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

Post-market lot-to-lot evaluation of the VivaDiag serological assay for COVID-19

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Office of Health Protection, Commonwealth Government of Australia

The Therapeutics Goods Administration (TGA) of Australia

Report prepared by:

Mr Wayne Dimech

Ms Shannon Curley

Dr Katherine Bond

Ms Tuyet Hoang

Dr Mike Catton

Professor Benjamin Howden

Professor Deborah Williamson

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Executive Summary

Here, we present results of the post-market lot-to-lot evaluation of the VivaDiag serological assay for the detection of SARS-CoV-2. A previous full post-market evaluation was undertaken by the Doherty Institute using a cohort of stored serum prior to the COVID-19 outbreak in Australia, and on serum specimens collected from patients with SARS-CoV-2 infection confirmed by molecular testing. The previous VivaDiag Report containing this data analysis was issued in April 2020. In September 2020, the Doherty Institute established a collaboration with the National Serology Reference Laboratory, Australia (NRL) to undertake future post-market evaluations using a different set of samples. The new panel of specimens have high volume, are well-characterised and will allow for comparison of performance across test kits.

It must be noted that the panel of specimens used in this study are plasma rather than serum. All positive specimens used in the current study were obtained from RT-PCR positive patients with the plasma specimens taken at least 14 days after symptom onset.

1. Introduction

This work continues the post-market validation work first reported on 29th April by the Doherty Institute. Following the initial laboratory responses and release of the viral whole genome sequence by Chinese investigators in early January 2020, there was a rapid development of serological assays for COVID-19.[1–3] The first serological tests for COVID-19 were 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 undergone an expedited assessment from the Australian Therapeutic Goods Administration (TGA). As such,

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post-market validation of COVID-19 diagnostic kits that are listed on the Australian Register of Therapeutic Goods (ARTG) has been undertaken. Here, we present the results of the VivaDiag COVID-19 IgM/IgG Rapid Test kit (listed on the ARTG) lot-to-lot variation evaluation.

2. Methods

2.1   Establishment of patient cohorts and serum samples

Specimens acquired by NRL have been used in this study and will be used in future post market evaluations.

Lot-to-lot variation

A cinical sensitivity panel of 100 plasma specimens were obtained from 100 unique patients with SARS-CoV-2 detected by RT-PCR from upper and / or lower respiratory tract specimens. The plasma specimens were collected no less than 14 days post infection. This time-period allows for the development of an immune response. Detectable SARS-CoV-2 IgG was confirmed in each sample using a chemiluminescent immunoassay. Two specimens in the clinical sensitivity panel were used to create a doubling-dilution series in negative plasma to determine lot-to-lot variation across all reagent batch lots.

2.2   Test Kits

There were four reagent batch lots of the VivaDiag COVID-19 IgM/IgG Rapid Test kits (VivaDiag) included in this study (Table 1). All test kits were stored in a temperature and

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humidity-controlled environment. All testing was performed according to the manufacturers' instructions for use (IFU). The same panel of specimens were tested on each test kit.

This test kit is a lateral flow serological assay and similar to other POCTs evaluated, include the following features:

    i.   single use immunochromatographic lateral flow test, for the detection of IgM and IgG in serum, plasma or whole blood

    ii.   the specific SARS-CoV-2 recombinant antigen(s) incorporated into the assay is not described

    iii.   IFU indicates that test results should not be used as the sole basis for clinical management decisions, requiring interpretation alongside clinical features.

Table 1. VivaDiag reagent batch numbers tested in the post market study.

Test Kit	Manufacturer	Batch Numbers #
VivaDiag	Vivachek Biotech (Hangzhou) *Stonestar Wholesale*	E2005005
		E2005006
	Vivachek Biotech (Hangzhou) *Device Technologies Australia*	E2005033
		E2003011

Immunochromatographic assays involve the detection of anti-SARS-CoV-2 IgM and/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.

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2.3   Testing protocol

Testing of the lateral flow assays was performed at NRL by three laboratory technicians, all of whom have undergone previous training in the use of lateral flow assays. Testing was performed exactly as per the IFU. For all testing, lateral flow test strips were each read by two different technicians. A third read by a third technician was undertaken if the first two reads were discordant, with the third read taken as the final result. Reading by all three scorers were made within the time-frame specified by the manufacturer.

Any specimen with an invalid test result was repeated on the same lot number, if sufficient test devices were available. If the subsequent test reported a valid result, that result was used as the final result. If the specimen was repeatedly invalid that specimen was removed from analysis, but the total number of invalid and discordant results were noted.

2.4   Statistical analysis

Lot-to-lot variation – The highest dilution of two samples having a reactive test result was compared across four reagent batch lots.

2.5   Ethics

The specimens used in this study were provided to NRL by third-party organisations, including national blood transfusion services. All specimens were collected from individuals with informed consent under various ethics approvals.

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3. Results

3.1 Lot-to-lot analysis

The IgG and IgM results from testing the dilution series in four reagent lots are summarised in Table 2.

Table 2. Results of testing dilution series of two positive specimens to determine lot- to-lot comparison of the VivaDiag COVID-19 IgG/IgM Rapid Test. The highest dilution recording a positive test result was determined as the end point.

VivaDiag Assay [Lot number]	Test Results Sample 1		Test Results Sample 2	
	IgG	**IgM**	**IgG**	**IgM**
E2005005	1:32	1:256	1:4	>1:1024
E2005006	1:32	1:256	1:8	1:64
E2005033	1:32	1:128	1:4	1:4
E2003011	1:32	1:128	1:8	1:64

4. Discussion

Results of the lot-to-lot post-market evaluation of four VivaDiag anti-SARS-Cov-2 rapid test kit reagent batches are presented. This study was performed by NRL, using a different panel of specimens than the previous Doherty reports.

There was no significant lot-to-lot variation detected for IgG in each sample across the four reagent lots. There was no significant lot-to-lot variation detected for IgM in Sample 1 across

the four reagent lots however there was some variation detected for IgM in Sample 2 across the four reagent lots.

5. Acknowledgements

We thank the NRL scientific and technical staff who compiled the panel of specimens and performed the testing.

6. References

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