Manufacturer Report – SARS-CoV-2 Serology Test Kit Evaluation Results

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Report Prepared for the:

VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total

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Introduction

In November 2019, a novel acute respiratory disease (COVID-19) caused by a new coronavirus (SARS-CoV-2) was first recognized. Since this time, a major global pandemic has ensued, causing significant mortality and morbidity and economic disruption. Due to the high level of concern regarding the spread of SARS-CoV-2 infection, regulators in many countries removed their usual strict regulatory requirement that *In-vitro* diagnostic device (IVD) manufacturers demonstrate evidence of adequate performance, safety and quality. Most, if not all regulators allowed use of IVDs under emergency use conditions requiring limited pre-market evidence of performance. Within six months, more than 700 IVDs used for the diagnosis of SARS-CoV-2 infection were available on the market. In parallel, studies were performed assessing the performance of these IVDs. Many studies were poorly structured, had inappropriate interpretation of test results and assessed small numbers of test kits in each study. Until recently, few findings were published in peer-reviewed journals.

Due to the seriousness of the situation, the National Serology Reference Laboratory, Australia (NRL), a WHO Collaborating Center and authorized WHO IVD Prequalification Evaluation Laboratory, established an evaluation protocol for serology tests for SARS-CoV-2. The protocol was designed to assess the performance characteristics of the IVDs using a large and diverse set of carefully selected samples. The samples were acquired in sufficient volume to conduct head-to-head evaluations of large numbers of test kits.

Aims

The primary aims of this study are to:

- Produce statistically significant and scientifically robust assessment of the performance of test kits in different formats, designed to detect antibodies to SARS-CoV-2;
- Compare performance data from a range of commercially available IVDs by testing the same panel of samples;
- Create a database of IVD performance results to inform the selection of test kits used for screening, confirmation, and possibly, diagnosis of SARS-CoV-2 infection;

- Collect testing data that may allow for the assessment of analytes such as IgA and IgM, which do not have reference or "gold standard" methods available.

This report summarizes the analysis of results when testing the performance panel using the IVD detailed below.

Product Details

Name of Test Kit	VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total
Product Number	619 9922
Name of Manufacturer	Ortho-Clinical Diagnostics, Inc.
Test Kit Type	ChLIA
Antibody Class Detected	Total Antibody (including IgM/ IgG/ IgA)
Assay IFU Version	GEM1293_US_EN (Version 3.2)
Reagent Lots Evaluated	330 & 351

Method

A detailed description of the method was published and distributed to participating manufacturers prior to the study. In brief, the protocol assesses the performance of tests according to several key criteria summarized below.

- Sensitivity
- Specificity
- Analytical Sensitivity
- Lot-to-lot Variation
- Seroconversion
- Cross-reactivity
- Interference
- Repeatability (Semi / Quantitative assays only)

Sensitivity: A total of 199 serum or plasma specimens obtained from SARS-CoV-2 infected individuals confirmed positive by SARS-CoV-2 nucleic acid testing (NAT) or clinical infection were used as the sensitivity panel. These samples were collected from time periods that ranged from 14 days to 71 days post onset of symptoms or post NAT positive result. It is noted that for the shorter timeframes there may be the possibility of some individuals not developing an IgG response in this time. If this is the case, all test kits will be subjected to the same inconsistency. Future analysis of all test kit results may help elucidate this situation if it occurs. Also note, there are no reference tests for IgM and IgA specific antibodies to SARS-CoV-2 and the immune reaction to these antibody classes are yet to be confirmed. Therefore "sensitivity" of assays specifically for IgM, IgA and combined IgG/IgM should be interpreted with these caveats in mind. Approximately half of the sensitivity panel samples were tested on one reagent lot and the other half, tested on a separate reagent lot which were provided by the manufacturer.

Specificity: A total of 300 plasma specimens obtained from NRL's sample bank, having been collected prior to November 2019, were used as the specificity panel. Given the emergence of SARS-CoV-2 cases in late 2019, these specimens are assumed negative for SARS-CoV-2 antibodies and no further confirmation testing was performed. Approximately half the panel of samples were tested on one reagent lot and the other half tested on a separate reagent lot, which were provided by the manufacturer.

Analytical Sensitivity/Lot-to-lot Variation: Three of the samples included in the sensitivity panel each had 10 doubling dilutions prepared in human plasma negative for SARS-CoV-2 antibodies. All dilutions were tested on two reagent lots and the results of reactivity compared. The lowest concentration with a reactive result in each dilution series, irrespective of the lot number, was deemed the limit of detection for that test kit.

Cross-reactivity: A total of 55 plasma or serum samples known to contain potentially cross-reacting analytes were tested in a single reagent lot, that was provided by the manufacturer. A summary of the analytes included in the cross-reaction study are presented in Annex 1.

Interfering substances: A total of 34 plasma samples known to contain potentially interfering substances were tested in a single reagent lot. The interfering substance panel consisted of:

- Five icteric samples;
- Five haemolysed samples;
- Six samples with high levels of bilirubin;
- Five lipaemic samples;
- Five samples with anti-nuclear antibodies;
- Three samples positive for antibodies to double-stranded DNA (Lupus);
- Five samples positive for rheumatoid factor.

In addition, two positive samples, obtained from the sensitivity panel, were each diluted 1:2 in icteric, lipaemic and haemolysed samples (derived from the 34 interfering samples above). These six spiked samples were tested to detect interference to reactivity caused by interfering substances.

Reverse seroconversion panels: The reverse seroconversion panel was comprised of 47 plasma samples, taken at varying intervals commencing 18 days or later, from 10 different symptomatic individuals. The purpose of this panel is to demonstrate the decline in antibody titre over time, in particular the IgM response.

Seroconversion panels: Consisted of a total of 60 plasma samples, collected from five different SARS-CoV-2 NAT positive individuals at regular intervals from early infection to approximately 8 weeks post symptoms. Results of testing were used to determine the number of days post infection the test kit first detected reactivity.

Repeatability: For repeatability studies, a selected specimen/commercial anti-SARS-CoV-2 quality control (QC) sample will be tested 30 times in the same test run and presented as the percentage coefficient of variation (%CV).

Testing

NRL provided the anti-SARS-CoV-2 sample panel to the manufacturer and testing was performed independently (external to NRL). The manufacturer was blinded to the reference results and the subsets of the samples being tested. All samples were tested in singlicate (sample volume permitting).

Results

Validation of results

Valid test results were determined according to the acceptance criteria contained in the test kit IFU.

Analysis of the data was performed by NRL.

Panel Test Results

The results of testing the panel of samples using the VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total are summarized below:

Sensitivity

The results of the sensitivity panel of 199 positive samples are presented in Table 1.

Table 1: Number of samples in the sensitivity panel with reactive / non-reactive results

No. of total antibody reactive	No. of total antibody non-reactive
199	0

Concordance of total antibody reactivity with infection was **100%** [95% CI: 97.6 - 100].

Specificity

The results of the specificity panel of 300 negative samples are presented in Table 2.

Table 2: Number of samples in the specificity panel with non-reactive / reactive results

No. of total antibody non-reactive	No. of total antibody reactive
290	3

There were seven samples in the specificity panel that had insufficient volume to complete testing. These samples have been removed from the analysis.

The specificity of total antibody reactivity with expected negative sample results was **99.0%** [95% CI:96.8 – 99.7].

Analytical Sensitivity / Lot-to-lot Variation

The serial doubling dilution of three SARS-CoV-2 antibody positive samples were tested in two reagent lots. The highest doubling dilution having any level of detectable reactivity (including equivocal test results) are presented in Table 3.

Table 3: Analytical Sensitivity of three SARS-CoV-2 antibody positive samples

Reagent Lot No.	330	351
Positive Sample-461	>1:1024	>1:1024
Positive Sample-491	1:128	1:128
Positive Sample-492	>1:1024	>1:1024

Cross-reactivity

Of the 55 samples having potentially cross-reacting analytes, there was insufficient volume to complete testing for one sample. Of the remaining 54 samples, no samples demonstrated false reactivity for total antibody.

Interference

Of the 34 samples having potentially interfering substances, there were no samples that yielded a false total antibody result.

Two antibody positive samples obtained from the Sensitivity Panel were each diluted 1:2 with a) icteric b) lipaemic and c) haemolysed samples (total of six spiked samples).

There were no false negative results detected in the six spiked positive samples.

Reverse seroconversion panels

The results of testing the reverse seroconversion panels are summarized in Table 4.

Table 4: Reverse seroconversion panel sample results

Donor ID	Sample	Days post symptoms	Total antibody result
13916	1	35	Insufficient sample volume
	2	39	R
	3	45	R
	4	49	R
13921	1	24	R
	2	27	R

	3	32	R
	4	35	R
	5	39	R
	6	42	R
13941	1	27	R
	2	34	R
	3	37	R
	4	40	R
	5	44	R
	6	47	R
13947	1	24	R
	2	32	R
	3	35	R
13954	2	31	R
	3	34	R
	4	37	R
	5	41	R
13980	1	26	R
	2	31	R
	3	34	R
	4	37	R
	5	41	R
	6	44	R
14000	1	37	R
	2	40	R
	3	43	R
	4	47	R
	5	50	R
14021	1	18	R
	2	21	R
	3	24	R
	4	27	R
	5	31	R
14041	1	34	R
	2	39	R
	3	42	R
	4	45	R
14066	1	37	R
	2	41	R
	3	44	R
	4	47	R

Key: R-Reactive

Seroconversion panels

The results of the 60 seroconversion panel samples tested are presented in Table 5.

Table 5. Seroconversion panel sample results

Donor ID	Sample	Days post symptoms	Total antibody result
COV-501	1	2	NR
	2	5	NR
	3	10	R
	4	14	R
	5	18	Insufficient sample volume
	6	22	R
	7	26	R
	8	30	R
	9	33	R
	10	37	R
	11	46	R
COV-504	1	6	NR
	2	9	NR
	3	16	R
	4	21	R
	5	27	R
	6	34	R
	7	37	R
	8	42	R
	9	48	R
	10	52	R
COV-506	1	1	R
	2	6	R
	3	9	R
	4	12	R
	5	15	R
	6	19	R
	7	26	R
	8	28	R
	9	51	R
COV-510	1	-8	NR
	2	-4	R
	3	-1	R
	4	3	R
	5	6	R
	6	10	R
	7	13	R

	8	17	R
	9	20	R
	10	24	R
	11	28	R
	12	32	R
	13	35	R
	14	38	R
	15	41	R
	16	52	R
COV-512	1	3	R
	2	6	R
	3	13	R
	4	17	R
	5	20	R
	6	24	R
	7	27	R
	8	31	R
	9	34	R
	10	38	R
	11	41	R
	12	44	R
	13	47	R
	14	51	R

Key: R-Reactive, NR-Non-reactive

Repeatability

The precision of the QC sample, measured as the percentage coefficient of variation (CV%), for repeatability (within run, using Lot:330) was **1.9%**.

Conclusion

The results in this report present a snapshot of the performance characteristics of two reagent lots. Ongoing monitoring of the performance of the test kits using quality assurance programs such as External Quality Assessment Schemes and/or quality controls programs are highly recommended. The assessment provides the manufacturer independent evidence of the test kit performance. Similar performance testing of other test kits, using the same panel of samples, protocol and analysis will be published, allowing the manufacturer and users to compare results.

It should be noted that when comparing results, the different formats and uses of the test kits should be taken into consideration. Each test kit will detect different antibody isotypes to different antigens and have varying sensitivity and specificity. Some tests are expected to detect antibody responses early whereas others may be reactive later in disease. Knowledge of the performance characteristics will allow users to determine whether the test kit is fit for their intended purpose. Therefore, differences in test kit performance can be beneficial attributes.

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Annex 1. Cross-reacting sample panel composition

A total of 55 samples were included in the cross-reacting sample panel. Table 6 below demonstrates the 55 samples that contain potentially cross-reacting analytes, noting that some samples contained multiple analytes.

Table 6: Composition of Cross-reacting Panel

Analyte	Number of Samples
CMV IgM positive	4
EBV VCA IgM positive	2
Influenza A positive	3
Influenza B positive	3
Hepatitis A IgM positive	1
Hepatitis B e antigen positive	3
Hepatitis B surface antigen	5
Hepatitis B surface antigen/ Hepatitis B c IgM positive	1
Hepatitis B surface antigen/ Hepatitis B c IgM / Hepatitis B e antigen positive	1
Hepatitis C virus antibody positive	4
HIV antibody positive	8
Malaria antibody positive	5
Mycoplasma IgM positive	1
Parainfluenza positive	1
Parvovirus IgM positive	2
Psittacosis positive	1
Rubella IgM positive	1
Syphilis positive	6
Toxoplasma IgM positive	3

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