiredness and dry cough, and possibly leading to severe sickness and death. Most patients recover without special treatment. Around 1 out of every 6 patients who get COVID-19 become seriously ill and develop difficulty breathing. Older people and those with underlying medical problems, like high blood pressure, heart problems or diabetes, are more likely to develop serious illness.

Human-to-human transmission of the virus has been confirmed and occur primarily via respiratory droplets from coughs and sneezes within a range of about 6 feet (1.8 m). Viral RNA has also been found in stool samples from patients. It's possible that the virus can be infectious even during the incubation period, but this has not been proven. WHO stated on February 1, 2020 that at this time, "transmission from asymptomatic cases is likely not a major driver of transmission[2-5].

Currently, the laboratory method for detecting SARS-CoV-2 infection is RT-PCR. However, this method requires sophisticated equipment and highly trained laboratory technicians. Moreover, viral load decreases rapidly 9 or 10 days after onset of symptoms. During the acute phase of infection, the titer of IgM to SARS-CoV-2 rises rapidly and peaks around 2-3 weeks after the infection. SARS-CoV-2 specific IgG antibodies appear shortly after IgM and persist for months[6]. It is unknown if SARS-CoV-2 infection leads to lifetime immunity or come with a 2nd infection. Nevertheless, the SARS-CoV-2 specific antibodies are useful markers for diagnosis and epidemiologic survey.

The *OnSite* COVID-19 IgG/IgM Rapid Test detects anti-SARS-CoV-2 IgG and IgM antibodies in human serum, plasma or whole blood. The test can be performed within 15 minutes by minimally skilled personnel without the use of cumbersome laboratory equipment.

TEST PRINCIPLE

The *OnSite* COVID-19 IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay. The test strip in the cassette consists of: 1) a colored conjugate pad containing SARS-CoV-2 recombinant antigens conjugated with colloidal gold (SARS-CoV-2 conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The G line is pre-coated with antibodies for the detection of anti-SARS-CoV-2 IgG, the M line is pre-coated with antibodies for the detection of anti-SARS-CoV-2 IgM, and the C line is pre-coated with a control line antibody.

When an adequate volume of specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action along the cassette strip. Anti-SARS-CoV-2 IgG, if present in the specimen, will bind to the SARS-CoV-2 conjugates. The immunocomplex is then captured by the pre-coated anti-human IgG, forming a colored G line, indicating an anti- SARS-CoV-2 IgG positive test result, suggesting a recent infection or a past infection. Anti-SARS-CoV-2 IgM, if present in the specimen, will bind to the SARS-CoV-2 conjugates. The immunocomplex is then captured by the pre-coated anti-human IgM, forming a colored M line, indicating an anti-SARS-CoV-2 IgM positive test result and suggesting an acute SARS- CoV-2 infection. An IgM and IgG double positive result suggests a late acute infection.

Absence of any of the test lines (G or M) suggests a negative result. Each test contains an internal control (C line) which should exhibit a colored line of the control antibodies regardless of color development on any of the test lines. If the C line does not develop, the test result is invalid, and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. Plastic droppers
3. Detection buffer (REF SB-R0180, 3 mL/bottle)
4. Instructions for Use

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. Lancing device for whole blood test

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

1. Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Once the pouch is opened, it should be used within 30 minutes to avoid failure caused by the moisture absorption.
4. Do not use expired devices or components.
5. Do not use the components of any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood specimen for testing.
7. Use only one specimen per device. Do not combine specimens.
8. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
9. Follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
10. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
11. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
12. Handle external controls in the same manner as patient specimens.
13. Read test results 10-15 minutes after a specimen is applied to the sample well of the device. Reading the test result after 15 minutes should be considered invalid and must be repeated.
14. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2-30°C. If stored at 2-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable until the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperatures above 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them with standard bio-safety procedures.

Plasma/Serum

Step 1: Collect blood specimen into collection tube containing EDTA or citrate **(not Heparin)** for plasma or collection tube containing no anticoagulants for serum by venipuncture.

Step 2: A) To prepare plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.

B) To prepare serum specimen, allow blood to clot, then centrifuge collected specimens and carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. If not tested immediately, specimens can be stored at 2-8°C for up to 3 days, or frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not use specimens demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid possible interference with result interpretation.

Whole Blood

Step 1: Whole blood can be obtained by either fingertip puncture or by venipuncture. Collect venous blood specimen into a collection tube containing EDTA or citrate **(not Heparin).** Do not use hemolyzed blood for testing.

Whole blood specimens should be stored in refrigeration (2-8°C), if not tested immediately. The specimens must be tested within 24 hours of collection.

Note: Do not test specimens demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

ASSAY PROCEDURE

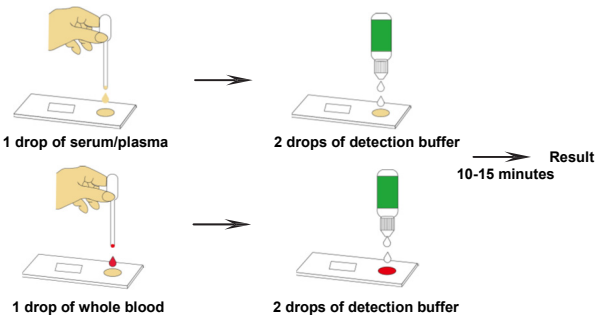
Step 1: Ensure that specimen and test components are equilibrated to room temperature. If frozen, mix the specimen well after thawing, prior to performing the assay.

Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Label the device with specimen's ID number.

Step 4: Fill the plastic dropper with the specimen.
Holding the dropper vertically, dispense 1 drop (~10 µL) of serum, plasma or whole blood (~15 µL) into the S well of the test cassette. Ensure there are no bubbles.

Step 5: Immediately add 2 drops (~70-100 µL) detection buffer into the S well of the test cassette. Ensure there are no bubbles.



Step 6: Set up timer.

Step 7: Read results at 10-15 minutes. Positive results may be visible as soon as 1 minute. Negative results must be confirmed at the end of 15 minutes. ***Any results interpreted outside 10-15 minutes window should be considered invalid and must be repeated. Discard used device after interpreting the results following local laws governing the disposal of device.***

QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen extract. Otherwise, review the whole procedure and repeat test with a new device.

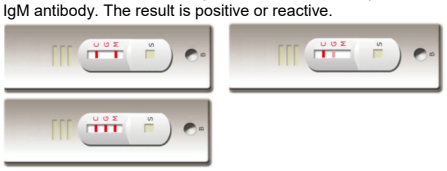
2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kit is used.
   c. A new shipment of kits is used.
   d. The temperature used during storage of the kit falls outside of 2-30°C.

e. The temperature of the test area falls outside of 15-30°C.
f. To verify a higher than expected frequency of positive or negative results.
g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

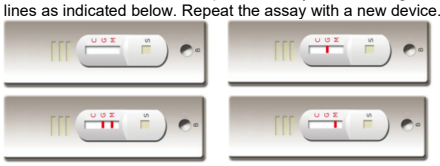
1. **NEGATIVE RESULT:** If only the C line is present, the absence of any color in both test lines (M and G) indicates that there is no SARS-CoV-2 IgG or IgM antibodies detected. The result is negative or non-reactive.

2. **POSITIVE RESULT:** In addition to the presence of the C line, if the G or M line develops, or both G and M lines develop, the test indicates the presence of SARS-CoV-2 IgG and/or IgM antibody. The result is positive or reactive.



Specimens with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis decision is made.

3. **INVALID:** If no C line develops, the assay is invalid regardless of any color in the test lines as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

1. **Clinical Performance**

A total of 551 specimens were collected from susceptible subjects and tested with the *OnSite* COVID-19 IgG/IgM Rapid Test and by a commercial PCR kit. Comparison for all subjects is shown in the following table:

	OnSite COVID-19 IgG/IgM Rapid Test IgG Results		*OnSite* COVID-19 IgG/IgM Rapid Test IgM Results	
PCR Test	**Positive**	**Negative**	**Positive**	**Negative**
Positive	216	7	174	49
Negative	0	328	2	326
Total	216	335	176	375

Relative IgG Sensitivity: 96.86% (95% CI: 93.66%-98.47%), Relative IgG Specificity: 100% (95% CI: 98.84%-100%)

Relative IgM Sensitivity: 78.03% (95% CI: 72.14%-82.96%), Relative IgM Specificity: 99.39% (95% CI: 97.80%-99.83%)

Relative Test Sensitivity: 96.86% (95% CI: 93.66%-98.47%), Relative Test Specificity: 99.39% (95% CI: 97.80%-99.83%), Overall Agreement: 98.37% (95% CI: 96.93%-99.14%)

2. **Cross reactivity**

No false positive anti-SARS-CoV-2 virus IgG and IgM test results were observed on at least 5 specimens from patients negative for COVID-19, but presenting similar clinical symptoms, as well as 2-5 specimens from the following disease states or specific conditions:

HBV	HCV	HIV	Pneumonia mycoplasma
Tuberculosis	Syphilis	Dengue	Pneumonia chlamydia
Zika	Chikungunya		

3. **Interference**

No interference was observed with the potentially interfering substances listed below at the indicated concentration:

Bilirubin	15 mg/dL	Triglycerides	400 mg/dL
Hemoglobin	20 g/dL	Rheumatoid factor	3250 IU/mL

LIMITATIONS OF TEST

1. The *OnSite* COVID-19 IgG/IgM Rapid Test is limited to the qualitative detection of anti-SARS-CoV-2 virus IgG and IgM in human serum, plasma and whole blood. The intensity of the test band does not have linear correlation with the antibody titer in the specimen.
2. The Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of antibodies to SARS-CoV-2 virus in serum, plasma and whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate results.
3. **Heparin potentially affects assay results; therefore, it should not be used as an anticoagulant.**
4. The *OnSite* COVID-19 IgG/IgM Rapid Test is not applicable for patients who have received vaccination or have been treated with antibody drug to SARS-CoV-2 coronavirus since the SARS-CoV-2 IgG/IgM antibodies may not be caused by virus infections in those cases.
5. The result is used as an aid to detection of SARS-CoV-2 infection only. A negative or non-reactive test result does not confirm the test subject does not carry the virus. It may be due to a poor immune response, the quantity of antibodies to SARS-CoV-2 virus present in the specimen is below the limits of detection, or if the antibodies are not present during the stage of disease in which a specimen is collected. Infection may progress rapidly. If the symptoms persist, while the result from *OnSite* COVID-19 IgG/IgM Rapid is negative or non-reactive, it is recommended to test with an alternative test method.
6. While positive test results only indicate that the test subject was infected before testing, it does not confirm that the test subject carries the virus. The test result must be carefully evaluated in conjunction with other methods. Take clinical symptoms into consideration.
7. It is possible that patients who were exposed to other viruses may show some level of reactivity with this test, due to potential cross-reactivity. Unusually high titer of heterophile antibodies or rheumatoid factor present in some specimens may affect the expected results[7, 8]. Factors, such as operational error can also potentially induce false results.

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Index of Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	Σ_N	Tests per kit
2°C 30°C	Store between 2-30°C	EC REP	Authorized Representative		Do not reuse
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
13855 Stowe Drive
Poway, CA 92064, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany

PI-R0180C Rev. C
Date released: 2020-03-17
English version

For Export Only, Not For Re-sale in the USA.

COVID-19 IgM/IgG Rapid Test Package Insert

REF VID35-08-011 / VID35-08-012 / VID35-08-013 / VID35-08-014	English

PRINCIPLE AND INTENDED USE

VivaDiag™ COVID-19 IgM/IgG Rapid Test is an *in vitro* diagnostic test for the qualitative determination of COVID-19's IgM and IgG antibodies in human whole blood (from vein or fingertip), serum or plasma.
The test kit consists of test devices and buffer. It is for *in vitro* diagnostic use only, and can be used in point-of-care testing settings and central laboratories.
The test kit is based on immunoassay technology. The test devices contain: 1) Conjugate pad: recombinant SARS-CoV-2 antigen labeled with colloidal gold which linked FITC, FITC antibody and quality control antibody gold marker. 2) NC membrane: coated with two detection lines (IgG line and IgM line) and one quality control line (C line). The IgM line coated with mouse anti-human IgM monoclonal antibody detects the COVID-19 IgM antibody. The IgG line coated with mouse anti-human IgG monoclonal antibody detects the COVID-19 IgG antibody. The C line coated with quality control antibody.
When sample is added to the sample well of the test device, it will move forward along the test device. If the sample contains IgM antibodies, antibodies will bind to the virus antigen labeled with colloidal gold, then forms a sandwich complex with the coated anti-human IgM monoclonal antibody at IgM line, IgM line will appear purplish red, prompting the COVID-19 IgM antibody is positive.
If the sample contains IgG antibodies, antibodies will bind to the virus antigen labeled with colloidal gold, then forms a sandwich complex with the coated anti-human IgG monoclonal antibody at IgG line, IgG line will appear purplish red, prompting the COVID-19 IgG antibody is positive.
If either line IgG or IgM does not show color, the negative result will be displayed. The test device also contains a quality control line C, whether there is a test line or not, quality control line C should display purplish red. Test result will be invalid if quality control line C does not show color, this sample needs to be retested.

COMPOSITION

Each test kit contains the test device, buffer, pipette (optional) and package insert.
Materials required but not provided: safety lancet (for fingertip blood), alcohol pad, timer.

STORAGE AND HANDLING

- Store the test kit in a cool, dry place between 2-30°C (36-86°F). Keep away from light. Exposure to temperature and / or humidity outside the specified conditions may result in inaccurate results.
- Do not freeze or refrigerate.
- Use the test kits at temperatures between 18-25°C.
- Use the test kits between 10-90% humidity.
- Do not use your test kits beyond the expiry date (printed on the foil pouch and box label).

Note: *The test kit is recommended to store between 2-8°C if it not used in a short time.*
All expiry dates are printed in Year-Month format. 2021-06 indicates June, 2021.

TEST PROCEDURE

1. Take out the test kit and leave it at room temperature for minimum 30 minutes.
2. Put a test device on a dust-free clean surface.
3. Apply 10μL whole blood (from vein or fingertip), serum or plasma onto the sample well of a Test Device, and then apply 2 drops (about 60-80μL) of buffer onto the sample well of a Test Device.
4. Read the result after 15 minutes.

Note:

- *For in vitro diagnostic use.*
- *Avoid contact with eyes and skin. Flush abundantly with water upon disposal if reagents are spilled.*
- *If you apply fingertip blood with pipette, please wipe the rest of blood on the fingertip with alcohol pad.*

LIMITATIONS

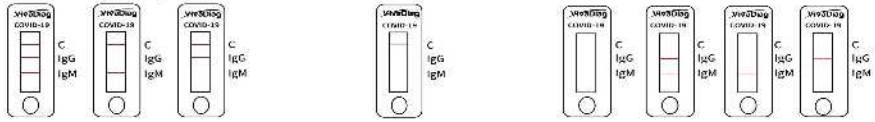
- The test result can not be used for diagnosis of COVID-19. If the result does not match the clinical evaluation, please do more testing.
- Please do not use highly hemolytic samples .
- Please do not reuse the test device.
- The test kit can only be used with whole blood (from vein or fingertip), serum or plasma. If use other samples, it may cause wrong results.
- Please make sure read the result at 15 minutes. If at other time, it may cause wrong results.
- Please follow the package insert when testing.

INTERPRETATION OF TEST RESULTS

1. Positive result:
1) The COVID-19 IgM antibody is detected if the quality control line C and the detection line IgM are both colored, and the detection IgG line is not colored, that means the COVID-19 IgM antibody is positive.
2) The COVID-19 IgG antibody is detected if the quality control line C and the detection IgG line are both colored, and the detection IgM line is not colored, that means the COVID-19 IgG antibody is positive.
3) The COVID-19 IgG and IgM antibodies are detected if the quality control line C, the detection line IgM and IgG are both colored, that means both the COVID-19 IgG and IgM antibodies are positive.

2. Negative result:
If there is only quality control line C is colored, IgG and IgM detection lines are not colored, the COVID-19 IgM/IgG antibody is not detected, that means the result is negative.

3. Invalid result:
If the quality control line C is not colored, no matter whether the detection line IgG/IgM is colored or not, the result is invalid and needs to be tested again.



Positive: At least one purplish red line (IgG, IgM line) and one purplish red quality control line (C) appear in the detection area.

Negative: Only the quality control line (C) appears in the detection area.

Invalid: No purplish red quality control line (C) appears in the detection area no matter the IgG/IgM line is colored or not.

PERFORMANCE

From the results of 350 negative and positive samples, we can confirm that VivaDiag™ COVID-19 IgM/IgG rapid test has good clinical performance.

Clinical Performance of negative sample			
Negative Cases (By PCR&CT)	Negative coincidence rate (VivaDiag™ COVID-19 IgM)	Negative coincidence rate (VivaDiag™ COVID-19 IgG)	Negative coincidence rate (Total)
200	200 (100%)	200 (100%)	200 (100%)
Clinical Performance of positive sample (infection time 4-10 days)			
Positive Cases (By PCR&CT)	Positive coincidence rate (VivaDiag™ COVID-19 IgM)	Positive coincidence rate (VivaDiag™ COVID-19 IgG)	Positive coincidence rate (Total)
80	65 (81.25%)	30 (37.5%)	65 (81.25%)
Clinical Performance of positive sample (infection time 11-24 days)			
Positive Cases (By PCR&CT)	Positive coincidence rate (VivaDiag™ COVID-19 IgM)	Positive coincidence rate (VivaDiag™ COVID-19 IgG)	Positive coincidence rate (Total)
70	68 (97.1%)	67 (95.7%)	68 (97.1%)

Relative Specificity: 100%
Relative Sensitivity (infection time 4-10 days, IgM & IgG): 81.25%
Relative Sensitivity (infection time 11-24 days, IgM & IgG): 97.1%
Accuracy (infection time 4-10 days, IgM & IgG): 94.6%
Accuracy (infection time 11-24 days, IgM & IgG): 99.3%
Total accuracy: 95.1%

INDEX OF SYMBOLS

	Consult instructions for use		Use by	Σ	Contains sufficient for <n> tests
IVD	For *in vitro* diagnostic use only	LOT	Lot number	REF	Catalog number
2°C 30°C	Storage temperature limitations		Manufacturer		Do not reuse
EC REP	Authorized Representative				

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